IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

NEVAKAR INJECTABLES, INC., *Plaintiff*, v. BAXTER HEALTHCARE CORP., *Defendant.*	C.A. No. 1:21-cv-01186-CJB **JURY TRIAL DEMANDED**

FIRST SUPPLEMENTAL COMPLAINT FOR PATENT INFRINGEMENT

Plaintiff Nevakar Injectables, Inc. ("Nevakar") hereby brings this action against Defendant Baxter Healthcare Corp. ("Baxter") for infringement of the claims of U.S. Patent Nos. 10,420,735 ("the '735 patent"); 10,471,026 ("the '026 patent"); 10,568,850 ("the '850 patent"); 10,646,458 ("the '458 patent"); and 11,602,508 ("the '508 patent") (collectively, "Patents-in-Suit").

NATURE AND SUMMARY OF THIS ACTION

1. This is an action for patent infringement arising under the patent laws of the United States, 35 U.S.C. § 1 *et seq*.

THE PARTIES

2. Plaintiff Nevakar is a corporation organized and existing under the laws of the state of Delaware, having a principal place of business at 1019 US Highway 202 #206, Bridgewater, NJ 08807.

3. Upon information and belief, Defendant Baxter is a corporation organized and existing under the laws of the state of Delaware, having a principal place of business at One

Baxter Parkway, Deerfield, IL 60015.

4. Upon information and belief, Defendant Baxter is a pharmaceutical company that develops, manufactures, markets and/or distributes pharmaceutical products around the world, including in this Judicial District.

JURISDICTION AND VENUE

5. This Court has subject-matter jurisdiction over this action pursuant to 28 U.S.C. §§ 1331 and 1338(a) because this action involves substantial claims arising under the United States Patent Act (35 U.S.C. §§ 1 *et seq.*), as well as under the Declaratory Judgment Act (28 U.S.C. §§ 2201 and 2202) because this action involves an actual case or controversy concerning the infringement of the Patents-in-Suit.

6. This Court has personal jurisdiction over Baxter because, among other reasons, it is incorporated in the state of Delaware and has designated its registered agent as The Corporation Trust Company, Corporation Trust Center, 1209 Orange Street, Wilmington, Delaware 19801.

7. Venue is proper in this judicial district under 28 U.S.C. § 1400(b) because Baxter is incorporated in the state of Delaware.

THE PATENTS-IN-SUIT

8. The ’508 patent, titled “Norepinephrine Compositions and Methods Therefor,” was duly and legally issued by the United States Patent and Trademark Office (“USPTO”) on March 14, 2023. Nevakar is the assignee of the ’508 patent. A true copy of the ’508 patent is attached as Exhibit A.

9. The ’735 patent, titled “Norepinephrine Compositions and Methods Therefor,” was duly and legally issued by the USPTO on September 24, 2019. Nevakar is the assignee of the ’735 patent. A true copy of the ’735 patent is attached as Exhibit B.

10. The ’026 patent, titled “Norepinephrine Compositions and Methods Therefor,” was duly and legally issued by the USPTO on November 12, 2019. Nevakar is the assignee of the ’026 patent. A true copy of the ’026 patent is attached as Exhibit C.

11. The ’850 patent, titled “Norepinephrine Compositions and Methods Therefor,” was duly and legally issued by the USPTO on February 25, 2020. Nevakar is the assignee of the ’850 patent. A true copy of the ’850 patent is attached as Exhibit D.

12. The ’458 patent, titled “Norepinephrine Compositions and Methods Therefor,” was duly and legally issued by the USPTO on May 12, 2020. Nevakar is the assignee of the ’458 patent. A true copy of the ’458 patent is attached as Exhibit E.

NEVAKAR’S NOREPINEPHRINE BITARTRATE IN 5% DEXTROSE PRODUCT

13. Nevakar is the holder of New Drug Application (“NDA”) No. 214628 for norepinephrine in sodium chloride injection, which has been approved by the FDA. There are three strengths of the Nevakar Products: 4 mg (16 µg/ml), 8 mg (32 µg/ml), and 16 mg (64 µg/ml) norepinephrine bitartrate, each of which is stored in 250 mL infusion bags. The Nevakar Products, once approved by FDA, will be indicated for the restoration of blood pressure in adult patients with acute hypotensive states. The Nevakar Products are ready-to-administer products that require no further dilution prior to infusion.

14. Norepinephrine is typically used during cardio-pulmonary resuscitation, in the treatment of cardiac arrest and profound hypotension, and for blood pressure control in certain acute hypotensive states. Currently-available norepinephrine drug products are not ready-to-administer, but rather are concentrated formulations requiring dilution prior to injection. Once diluted, the currently-available products degrade quickly and must be used within one day when stored at room temperature.

15. Nevakar undertook substantial efforts to address the limitations of currently-

available norepinephrine products. Based on intensive research and development starting in 2015, requiring specialized equipment and approximately 50% of Nevakar's research and development resources, Nevakar invented stable, ready-to-administer products. Consequently, Nevakar was awarded the Patents-in-Suit. Nevakar depends on its ready-to- administer norepinephrine products as a major driver of revenue in the near term.

BAXTER'S NOREPINEPHRINE BITARTRATE PRODUCTS

16. Baxter filed NDA No. 214313 under Section 505(b)(2) of the Federal Food Drug and Cosmetic Act ("FDCA") on March 16, 2020 for ready-to-administer formulations of norepinephrine bitartrate in 5% dextrose (the "Baxter Products"). FDA approved that NDA on January 15, 2021 "for restoration of blood pressure in adult patients with acute hypotensive states." (*See* Ex. F (true copy of FDA Approval Letter for NDA No. 214313, dated January 15, 2021); Ex. G at 1 (true copy of product label for Baxter Products).)

17. There are two strengths of the Baxter Products: 4 mg (16 µg/ml) and 8 mg (32 µg/ml) norepinephrine bitartrate, each of which is stored in a 250 mL infusion bag. (Ex. G at 1, 7; Ex. H at 3 (true copy of FDA Product Quality Review for NDA No. 214313).) The norepinephrine bitartrate used in the Baxter Products is a chiral compound in the R-configuration. (Ex. H at 3.) The Baxter Products' inactive ingredients include: 50 mg/ml dextrose monohydrate, water, and a combination of sodium hydroxide and hydrochloric acid. (Ex. G at 7.) The pH of the Baxter Products is 3.5-3.9 (*id.*), with an average pH of 3.8 (Ex. H at 21). The Baxter Products do not contain any antioxidants. (Ex. H at 18.)

BAXTER'S OFFER FOR SALE OF ITS INFRINGING PRODUCTS

18. Upon information and belief, Baxter has offered to enter and/or has entered a sales contract with Vizient, Inc. ("Vizient"), and other potential customers, for delivery of Baxter Products starting on or about September 1, 2021. On July 15, 2021, Vizient, a group purchasing

organization representing more than half of the healthcare organizations in the United States, sent Par Sterile a Request for Proposal ("RFP") stating that Vizient will entertain offers from pharmaceutical manufacturers to supply Norepinephrine Bitartrate in 5% dextrose injection from September 1, 2021 through March 31, 2024.

19. On September 23, 2021, the Baxter "announced the U.S. Food and Drug Administration (FDA) approval and commercial launch of premix Norepinephrine Bitartrate in 5% Dextrose Injection (norepinephrine)."

20. Baxter Products appeared in the Price Rx® database. Price Rx® provides pharmacy benefit managers and manufacturers current information regarding, *inter alia*, which pharmaceutical products have been offered for sale. The Price Rx® listing of the Baxter Products, complete with detailed information such as the number of doses per package, shows that Baxter is now infringing the Patents-in-Suit by offering to sell the Baxter Products..

21. By letter dated February 19, 2021, Endo, through its affiliate Par Sterile, (and formerly the exclusive licensee of the '735 patent, the '026 patent, the '850 patent, and the '458 patent) notified Baxter that the Patents-in-Suit cover ready-to-administer norepinephrine products. Endo requested that Baxter consider these patents before launching the Baxter Products and to respect Endo's intellectual property rights.

COUNT ONE
Baxter's Infringement of the '508 Patent

22. Plaintiff re-alleges and incorporates each of the preceding paragraphs as if fully set forth herein.

23. Baxter's commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States of the Baxter Products constitutes infringement of at least claim 1 of the '508 patent, both directly under 35 U.S.C. § 271(a) and indirectly under

35 U.S.C. §§ 271(b) and 271(c), literally and/or under the doctrine of equivalents.

24. Claim 1 of the '508 patent reads as follows:

> A ready-to-administer norepinephrine composition, comprising:
>
> an aqueous solution having a pH range of between 3.7 and 4.3, wherein the aqueous solution comprises:
>
> norepinephrine present at a concentration of between 10 µg/ml and 100 µg/ml, wherein the norepinephrine initially comprises at least 95% of R-isomer as determined by HPLC;
>
> a chelating agent comprising a tartrate bicarboxylic acid, wherein the chelating agent is present at a concentration of between 10 µg/ml and 100 µg/ml; and
>
> a tonicity agent,
>
> wherein the ready-to-administer norepinephrine composition is substantially free of antioxidants; and
>
> wherein after storage at 25±2° C. and 60±5% relative humidity over at least three months, the norepinephrine comprises at least 90% R-isomer as determined by HPLC.

25. Each of the Baxter Products is "[a] ready-to-administer norepinephrine composition." (*See, e.g.*, Ex. G at 2.)

26. Each of the Baxter Products contain "an aqueous solution having a pH range of between 3.7 and 4.3." (*See, e.g.*, Ex. H at 21.)

27. The "aqueous solution" of the Baxter Products also "comprises a chelating agent," namely the "bitartrate" component of the Baxter Products' active pharmaceutical ingredient. (*See, e.g.*, Ex. G at 1.) The '508 patent's specification discloses that "tartrate" is one example of a chelating agent. (Ex. A at 5:2-8.)

28. The "chelating agent" of the Baxter Products "is present in an amount of between 10 µg/ml and 100 µg/ml." The 4 mg strength of the Baxter Products contains roughly 15.9 µg/mL of bitartrate monohydrate (of which 14.10 µg/mL is the bitartrate concentration) which is the

difference between the 31.9 µg/mL of norepinephrine bitartrate monohydrate supplied and the resulting 16 µg/mL dose of norepinephrine base. (*See, e.g.*, Ex. G at 7.) The 8 mg strength of the Baxter Products contains roughly 31.8 µg/mL of bitartrate monohydrate (of which 28.20 µg/mL is the bitartrate concentration) which is the difference between the 63.8 µg/mL of norepinephrine bitartrate monohydrate supplied and the resulting 32 µg/mL dose of norepinephrine base. (*See, e.g.*, *Id.*)

29. The "norepinephrine" in the Baxter Products is "dissolved at a concentration suitable for administration to a patient in need thereof, wherein the norepinephrine is an R-isomer." (*See, e.g.*, Ex. H at 3.)

30. The Baxter Products are "substantially free of antioxidants." (*See, e.g.*, Ex. H at 18.)

31. On information and belief, the Baxter Products are formulated such that "after storage at 25±2° C. and 60±5% relative humidity over at least three months, the norepinephrine comprises at least 90% R-isomer as determined by HPLC.." (*See, e.g.*, Ex. H at 5-6.)

32. Plaintiff is entitled to a judgment that the commercial manufacture, use, offer to sell, or sale within the United States, and/or importation into the United States, of the Baxter Products, or the inducement of and/or contribution to the commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States, of the Baxter Products before expiration of the '508 patent by Baxter or its agents, constitutes infringement, inducement of infringement, and/or contributory infringement of the '508 patent under 35 U.S.C.§§ 271(a), (b), and/or (c).

33. Plaintiff will be irreparably harmed if Baxter is not enjoined from infringing, inducing, or contributing to infringement of the '508 patent. Plaintiff does not have an

adequate remedy at law to fully compensate Plaintiff for its damages.

34. Baxter's infringement of the '508 patent is willful, entitling Plaintiff to enhanced damages. Baxter knew that its Baxter Products would infringe the '508 patent no later than March 14, 2023.

35. This case is exceptional and Plaintiff is entitled to an award of reasonable attorney fees under 35 U.S.C. § 285.

COUNT TWO
Baxter's Infringement of the '735 Patent

36. Plaintiff re-alleges and incorporates each of the preceding paragraphs as if fully set forth herein.

37. Baxter's commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States of the Baxter Products constitutes infringement of at least claim 1 of the '735 patent, both directly under 35 U.S.C. § 271(a) and indirectly under 35 U.S.C. §§ 271(b) and 271(c), literally and/or under the doctrine of equivalents.

38. Claim 1 of the '735 patent reads as follows:

> A method of treating hypotension, comprising:
>
> administering a ready-to-administer norepinephrine composition at an initial dose per minute;
>
> administering the norepinephrine composition at a maintenance dose per minute, wherein the initial dose per minute is greater than the maintenance dose per minute;
>
> wherein the initial dose per minute is a dose of between 8 and 12 µg/min, and wherein the maintenance dose per minute is a dose of between 2 and 4 µg/min;
>
> wherein the norepinephrine composition comprises norepinephrine or a salt thereof at a concentration of between 10 µg/ml and 100 µg/ml in an aqueous acidic solution having a pH range of between 3.7 and 4.3, wherein the aqueous acidic solution further comprises a chelating agent at a concentration of between 1 µg/ml and 100 µg/ml and a tonicity agent;

> wherein the norepinephrine composition is substantially free of antioxidants; and
>
> wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

39. Baxter instructs and actively induces physicians to use the Baxter Products to "treat[] hypotension." (*See, e.g.*, Ex. G at 1.)

40. Baxter instructs and actively induces physicians to "administer[] a ready-to-administer norepinephrine composition [i.e., the Baxter Products] at an initial dose per minute" followed by "administering the norepinephrine composition at a maintenance dose per minute, wherein the initial dose per minute is greater than the maintenance dose per minute[,] wherein the initial dose per minute is a dose of between 8 and 12 μg/min, and wherein the maintenance dose per minute is a dose of between 2 and 4 μg/min." (*See, e.g.*, Ex. G at 2-3.)

41. The Baxter Products contain "norepinephrine or a salt thereof at a concentration of between 10 μg/ml and 100 μg/ml in an aqueous acidic solution having a pH range of between 3.7 and 4.3." (*See, e.g.*, Ex. G at 1, 7; Ex. H at 21.)

42. "[T]he aqueous acidic solution" of the Baxter Products "further comprises a chelating agent at a concentration of between 1 μg/ml and 100 μg/ml and a tonicity agent."

43. The "aqueous acidic solution" of the Baxter Products also "comprises a chelating agent," namely the "bitartrate" component of the Baxter Products' active pharmaceutical ingredient. (See, e.g., Ex. G at 1.) The '735 patent's specification discloses that "tartrate" is one example of a chelating agent. (Ex. B at col. 4:2-col. 6:2.)

44. The "aqueous acidic solution" of the Baxter Products also comprises "a pharmaceutically acceptable salt." The Baxter Products contain NaCl, which is created when hydrochloric acid and sodium hydroxide used to adjust the Baxter Products' pH are put into the

formulation. (See, e.g., Ex. H at 18.) The norepinephrine bitartrate in the Baxter Products is also "a pharmaceutically acceptable salt."

45. The "chelating agent" of the Baxter Products "is present in an amount of between 1 µg/ml and 100 µg/ml." The 4 mg strength of the Baxter Products contains roughly 15.9 µg/mL of bitartrate, which is the difference between the 31.9 µg/mL of norepinephrine bitartrate supplied and the resulting 16 µg/mL dose of norepinephrine base. (See, e.g., Ex. G at 7.) The 8 mg strength of the Baxter Products contains roughly 31.8 µg/mL of bitartrate, which is the difference between the 63.8 µg/mL of norepinephrine bitartrate supplied and the resulting 32 µg/mL dose of norepinephrine base. (*See, e.g., id.*)

46. As discussed *supra* ¶ 45, the Baxter Products contain either 15.9 µg/mL or 31.8 µg/mL chelating agent. The Baxter Products also contain two tonicity agents. First, the '735 patent discloses that NaCl (i.e., table salt, saline) "can be used to increase tonicity." (*See, e.g.*, Ex. B at 4:50-51; 7:9-15.) The Baxter Products contain NaCl, which is created when hydrochloric acid and sodium hydroxide used to adjust the Baxter Products' pH are put into the formulation. (*See, e.g.*, Ex. H at 18.) The Baxter Products also contain 50 mg/mL dextrose, which the '735 patent also discloses as a "suitable tonicity agent." (Ex. G at 7; Ex. B at 7:16-19.)

47. The Baxter Products are "substantially free of antioxidants." (*See, e.g.*, Ex. H at 18.)

48. "[T]he norepinephrine or a salt thereof in the [Baxter Products] comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC." The norepinephrine bitartrate used in the Baxter Products is the R-isomer of norepinephrine. (*See, e.g.*, Ex. H at 3.) Stability testing demonstrated that after 7.4 months stored at 25ºC and 40% relative humidity, 90% of the norepinephrine remained in the R-configuration, which indicates that the Baxter Products are

stable at the more mild condition of 60% relative humidity. (*See, e.g.*, *id.* at 5-6.) Moreover, it is well-known that isomerization—if it occurs at all—increases over time. So the fact that no more than 10% of the R-isomer converted to the L-isomer after 7.4 months shows that even less than 10% will convert after only three months.

49. Plaintiff is entitled to a judgment that the commercial manufacture, use, offer to sell, or sale within the United States, and/or importation into the United States, of the Baxter Products, or the inducement of and/or contribution to the commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States, of the Baxter Products before expiration of the '735 patent by Baxter or its agents, constitutes infringement, inducement of infringement, and/or contributory infringement of the '735 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

50. Plaintiff will be irreparably harmed if Baxter is not enjoined from infringing, inducing, or contributing to infringement of the '735 patent. Plaintiff does not have an adequate remedy at law to fully compensate Plaintiff for its damages.

51. Baxter's infringement of the '735 patent is willful, entitling Plaintiff to enhanced damages. Baxter knew that its Baxter Products would infringe the '735 patent no later than February 19, 2021.

52. This case is exceptional and Plaintiff is entitled to an award of reasonable attorney fees under 35 U.S.C. § 285.

COUNT THREE
Baxter's Infringement of the '026 Patent

53. Plaintiff re-alleges and incorporates each of the preceding paragraphs as if fully set forth herein.

54. Baxter's commercial manufacture, use, offer for sale, or sale within the United

States, and/or importation into the United States of the Baxter Products constitutes infringement of at least claim 1 of the '026 patent, both directly under 35 U.S.C. § 271(a) and indirectly under 35 U.S.C. §§ 271(b) and 271(c), literally and/or under the doctrine of equivalents.

55. Claim 1 of the '026 patent reads as follows:

> A method of controlling S-isomer content in a ready-to-administer norepinephrine composition comprising:
>
> admixing an R-isomer of norepinephrine or salt thereof, a chelating agent and a tonicity agent into an aqueous acidic solution having a pH between 3.7 and 4.3;
>
> wherein the chelating agent is present in an amount of between 1 µg/ml and 100 µg/ml, and wherein the tonicity agent is present in an amount of between 0.6 wt % and 1.2 wt %; and
>
> wherein the concentration of norepinephrine or salt thereof is between 10 µg/ml and 100 µg/ml, and wherein the composition is substantially free of anti-oxidants.

56. The Baxter Products are made via "[a] method of controlling S-isomer content in a ready-to-administer norepinephrine composition." (*See, e.g.*, Ex. G at 2; Ex. H at 3.)

57. The process of making the Baxter Products includes "admixing an R-isomer of norepinephrine or salt thereof, a chelating agent and a tonicity agent into an aqueous acidic solution having a pH between 3.7 and 4.3." The norepinephrine bitartrate used in the Baxter Products is an isomer of norepinephrine in the R-configuration. (*See, e.g.*, Ex. H at 3.) As discussed *supra* ¶¶ 28, 30 and 45, the Baxter Products also contain a chelating agent and tonicity agents. The pH of the Baxter Products is 3.8. (*See, e.g.*, Ex. H at 21.)

58. "[T]he chelating agent [of the Baxter Products] is present in an amount of between 1 µg/ml and 100 µg/ml," as discussed *supra* ¶¶ 28 and 30. On information and belief, the NaCl "tonicity agent" of the Baxter Products, discussed *supra* ¶ 45, "is present in an amount of between 0.6 wt % and 1.2 wt %."

59. "[T]he concentration of norepinephrine or salt thereof [of the Baxter Products] is

between 10 µg/ml and 100 µg/ml." (*See, e.g.*, Ex. G at 1, 7; Ex. H at 21.)

60. The Baxter Products are also "substantially free of anti-oxidants." (*See, e.g.*, Ex. H at 18.)

61. Plaintiff is entitled to a judgment that the commercial manufacture, use, offer to sell, or sale within the United States, and/or importation into the United States, of the Baxter Products, or the inducement of and/or contribution to the commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States, of the Baxter Products before expiration of the '026 patent by Baxter or its agents, constitutes infringement, inducement of infringement, and/or contributory infringement of the '026 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

62. Plaintiff will be irreparably harmed if Baxter is not enjoined from infringing, inducing, or contributing to infringement of the '026 patent. Plaintiff does not have an adequate remedy at law to fully compensate Plaintiff for its damages.

63. Baxter's infringement of the '026 patent is willful, entitling Plaintiff to enhanced damages. Baxter knew that its Baxter Products would infringe the '026 patent no later than February 19, 2021.

64. This case is exceptional and Plaintiff is entitled to an award of reasonable attorney fees under 35 U.S.C. § 285.

COUNT FOUR
Baxter's Infringement of the '850 Patent

65. Plaintiff re-allegse and incorporates each of the preceding paragraphs as if fully set forth herein.

66. Baxter's commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States of the Baxter Products constitutes infringement

of at least claim 1 of the '850 patent, both directly under 35 U.S.C. § 271(a) and indirectly under 35 U.S.C. §§ 271(b) and 271(c), literally and/or under the doctrine of equivalents.

67. Claim 1 of the '850 patent reads as follows:

> A sterile, ready-to-administer, packaged norepinephrine composition, comprising:
>
> a container filled with a sterile, ready-to-administer norepinephrine composition and packaged in a secondary container;
>
> wherein the sterile, ready-to-administer norepinephrine composition comprises norepinephrine or a salt thereof in an amount of between 10 µg/ml and 100 µg/ml, a chelating agent in an amount of between 1 µg/ml and 100 µg/ml, a tonicity adjusting agent in an amount of between 0.6 wt % and 1.2 wt %, and an aqueous acidic solution, wherein the norepinephrine comprises at least 95% of R-isomer of norepinephrine;
>
> wherein the sterile, ready-to-administer norepinephrine composition is substantially free of antioxidants;
>
> wherein the sterile, ready-to-administer norepinephrine composition has a pH of between 3.7 and 4.3; and
>
> wherein the sterile, ready-to-administer, packaged norepinephrine composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

68. Each of the Baxter Products is "[a] sterile, ready-to-administer, packaged norepinephrine composition, comprising[] a container filled with a sterile, ready-to-administer norepinephrine composition and packaged in a secondary container." (*See, e.g.*, Ex. G at 2, 7.) The Baxter Products are stored within "250 ml Viaflo container closure system[s] (i.e., [] infusion bag[s])," (*see, e.g.*, Ex. H at 5), which are then stored within cartons, (*see, e.g.*, Ex. F at 2).

69. The Baxter Products contain "norepinephrine or a salt thereof in an amount of between 10 µg/ml and 100 µg/ml." (*See, e.g.*, Ex. G at 1, 7; Ex. H at 21.)

70. The Baxter Products also contain "a chelating agent in an amount of between 1

µg/ml and 100 µg/ml, as discussed *supra* ¶¶ 28 and 30. On information and belief, the Baxter Products contain "a tonicity adjusting agent in an amount of between 0.6 wt % and 1.2 wt %," as discussed *supra* ¶¶ 45 and 57.

71. The Baxter Products further contain "an aqueous acidic solution, wherein the norepinephrine comprises at least 95% of R-isomer of norepinephrine." (*See, e.g.*, Ex. G at 7; Ex. H at 21.)

72. The Baxter Products are also "substantially free of antioxidants." (*See, e.g.*, Ex. H at 18.)

73. The Baxter Products have "a pH of between 3.7 and 4.3." (*See, e.g.*, Ex. H at 21.)

74. The Baxter Products also "comprise[] at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC," as discussed *supra* ¶ 47.

75. Plaintiff is entitled to a judgment that the commercial manufacture, use, offer to sell, or sale within the United States, and/or importation into the United States, of the Baxter Products, or the inducement of and/or contribution to the commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States, of the Baxter Products before expiration of the '850 patent by Baxter or its agents, constitutes infringement, inducement of infringement, and/or contributory infringement of the '850 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

76. Plaintiff will be irreparably harmed if Baxter is not enjoined from infringing, inducing, or contributing to infringement of the '850 patent. Plaintiff does not have an adequate remedy at law to fully compensate Plaintiff for its damages.

77. Baxter's infringement of the '850 patent is willful, entitling Plaintiffs to enhanced damages. Baxter knew that its Baxter Products would infringe the '850 patent no later than

February 19, 2021.

78. This case is exceptional and Plaintiff is entitled to an award of reasonable attorney fees under 35 U.S.C. § 285.

COUNT FIVE
Baxter's Infringement of the '458 Patent

79. Plaintiff re-alleges and incorporates each of the preceding paragraphs as if fully set forth herein.

80. Baxter's commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States of the Baxter Products constitutes infringement of at least claim 1 of the '458 patent, both directly under 35 U.S.C. § 271(a) and indirectly under 35 U.S.C. §§ 271(b) and 271(c), literally and/or under the doctrine of equivalents.

81. Claim 1 of the '458 patent reads as follows:

> A method of preparing a sterile, ready-to-administer norepinephrine composition, comprising the steps of:
>
> (a) combining norepinephrine or a salt thereof, a chelating agent, a tonicity adjusting agent, and an aqueous acidic solution to form a liquid parenteral composition, wherein the norepinephrine comprises at least 95% of R-isomer of norepinephrine, wherein the norepinephrine or salt thereof is present in the liquid parenteral composition in an amount of between 10 µg/ml and 100 µm/ml, wherein the chelating agent is present in the liquid parenteral composition in an amount of between 1 µg/ml and 100 µm/ml, and wherein the tonicity adjusting agent is present in the liquid parenteral composition in an amount of between 0.6 wt % and 1.2 wt %;
>
> (b) adjusting the pH of the liquid parenteral composition to a pH range of between 3.7 and 4.3;
>
> (c) filling the liquid parenteral composition into a container; and
>
> (d) heat sterilizing the liquid parenteral composition in the container to sterility to form the sterile, ready-to-administer norepinephrine composition;
>
> wherein the sterile, ready-to-administer norepinephrine

composition is substantially free of antioxidants; and

wherein the sterile ready-to-administer norepinephrine composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

82. Plaintiff is entitled to a judgment that the commercial manufacture, use, offer to sell, or sale within the United States, and/or importation into the United States, of the Baxter Products, or the inducement of and/or contribution to the commercial manufacture, use, offer for sale, or sale within the United States, and/or importation into the United States, of the Baxter Products before expiration of the '458 patent by Baxter or its agents, constitutes infringement, inducement of infringement, and/or contributory infringement of the '458 patent under 35 U.S.C. §§ 271(a), (b), and/or (c).

83. The Baxter Products are made via "[a] method of preparing a sterile, ready-to-administer norepinephrine composition." (*See, e.g.*, Ex. G at 2, 7.)

84. The method of making the Baxter Products includes "combining norepinephrine or a salt thereof, a chelating agent, a tonicity adjusting agent, and an aqueous acidic solution to form a liquid parenteral composition." (*See, e.g.*, Ex. G. at 7; Ex. H at 21; *supra* ¶ 28 regarding the Baxter Product's "chelating agent" and ¶ 45 regarding the Baxter Product's tonicity adjusting agent.)

85. "[T]he norepinephrine [of the Baxter Products] comprises at least 95% of R-isomer of norepinephrine, wherein the norepinephrine or salt thereof is present in the liquid parenteral composition in an amount of between 10 µg/ml and 100 µm/ml." (*See, e.g.*, Ex. H at 3, 21; Ex. G at 1, 7.)

86. "[T]he chelating agent [of the Baxter Products] is present in the liquid parenteral composition in an amount of between 1 µg/ml and 100 µm/ml," and, on information and belief,

"the tonicity adjusting agent [of the Baxter Products] is present in the liquid parenteral composition in an amount of between 0.6 wt % and 1.2 wt %." (*See supra* ¶¶ 28 and 30 regarding the Baxter Product's "chelating agent" and ¶¶ 45 and 57 regarding the Baxter Product's tonicity adjusting agent.)

87. The method of making the Baxter Products includes "adjusting the pH of the liquid parenteral composition to a pH range of between 3.7 and 4.3." (*See, e.g.*, Ex. H at 18, 21.)

88. The method of making the Baxter Products includes "filling the liquid parenteral composition into a container." (*See, e.g.*, Ex. H at 5.)

89. On information and belief, the method of making the Baxter Products includes "heat sterilizing the liquid parenteral composition in the container to sterility to form the sterile, ready-to-administer norepinephrine composition." (*See, e.g.*, Ex. G at 7.)

90. The Baxter Products are "substantially free of antioxidants." (*See, e.g.*, Ex. H at 18.)

91. The Baxter Products have "at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC," as discussed *supra* ¶ 47.

92. Plaintiff will be irreparably harmed if Baxter is not enjoined from infringing, inducing, or contributing to infringement of the '458 patent. Plaintiff does not have an adequate remedy at law to fully compensate Plaintifs for its damages.

93. Baxter's infringement of the '458 patent is willful, entitling Plaintiff to enhanced damages. Baxter knew that its Baxter Products would infringe the '458 patent no later than February 19, 2021.

This case is exceptional and Plaintiff is entitled to an award of reasonable attorney fees under 35 U.S.C. § 285.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff respectfully requests that the Court:

A. adjudge that Baxter has infringed directly, by inducement, and by contribution, one or more claims of the '508 patent, and that the manufacture, use, sale, offer for sale and/or importation of the Baxter Products infringe one or more claims of the '508 patent;

B. adjudge that Baxter has infringed directly, by inducement, and by contribution, one or more claims of the '735 patent, and that the manufacture, use, sale, offer for sale and/or importation of the Baxter Products infringe one or more claims of the '735 patent;

C. adjudge that Baxter has infringed directly, by inducement, and by contribution, one or more claims of the '026 patent, and that the manufacture, use, sale, offer for sale and/or importation of the Baxter Products infringe one or more claims of the '026 patent;

D. adjudge that Baxter has infringed directly, by inducement, and by contribution, one or more claims of the '850 patent, and that the manufacture, use, sale, offer for sale and/or importation of the Baxter Products infringe one or more claims of the '850 patent;

E. adjudge that Baxter has infringed directly, by inducement, and by contribution, one or more claims of the '458 patent, and that the manufacture, use, sale, offer for sale and/or importation of the Baxter Products infringe one or more claims of the '458 patent;

F. permanently enjoin Baxter, its officers, agents, servants and employees, and those in active concert or participation with any of them, from infringing any of the Patents-

in-Suit, either directly, by inducement, or by contribution;

G. award Plaintiff compensatory damages for Baxter's infringement of the Patents-in-Suit;

H. award Plaintiff increased damages under 35 U.S.C. § 284 for Baxter's willful and deliberate infringement of the Patents-in-Suit;

I. declare this to be an exceptional case under 35 U.S.C. § 285;

J. award Plaintiff its attorney fees and costs incurred in prosecuting this action, together with pre-judgment and post-judgment interest; and

K. grant Plaintiff such other and further relief as this Court deems just and proper.

Dated: April 21, 2023

/s/ Kenneth L. Dorsney

MORRIS JAMES LLP
Kenneth L. Dorsney (#3726)
Cortlan S. Hitch (#6720)
500 Delaware Avenue, Suite 1500
Wilmington, DE 19801-1494
(302) 888-6800
kdorsney@morrisjames.com
chitch@morrisjames.com

Attorneys for Plaintiff
Nevakar Injectables, Inc

OF COUNSEL:

Alexandra Haner, Ph.D., Esq.
ADRIANO & ASSOCIATES
500 Seventh Avenue, 8th Floor
New York, NY 10018
Telephone: (908) 403-8312
Facsimile: (626) 449-0402
ahaner@adrianoassociates.com

EXHIBIT A

US011602508B2

(12) **United States Patent**
Hingorani et al.

(10) **Patent No.: US 11,602,508 B2**
(45) **Date of Patent: *Mar. 14, 2023**

(54) **NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR**

(71) Applicant: **Nevakar Injectables Inc.**, Bridgewater, NJ (US)

(72) Inventors: **Tushar Hingorani**, Bridgewater, NJ (US); **Kumaresh Soppimath**, Skillman, NJ (US)

(73) Assignee: **NEVAKAR INJECTABLES INC.**, Bridgewater, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **17/872,450**

(22) Filed: **Jul. 25, 2022**

(65) **Prior Publication Data**

US 2022/0362175 A1 Nov. 17, 2022

Related U.S. Application Data

(62) Division of application No. 16/839,450, filed on Apr. 3, 2020, now Pat. No. 11,413,259, which is a division of application No. 16/239,465, filed on Jan. 3, 2019, now Pat. No. 10,646,458, which is a division of application No. 15/883,798, filed on Jan. 30, 2018, now Pat. No. 10,226,436.

(60) Provisional application No. 62/452,220, filed on Jan. 30, 2017.

(51) **Int. Cl.**

A61K 31/137	(2006.01)
A61K 9/00	(2006.01)
A61K 47/18	(2017.01)
A61P 9/02	(2006.01)
A61K 47/12	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/137*** (2013.01); ***A61K 9/0019*** (2013.01); ***A61K 47/12*** (2013.01); ***A61K 47/183*** (2013.01); ***A61P 9/02*** (2018.01)

(58) **Field of Classification Search**

CPC ... A61K 31/137; A61K 47/183; A61K 9/0019

See application file for complete search history.

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Primary Examiner — Theodore R. Howell

(74) *Attorney, Agent, or Firm* — Umberg Zipser LLP

(57) **ABSTRACT**

The inventive subject matter is directed to compositions and methods for ready-to-inject norepinephrine compositions with improved stability. Most preferably, compositions presented herein are substantially antioxidant free and exhibit less than 10% isomerization of R-norepinephrine and exhibit less than 5% degradation of total norepinephrine.

10 Claims, No Drawings

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NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR

This application is a divisional application of allowed U.S. non-provisional application with Ser. No. 16/839,450, which was filed Apr. 3, 2020, which claims priority to U.S. Pat. No. 10,646,458, which was filed Jan. 3, 2019, which claims priority to U.S. Pat. No. 10,226,436, filed Jan. 30, 2018, which claims priority to US provisional application with Ser. No. 62/452,220, filed Jan. 30, 2017.

FIELD OF THE INVENTION

The field of the invention is pharmaceutical compositions comprising norepinephrine, especially as it relates to storage stable, ready-to-inject, antioxidant free compositions, and method of manufacturing such compositions.

BACKGROUND

The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

Norepinephrine is often used during CPR (cardio-pulmonary resuscitation), and in the treatment of cardiac arrest and profound hypotension. Norepinephrine is also used for blood pressure control in certain acute hypotensive states, including for example sympathectomy, poliomyelitis, pheochromocytomectomy, spinal anesthesia, myocardial infarction, blood transfusion, and septicemia.

Currently, norepinephrine is marketed as Levophed®, which is a concentrated 4 mg per 4 mL norepinephrine bitartrate formulation to be administered by intravenous infusion following dilution with dextrose or dextrose and sodium chloride injection. Norepinephrine is also marketed by Baxter which supplies as a norepinephrine concentrate that is free of sodium metabisulfite and packaged under nitrogen. Unfortunately, most, if not all diluted commercially available norepinephrine formulations lack storage and should therefore be discarded within one day after reconstitution when stored at room temperature. Consequently, risk for microbial contamination and dilution errors is present. In addition, Levophed also contains sodium metabisulphite as an antioxidant, and carries a warning label that sulfite may cause allergic type reactions including anaphylactic shock and life threating or less severe asthmatic episodes in susceptible people. Table 1 depicts ingredients of currently marketed norepinephrine compositions.

TABLE 1

Composition of currently marketed Norepinephrine Bitartrate Products.

Ingredient	Levophed ® (Hospira)	Norepinephrine Bitartarate (Baxter)
Norepinephrine Bitartrate equivalent to Norepinephrine Base	1 mg/mL	1 mg/mL
Sodium Chloride	Isotonic	Isotonic
Sodium Metabisulphite	0.2 mg/mL	—
pH	3-4.5	3-4.5
Water for injection	q.s. 1 mL	q.s 1 mL

Stability of Levophed® and Norepinephrine bitartrate injection (Baxter), in normal saline solutions is presented in Table 2 and Table 3 where norepinephrine was diluted to a concentration of 16 μg/ml. Stability was assessed in 250 ml saline at accelerated (i.e., 40±2° C. and 75±5% relative humidity, duration as indicated) and long term stability (i.e., 25±2° C. and 60±5% relative humidity, duration as indicated) storage conditions.

TABLE 2

Stability study of Levophed ® diluted in 0.9% Saline (Hospira) at 16 μg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	97.3	98.9	97.9	91.9	98.8	96.5	80.2	71.9
Total Impurities	0.05	—	0.71	8.08	0.03	1.96	5.29	9.73

TABLE 3

Stability study of Norepinephrine bitartrate injection [Baxter] diluted in 0.9% Saline (Hospira) at 16 μg/mL

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	99.9	99.7	97.0	92.2	99.4	91.5	82.9	77.6
Total Impurities	0.08	1.73	2.68	10.17	0.10	2.34	4.46	6.71

As can be seen from the results, the norepinephrine at ready-to-inject concentrations underwent significant degradation. Oxidative degradation could possibly be reduced or even prevented by addition of effective amounts of sodium metabisulphite to the ready-to-inject norepinephrine solution. However, the quantities of sodium metabisulphite that would be administered by injection of 250 ml of the ready-to-inject solution would be substantial and detrimental to the patient. To avoid issues associated with sodium metabisulphite, efforts have been made to provide norepinephrine formulations with a non-sulfite anti-oxidant. For example US 2016/0058715 teaches a ready-to-inject dosage form of norepinephrine that uses butylated hydroxyl anisole as an anti-oxidant. While generally deemed safe for topical and cosmetic use, butylated hydroxyl anisole was shown to produce some renal and hepatic damage (e.g., *Int J Toxicol.* 2002; 21 Suppl 2:19-94).

In other attempts to provide ready-to-administer norepinephrine formulations with increased storage stability and reduced risk of human error, the pH on the injectable solution was reduced to between 3.2 and 3.6 with 40-200 μg/ml norepinephrine as is described in WO 2015/128418. While such formulations exhibited reduced degradation as compared to higher pH formulations, significant discomfort can occur at the injection site. Worse yet, at the pH used, norepinephrine isomerized relatively quickly from the active R (−) isomer to the inactive S (+) isomer. Isomerization is also encountered at exposure of norepinephrine to higher temperatures.

Therefore, there is a need for improved stable, low concentration, ready-to-inject and antioxidant free norepinephrine formulations, and methods of manufacturing and storing the same.

SUMMARY OF THE INVENTION

The inventive subject matter is directed to antioxidant free sterilizable/autoclavable ready-to-inject norepinephrine compositions having improved stability and a physiologically acceptable pH.

In one aspect of the inventive subject matter, the inventors contemplate a ready to ready-to-inject norepinephrine composition that comprises an aqueous acidic buffer having a pH range of between 3.7 and 4.3, wherein the aqueous buffer further comprises a chelating agent and a pharmaceutically acceptable salt. Most typically, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and the pharmaceutically acceptable salt is present in an amount of between 0.6 wt % and 1.2 wt %. Norepinephrine (typically enantiomerically pure (i.e., at least 98%) R-isomer) is dissolved at a concentration that is suitable for administration to a patient in need thereof. In further preferred aspects, the ready-to-administer norepinephrine composition is substantially free of antioxidants, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As used herein, reference to the term norepinephrine should be interpreted broadly to include pharmaceutically acceptable salts and prodrugs thereof.

Therefore, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes a step of formulating a liquid parenteral composition that contains in an aqueous acidic buffer norepinephrine as an R-isomer such that (a) the formulation exhibits less than 10% of isomerization of the R-isomer to an S-isomer after three months of storage of the liquid composition, and (b) the formulation exhibits equal or less than 5% degradation of total norepinephrine after three months of storage of the liquid composition. The aqueous acidic buffer will typically have a pH range of between 3.7 and 4.3, and the aqueous buffer will further comprise a chelating agent and a pharmaceutically acceptable salt. In such methods, the total norepinephrine is present in the liquid parenteral composition at a concentration of between 10 μg/ml and 100 μg/ml, and the ready-to-inject norepinephrine composition is substantially free of antioxidants.

Viewed form a different perspective, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes the steps of preparing an aqueous acidic buffer at a pH range of between 3.7 and 4.3, wherein the aqueous buffer also includes a chelating agent and a pharmaceutically acceptable salt. Preferably, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and tonicity is adjusted with the pharmaceutically acceptable salt (e.g., NaCl). In a further step, norepinephrine (preferably enantiomerically pure R-isomer) is dissolved at a concentration suitable for administration to a patient in need thereof, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As before, it is generally preferred that the ready-to-administer norepinephrine composition is substantially free of antioxidants. In yet another step, the composition is autoclaved to sterility.

Most typically, but not necessarily, the aqueous acidic buffer is a citrate buffer and/or preferably has a concentration of between 5 mM and 20 mM. Furthermore, preferred aqueous acidic buffers will have a pH of between 3.8 and 4.2. With respect to the chelating agent it is contemplated

that such agents are a bicarboxylic acid (e.g., optionally hydroxylated, tartrate), a tricarboxylic acid (e.g., aconitic acid, trimesic acid, citric acid), and/or an aminopolycarboxylic acid (e.g., EDTA, EGTA, etc.), and that such chelating agents are present at low concentrations, preferably between 1 μg/ml and 10 μg/ml, or between 10 μg/ml and 100 μg/ml. The norepinephrine is typically present at a concentration of between 10 μg/ml and 100 μg/ml, for example, at a concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), or 64 μg/ml (+/−10%). Contemplated methods may also include a step of autoclaving the compositions.

With respect to stability it is contemplated that the storage condition is over at least three months at 40° C. and 75% (+/−5) relative humidity, that equal or less than 6% of the R-isomer form will isomerize to the S-isomer, and/or that equal or less than 3.5% of the total norepinephrine will degrade to degradation products.

Where desired, contemplated compositions have a dissolved oxygen concentration of equal or less than 1 ppm (e.g., by formulating the liquid parenteral composition using deoxygenated water), and/or or by packaging the composition together with a (preferably metal free) oxygen scavenger. Packaging may further make use of a container that is configured (e.g., aluminized or otherwise treated) to reduce light-mediated oxidation of the norepinephrine.

DETAILED DESCRIPTION OF THE INVENTION

The inventive subject matter is directed to stable aqueous pharmaceutical preparations of norepinephrine (and pharmaceutically acceptable salts thereof) in a ready-to-inject form that are sterile and preferably substantially free of antioxidants. Most preferably, stability of such compositions is characterized by low (oxidative and photo-induced) degradation as well as low isomerization.

More specifically, the inventors have discovered that formulations can be prepared that will exhibit less than 8%, more typically less than 6%, even more typically less than 4%, and most typically less than 3% of degradation as determined by HPLC-UV, and that will exhibit less than 10%, more typically less than 8%, even more typically less than 6%, and most typically less than 4% of isomerization from R- to S-configuration as determined by HPLC-UV. Most notably, such formulations were found to be stable over extended periods without antioxidants (e.g., at least 1 month, or at least two months, or at least three months), even at elevated storage temperatures (e.g., accelerated storage conditions such as 40° C. and 75% relative humidity (+/−5%)). Even more remarkable, such formulations could also be subjected to thermal sterilization, and particularly sterilizing to sterility (e.g., over at least 5 min, or at least 10 min, or at least 15 min at 121° C.), without substantial increase (i.e., >1.5%, or >1.0%, or >0.7%) of the S-isomer of norepinephrine.

Additionally, it should be appreciated that contemplated formulations can be filled in a polymer bag (e.g., polypropylene) or other container that may subsequently be placed into a secondary container together with an oxygen scavenger, and especially a metal-free oxygen scavenger. Most typically, at least one of the polymer bag and the secondary container may be impervious to light in general or light of a wavelength that promotes photo-initiated degradation. For example, containers may be metalized (e.g., aluminized) or combined or coated with carbonaceous materials or other dye(s). If desired, contemplated formulations are sufficiently stable to also allow filling into containers using a blow-fill-seal (BFS) process.

Therefore, contemplated norepinephrine formulations of the inventive subject matter can advantageously be provided in a ready-to-inject form to thereby avoid the inconvenience associated with diluting concentrated small volume norepinephrine parenteral formulations into diluents prior to infusion. Thus, the ready-to-inject formulations also eliminate microbial contamination risks and calculation errors associated with dilution. Most typically, contemplated formulations will be available in a range of concentrations commonly required by medical practitioners for emergency restoration of blood pressure, for example in cases of acute hypotension. Consequently, norepinephrine will typically be present in formulations at a concentration of between 10 μg/ml and 100 μg/ml, including concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), and 64 μg/ml (+/−10%).

As will be readily appreciated, the norepinephrine for preparation of contemplated formulations is preferably (R)-Norepinephrine, or enantiomerically pure (i.e., at least 98% R-isomer) norepinephrine. However, in less preferred aspects, isomeric purity can also be between 95-98%, or even between 90-95%. Of course, it should also be appreciated that the norepinephrine may be a salt of any suitable and pharmaceutically acceptable form, including mineral salts (e.g., HCl salt) and organic salts (e.g., bitartrate). Similarly, where desired, the norepinephrine may also be used in any suitable prodrug form (e.g., β,3-dihydroxytyrosine, L-dihydroxyphenylserine, etc.).

Suitable buffers are generally buffers that stabilize the pH of the contemplated liquid formulations in an acidic pH range and will therefore include glycine buffers, citrate buffers, citrate/phosphate buffers, acetate buffers, etc. However, the inventors have further discovered that where the norepinephrine is provided as the norepinephrine bitartrate salt, a buffer can advantageously be omitted and the pH can be adjusted with suitable acid and/or base as is well known in the art. Notably, the bitartrate appeared to act as a weak buffer in the stability range for the norepinephrine as is shown in more detail below. Most typically the pH of the formulation will be less than 5.0 and more typically less than 4.5, and most typically less than 4.3, but higher than 3.0, more typically higher than 3.5, and most typically higher than 3.7. For example, suitable buffers will have a pH in the range of between 3.7 and 4.3, or between 3.7 and 4.0, or between 3.8 and 4.1, or between 3.9 and 4.2, or between 4.0 and 4.2. Notably, such pH range provided remarkable stability for low concentrations of norepinephrine, especially when in combination with a chelator and a salt. While not limiting to the inventive subject matter, the buffer strength is typically relatively low, for example, equal or less than 100 mM, and more typically equal or less than 50 mM, and most typically between 5 mM and 20 mM (e.g., 10 mM).

Moreover, in further contemplated aspects, the formulation will also include one or more chelating agents, and particularly metal ion chelators. For example, suitable chelators include various bicarboxylic acids, tricarboxylic acids, and aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and penta(carboxymethyl)diethylenetriamine (DTPA), and salts and hydrates thereof. While not limiting to the inventive subject matter, it is contemplated that the metal ion chelators will slow down both the baseline and metal ion-stimulated autoxidation of norepinephrine. Remarkably, the inventors unexpectedly observed that the desirable effect of the chelators was observable at relatively low concentrations of the chelators. For example, reduction of the baseline and metal ion-stimulated autoxidation of norepinephrine was observed at chelator concentrations of between 1 μg/ml and 10 μg/ml, and between 10 μg/ml and 100 μg/ml. Interestingly, the chelators, and especially the aminopolycarboxylic acids retained stabilizing effect despite the relatively low pH favoring protonated forms of the chelators.

With respect to suitable salts it is contemplated that the salt is a pharmaceutically acceptable salt that can be used to increase tonicity. Therefore, pharmaceutically acceptable

salts are contemplated, and especially NaCl, at a concentration of at least 0.6 wt %, or at least 0.7 wt %, or at least 0.8 wt %, or at least 0.9 wt %. For example, suitable salt concentrations are between 0.6 wt % and 1.2 wt %. Depending on the particular salt concentration, additional tonicity agents may be added and suitable tonicity agents include glycerol, thioglycerol, mannitol, lactose, and dextrose. The amount of tonicity adjusting agent used can be adjusted to obtain osmolality of the formulations in the range of 260 to 340 mOsm/kg. An osmometer can be used to check and adjust the amount of tonicity adjusting agent to be added to obtain the desired osmolality.

It should further be appreciated that contemplated compositions are substantially free of antioxidants (i.e., do not include antioxidants in an amount effective to reduce degradation of total norepinephrine by at least 1% when stored over a period of at least three months at 25° C. Indeed, the inventors unexpectedly discovered that some formulations with antioxidants (particularly with ascorbic acid) had decreased stability. Notably, contemplated formulations were stable as described in more detail below, even in the absence of effective quantities of antioxidants, especially where deoxygenated solvents (e.g., typically water and/or buffer) were employed. Deoxygenation (i.e., reduction of molecular dissolved oxygen) can be achieved in numerous manners, including sparging with inert gases (e.g., helium, various freons, argon, xenon), agitation under vacuum, and/or using enzymatic systems that deplete a solution of dissolved oxygen (see e.g., U.S. Pat. No. 9,187,779). Additionally, or alternatively, ingress of molecular oxygen into the formulation can also be reduced by co-packaging a container with the formulation in a secondary container that includes an oxygen scavenger, and especially a metal-free oxygen scavenger (e.g., GLS100, Ageless®, Pharmakeep®, all commercially available from Mitsubishi Gas Chemical America).

With respect to the sterilization of contemplated formulations it should be appreciated that contemplated formulations may be sterilized using all known manners of sterilization, including filtration through 0.22 micron filters, heat sterilization, autoclaving, radiation (e.g., gamma, electron beam, microwave). Unexpectedly, and as shown in more detail below, the inventors have also discovered that contemplated formulations were heat stable and did not undergo significant isomerization, even under conditions of sterilization (exposure to high-pressure saturated steam) at 121° C. for at least 5, or at least 10, or at least 15 minutes.

Based on the unexpected heat stability, the formulations contemplated herein can also be filtered through a 0.22 micron filter, and filled in to a polyethylene, polypropylene or low-density polyethylene containers in a blow-fill-seal (BFS) process. BFS is a form of advanced aseptic manufacturing wherein the container is formed, filled, and sealed in one continuous, automated system not requiring human intervention. The process begins with the extrusion of plastic granules in the form of a hot hollow pipe of molten plastic called a parison. The next step is the blow molding of the container with an open top through which the container is filled, all while the plastic remains hot and in a molten state. Once filled, the container is hermetically sealed and cooled. The blow-fill seal process can take several seconds, and contemplated ready-to-inject compositions advantageously are formulated to withstand the temperature and pressure requirements without substantial degradation of norepinephrine (e.g., less than 5 wt %, less than 3 wt %, less than 2 wt %, less than 1 wt % degradation).

Once the norepinephrine formulations are filled in large volume polymeric, semi-permeable infusion containers (e.g., BFS container or flexible IV bags), the containers can optionally be layered or covered with a secondary packaging system including an aluminum pouch or other oxygen scavenger. For example, the BFS containers can further be sealed in an oxygen and moisture barrier blister packaging. The blister packaging can comprise one or more layers, and the one or more layers can include aluminum foil or other oxygen absorber having an Oxygen Transmission Rate (OTR) between 0.0005 to 5.00 cc/100 in^2/24 hrs. Additionally or alternatively, one or more oxygen absorbers (metal or metal free, organic material) can be incorporated into any portion of the BFS container, the secondary packaging system, or between the two (e.g., between the BFS container and the multi-layer packaging) such that the oxygen absorber removes at least a portion of oxygen from the air surrounding said oxygen-sensitive drug. A beneficial feature of the oxygen absorber is the absorbance and removal of oxygen present in the primary packaging and in the liquid drug itself. Notably, it was found that the oxygen absorber also removed residual headspace oxygen in the primary packaging and also dissolved oxygen in the liquid over time, thereby further improving stability of norepinephrine.

The following examples are provided for illustrative purposes only and should not be interpreted as limiting the present invention.

EXAMPLES

The following examples illustrate some of the experiments leading to the formulations according to the inventive subject matter, however, should not be construed to limit the scope of the claims in any way.

Stability and Isomerization: The ionization behavior of norepinephrine in aqueous solution is complex. Common with other o-hydroquinone systems, norepinephrine in aqueous solution is susceptible to oxidation to form the corresponding o-quinone, which can then also undergo various secondary reactions, which also becomes more prevalent as the pH becomes more alkaline. Norepinephrine may further isomerize to the pharmacologically less active S-enantiomer at low pH values, corresponding to protonation of the hydroxyl group at the benzylic chiral center. Therefore, to prevent norepinephrine cyclization reactions pH values less than 6.0 are desired. A pH range of 3.0 to 6.2 was screened to determine pH of optimum stability. Composition of norepinephrine bitartrate equivalent to 16 μg/mL norepinephrine base at various pH values were prepared are described below, with Table 4 listing compositions of norepinephrine bitartrate in citrate buffer (10 mM),

For preparation of the solutions, about 90% of the final quantity of water was collected in a glass media bottle. Nitrogen (N_2) gas was purged for about thirty minutes to reduce the dissolved oxygen levels. Sodium chloride was added and the solution was stirred until a homogenous solution was obtained. Citric acid was added and the solution was stirred until a homogenous solution was obtained. The pH of the bulk solutions was adjusted to pH 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, and 6.2, respectively for each formulation composition using sufficient quantity of 10% w/v sodium hydroxide or 10% w/v hydrochloric acid. Norepinephrine bitartrate was added and the solution was stirred for approximately 10 minutes until a clear solution was formed. Solutions were made up to volume with water. The solutions were filled into 10 mL glass vials, overlaid with nitrogen, stoppered, and sealed. The stability was studied at 4° C., 25° C., and 60° C. by assay. Samples were observed visually for precipitation and change in color for a period of 7 days. Data are presented in Table 5.

TABLE 4

Compositions of Norepinephrine Bitartrate for pH dependent stability in Citrate Buffer (10 mM).

	Concentration (mg/mL)								
Ingredients	I	II	III	IV	V	VI	VII	VIII	IX
Norepinephrine Bitartarate equivalent to Norepinephrine base	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Sodium Chloride	9	9	9	9	9	9	9	9	9
Citric acid	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Sodium Citrate	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
HCl/NaOH (q.s. pH)	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2
Water for Injection (q.s. mL)	1	1	1	1	1	1	1	1	1

TABLE 5

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
4° C.	I	96.4	96.5	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	98.5	3.8	No	No
	IV	99.1	98.4	4.2	No	No
	V	98.1	98.6	4.6	No	No
	VI	98.4	98.1	5.0	No	No
	VII	97.1	96.6	5.4	No	No
	VIII	97.8	97.5	5.8	No	No
	IX	91.5	91.2	6.2	No	No
25° C.	I	96.4	96.4	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	97.9	3.8	No	No
	IV	99.1	97.7	4.2	No	No
	V	98.1	97.3	4.6	No	No
	VI	98.4	97.3	5.0	No	No
	VII	97.1	95.9	5.4	No	No
	VIII	97.8	94.5	5.8	No	No
	IX	91.5	80.4	6.2	Yes	No
60° C.	I	96.4	95.2	3.0	No	No
	II	98.0	95.0	3.4	No	No
	III	99.0	95.2	3.8	No	No
	IV	99.1	93.2	4.2	No	No
	V	98.1	88.9	4.6	No	No
	VI	98.4	77.4	5.0	Yes	No
	VII	97.1	46.8	5.4	Yes	No
	VIII	97.8	NT	5.8	Yes	No
	IX	91.5	NT	6.2	Yes	No

No change in physical appearance was observed in the solutions stored at 4° C. In the solutions stored at 25° C., a change in color was observed at pH 6.2. Red brown color was observed in solutions stored at or above pH 5.0 at 60° C. Oxidation and color formation are very common with norepinephrine in unfavorable conditions and the speed of the reaction and the nature of the final products are dependent on the catalysts (e.g., metal ion impurities) and buffers employed. A pH range from 3.0 to 4.5 was selected for further testing.

Stability of Norepinephrine in selected pH ranges and formulations: The formulations for the next experiments are shown in Table 6 below, involving three different compositions of norepinephrine bitartrate at three different pH (3.5, 4.0, 4.5, and 5.0) values. Lab scale batches were prepared and subjected to lab scale stability tests at accelerated (40° C./75% RH) and long term stability (25° C./60% RH) storage conditions. The test results from the stability studies are presented in Table 7-Table 10, with CCS indicating Clear colorless solution; ND indicating Not Detected; NR indicating Not Reported (<0.05%); and NT indicating Not Tested.

TABLE 6

Formulation composition selected for further development activities and optimization

Ingredient	Quantity (mg/mL)			
Formulation	X	XI	XII	XIII
Norepinephrine Bitartrate	0.016	0.016	0.016	0.016
Edetate Sodium	0.10	0.10	0.10	0.10
Sodium chloride	9	9	9	9
HCl/NaOH	q.s. pH 3.5	q.s. pH 4.0	q.s. pH 4.5	q.s. pH 5.0

TABLE 6-continued

Formulation composition selected for further development activities and optimization				
Ingredient	Quantity (mg/mL)			
Formulation	X	XI	XII	XIII
Water for Injection Q.S.	1 mL	1 mL	1 mL	1 mL
Dissolved Oxygen (ppm)	<1	<1	<1	<1

TABLE 7

Stability study of Formulation X - Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 3.5).

	Storage Condition												
	25 ± 2° C./60 ± 5% RH							40 ± 2° C./75 ± 5% RH					
	Time Point												
	Initial	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.50	3.65	3.59	3.56	3.58	3.54	3.48	3.66	3.61	3.59	3.64	3.60	3.59
Assay	101.4	99.6	97.1	97.1	101.0	102.3	102.2	99.5	97.0	98.7	100.4	101.7	101.4
S-form	NT	NT	NT	NT	1.8	2.2	2.2	NT	NT	NT	7.6	8.1	9.8
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 8

Stability study of Formulation XI- Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 4.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH				
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.02	3.96	3.98	3.97	3.91	4.01	3.99	4.02	4.03
Assay	101.3	98.7	95.5	99.2	100.5	98.6	95.3	97.1	97.5
S-form	NT	NT	NT	NT	1.7	NT	NT	NT	7.8
Total Impurities	0.1	ND	0.06	ND	0.80	ND	0.06	0.1	0.79

TABLE 9

Stability study of Formulation XII - Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 4.5).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH				
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.50	4.35	4.36	4.32	4.33	4.33	4.40	4.39	4.29
Assay	100.1	98.9	95.5	98.2	97.9	97.1	92.5	93.7	77.2
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	2.9
Total Impurities	ND	0.32	0.79	0.52	3.41	1.18	0.38	5.59	10.38

TABLE 10

Stability study of Formulation XIII - Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 5.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	4 Month	1 Month	2 Month	3 Month	4 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.99	4.62	4.51	4.57	4.51	4.87	4.81	4.83	4.53
Assay	102.7	100.5	95.6	99.2	100.4	98.3	89.8	87.0	72.3
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	3.0
Total Impurities	ND	0.75	0.81	0.48	1.29	0.94	2.4	5.39	14.91

Based on the above considerations, the effect of different levels of EDTA on stability of norepinephrine was determined. Three batches at concentrations of 16 µg/mL, 32 µg/mL, and 64 µg/mL were made with EDTA concentrations of 100 µg/mL: Formulation XIV (16 µg/mL), Formulation XV (32 µg/mL), Formulation XVI (64 µg/mL). Two additional batches were made at 10 µg/mL EDTA Formulation XVII and 1 µg/mL EDTA (Formulation XVIII) at 64 µg/mL Norepinephrine. The composition of the batches is specified in Table 11. The drug product was compounded as described earlier and packaged in 250 mL in polypropylene bags. This was further packed into aluminum overwrap with an oxygen scavenger (GLS 100, Mitsubishi Gas Chemicals). The batches were then stored at room temperature and accelerated temperature conditions.

TABLE 11

Formulation composition selected with different level of EDTA concentrations.

Ingredient Formulation	Quantity (mg/mL)				
Number	XIV	XV	XVI	XVII	XVIII
Norepinephrine Bitartrate	0.016	0.032	0.064	0.064	0.064

TABLE 11-continued

Formulation composition selected with different level of EDTA concentrations.

Ingredient Formulation	Quantity (mg/mL)				
Number	XIV	XV	XVI	XVII	XVIII
Edetate Sodium	0.10	0.10	0.10	0.010	0.0010
Sodium chloride	9	9	9	9	9
Hydrochloric Acid/Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for Injection	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL

The resultant stability data on these formulations are presented in Table 12-Table 16 (CCS— Clear colorless solution; ND—Not Detected). The results of the stability studies at different amounts of EDTA at pH 4.0 indicates that both 0.01%, 0.001% of EDTA significantly prevented the degradation rate of norepinephrine in terms of known and unknown impurities. Moreover, with respect to isomerization from the R-isomer to the S-isomer it was notably observed that the amount of EDTA had substantially no influence on racemization or enantiomer formation during stability and after autoclaving.

TABLE 12

Stability study of Formulation XIV - Norepinephrine bitartrate injection (16 µg/mL); pH 4.0 at 100 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.99	3.96	4.08	4.08	4.02	4.08	4.08
Assay	98.5	100.4	100.1	99.7	100.3	100.0	99.5
S-form	0.9	1.1	1.4	1.3	1.9	2.9	4.2
Total Impurities	0.05	ND	ND	ND	ND	0.10	0.38

TABLE 13

Stability study of Formulation XV - Norepinephrine bitartrate injection (32 µg/mL); pH 4.0 at 100 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.01	3.99	4.08	4.08	4.02	4.08	4.08
Assay	101.0	102.9	97.1	100.7	102.9	99.4	100.6
S-form	0.9	1.1	1.3	1.4	1.9	3.0	4.1
Total Impurities	0.06	ND	ND	ND	ND	ND	0.14

TABLE 14

Stability study of Formulation XVI - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 100 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.99	4.08	4.08	3.98	4.07	4.07
Assay	98.4	103.2	98.7	100.2	104.6	99.3	99.8
S-form	0.9	1.1	1.3	1.3	2.0	3.2	4.2
Total Impurities	0.06	ND	0.12	ND	ND	ND	ND

TABLE 15

Stability study of Formulation XVII - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 10 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.06	4.06	3.99	4.05	4.05
Assay	102.7	105.7	103.4	104.3	107.8	103.6	103.9
S-form	0.9	1.1	1.2	1.5	2.0	3.3	4.3
Total	0.06	ND	ND	ND	ND	0.26	ND

TABLE 16

Stability study of Formulation XVIII - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 1 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.07	4.07	4.02	4.06	4.06
Assay	98.7	102.6	100.4	100.4	105.0	99.9	99.2
S-form	0.9	1.1	1.3	1.4	2.0	3.2	4.3
Total	0.06	ND	ND	ND	ND	ND	ND

Sterilization and Stability: The volume for ready-to-inject formulations is 250 mL and as such classifies as a large volume parenteral (LVP). To achieve a desired or required sterility assurance level of 10^{-6} for a LVP terminal sterilization via heat it is typically required. The inventors therefore investigated whether or not contemplated formulations could be terminally sterilized via autoclaving.

Formulations at a concentration 16 μg/mL and 64 μg/mL (Formulation XVII) Norepinephrine base were prepared substantially as shown in Table 11 above and packaged in secondary packaging of aluminum overwrap with an oxygen scavenger and shipped for terminal sterilization. The secondary packaging was removed and the bags were terminally sterilized using steam sterilizer (Fedegari, Model #FOB3) with an air over-pressure (AOP) sterilization cycle. The terminal sterilization was performed at 121° C. for 5, 10, and 15 min. Post completion of sterilization temperature, the bags underwent spontaneous cooling to 95° C. and forced cooling to 70° C. The total exposure time and calculated F_0 values were 11.09, 17.04, and 22.42 for 5 min, 10 min, and 15 min cycles respectively. The bags were then analyzed for assay, impurities, and S-isoform, and the results are shown in Table 17 and Table 18.

TABLE 17

Stability study of Norepinephrine bitartrate injection (16 μg/mL) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA; terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.76	3.85	3.78	3.77	3.76	3.76	3.78	3.76	3.75	3.76
Dissolved Oxygen	0.63	4.93	4.86	4.89	0.75	0.48	0.55	0.65	0.78	0.77
Assay	103.1	103.1	103.1	103.1	103.1	103.0	103.1	103.1	103.2	103.1
S-Form	1.0	3.0	3.0	3.0	3.8	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 18

Stability study of Norepinephrine bitartrate injection (64 μg/ml) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.74	3.74	3.75	3.73	3.74	3.74	3.76	3.74	3.73	3.74
Dissolved Oxygen	0.69	5.15	5.03	5.00	0.52	0.59	0.75	0.69	0.80	0.74
Assay	101.2	102.2	101.2	101.5	101.7	101.2	101.3	101.2	101.3	102.2
S-Form	1.0	3.0	3.0	3.0	3.7	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND

As can be seen from the data, the S-isoform appears to increase proportionally to time during the terminal sterilization cycle. No increase in reportable impurities was observed.

Test method—Determination of norepinephrine and degradation products: Separation of Norepinephrine and related compounds was performed using a gradient HPLC method with UV detection. Pentofluorophenylpropyl terminated silica was used as a stationary phase for chromatographic analysis. The mobile phase was prepared by mixing water and methanol, with both solvents containing formic acid. Related compounds were defined by their relative retention times (RRT) based on the NE peak retention time. Quantitation of related compounds was accomplished by comparing the corresponding peak area from a sample solution chromatogram to that of the NE peak from a Reference Standard (RS) solution of a known concentration. Relative Response Factors (RRF) were used to correct for chemical structure effects on the responses of the identified impurities. Chromatography was performed using parameters and methods as shown in Table 19.

TABLE 19

HPLC	Waters Alliance e2695
Column	Supelco Discovery HS F-5 Column, 3 μm, 4.6 × 150 mm
Column Temperature	35° C.
Sample Temperature	Ambient
Injection volume	85.0 μL

TABLE 19-continued

Flow Rate	0.8 mL/min		
Detection	Spectrum: 200-600 nm, resolution 12 nm Single channel: 280 nm, resolution 4.8 nm PDA Filter Time Constant: Normal Sampling rate: 5 points/sec		
Solution A	0.1% Formic acid in Water		
Solution B	0.1% Formic acid in Methanol		
Mobile Phase	Time (mins)	%Solution A	%Solution 8
	0	100	0
	3	100	0
	6	93	7
	8	93	7
	15	88	12
	30	2	98
	35	2	96
	36	100	0
	40	100	0

Test Method—Identification, Assay and Enantiomeric Purity of Norepinephrine: Identification and quantification of S-norepinephrine and R-norepinephrine was performed using an HPLC method with UV detection. HPLC-UV was used to separate and quantitate the amount of (R)- and (S)-enantiomers of norepinephrine (NE) present in the drug product with the NE concentrations of 16, 32 and 64 μg/ml. The comparison of the sum of (R)- and (S)-peak responses in a sample chromatogram versus a reference standard chromatogram gives the total amount of NE. The (S)-enantiomer was quantitated based on its peak response as the percentage of the total peak response of both enantiomers.

More specifically, determination of R- and S-enantiomers of norepinephrine in the drug product solution was performed using an isocratic reverse-phase HPLC method with UV detection. Separation was achieved by using a protein-based column with functional chiral selectors. The chiral selector is cellobiohydrolase (CBH), a stable enzyme that has been immobilized onto spherical silica particles. This enzyme preferentially separates compounds containing one or more basic nitrogen groups together with one or more hydrogen-accepting or hydrogen-donating groups. Chromatography was performed using parameters and methods as shown in Table 20.

TABLE 20

HPLC	Agilent 1260 Infinity
Column	Daicel Chiralpak CBH ™ column, 5 μm, 4.0 × 100 mm
Column Temperature	27° C. ± 2° C.
Sample Temperature	Ambient
Injection volume	20.0 μL for 16 mcg/mL, 10.0 μL for 32 mcg/mL, 5.0 μL for 64 mcg/mL
Flow Rate	0.9 mL/min
Detection	Single channel: 250 nm, resolution 4.8 nm Spectrum: 200-800 nm, resolution 1.2 nm
Mobile Phase:	Buffer/IPA 95:5 v/v Buffer: Sodium Phosphate, Disodium Edetate, pH 6.0
Run Time	8 min

While contemplated formulations can be administered following various protocols, the inventors contemplate that administration of the formulations, especially administration for treatment of hypotension, will follow a protocol that comprises at least two distinct steps, with an accelerated administration followed by a maintenance administration as exemplarily described in Table 21 below.

TABLE 21

		Initial Dose		Maintenance Dose	
Presentation (mg/mL)	Concentration (μg/mL)	Dose per Minute (μg/min)	Flow Rate (mL/min)	Dose per Minute (μg/min)	Flow Rate (mL/min)
16 μg/mL (4 mg in 250 mL)	16	8-12	0.500-0.750	2-4	0.125-0.250
32 μg/mL (8 mg in 250 mL)	32		0.250-0.375		0.062-0.125
64 μg/mL (16 mg in 250 mL)	64		0.125-0.187		0.031-0.062

As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

Moreover, in interpreting the disclosure all terms should be interpreted in the broadest possible manner consistent with the context. In particular the terms "comprises" and "comprising" should be interpreted as referring to the elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps can be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

What is claimed is:

1. A ready-to-administer norepinephrine composition, comprising:

an aqueous solution having a pH range of between 3.7 and 4.3, wherein the aqueous solution comprises:

norepinephrine present at a concentration of between 10 μg/ml and 100 μg/ml, wherein the norepinephrine initially comprises at least 95% of R-isomer as determined by HPLC;

a chelating agent comprising a tartrate bicarboxylic acid, wherein the chelating agent is present at a concentration of between 10 μg/ml and 100 μg/ml; and

a tonicity agent,

wherein the ready-to-administer norepinephrine composition is substantially free of antioxidants; and

wherein after storage at 25±2° C. and 60±5% relative humidity over at least three months, the norepinephrine comprises at least 90% R-isomer as determined by HPLC.

2. The composition of claim **1**, wherein the tonicity agent is present in an amount to obtain osmolality of the solution of between 260 mOsm/kg and 340 mOsm/kg.

3. The composition of claim **1**, wherein the tonicity agent comprises a pharmaceutically acceptable salt and is present in an amount of between 0.6 wt % and 1.2 wt %.

4. The composition of claim **1**, wherein the tonicity agent comprises dextrose.

5. The composition of claim **1**, wherein the tonicity agent comprises NaCl.

6. The composition of claim **1**, wherein the composition is sterile.

7. The composition of claim **1**, wherein the norepinephrine comprises one or more of norepinephrine acid, norepinephrine mineral salt and norepinephrine organic salt.

8. The composition of claim **1**, wherein the norepinephrine comprises a norepinephrine organic salt.

9. The composition of claim **1**, wherein the composition is free of added buffer.

10. The composition of claim **1**, wherein the chelating agent further comprises an aminopolycarboxylic acid.

* * * * *

EXHIBIT B

US010420735B2

(12) **United States Patent**
Hingorani et al.

(10) **Patent No.: US 10,420,735 B2**
(45) **Date of Patent: Sep. 24, 2019**

(54) **NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR**

(71) Applicant: **Nevakar, Inc.**, Bridgewater, NJ (US)

(72) Inventors: **Tushar Hingorani**, Bridgewater, NJ (US); **Prem Sagar Akasapu**, Edison, NJ (US); **Kumaresh Soppimath**, Skillman, NJ (US)

(73) Assignee: **NEVAKAR INC.**, Bridgewater, NJ (US)

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(52) **U.S. Cl.**

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(58) **Field of Classification Search**

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See application file for complete search history.

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Primary Examiner — Theodore R. West

(74) *Attorney, Agent, or Firm* — Umberg Zipser LLP

(57) **ABSTRACT**

The inventive subject matter is directed to compositions and methods for ready-to-inject norepinephrine compositions with improved stability. Most preferably, compositions presented herein are substantially antioxidant free and exhibit less than 10% isomerization of R-norepinephrine and exhibit less than 5% degradation of total norepinephrine.

22 Claims, No Drawings

NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR

This application is a divisional application of US non-provisional application with Ser. No. 15/883,798, which was filed Jan. 30, 2018, which claims priority to US provisional application with Ser. No. 62/452,220, which was filed Jan. 30, 2017.

FIELD OF THE INVENTION

The field of the invention is pharmaceutical compositions comprising norepinephrine, especially as it relates to storage stable, ready-to-inject, antioxidant free compositions, and method of manufacturing such compositions.

BACKGROUND

The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

Norepinephrine is often used during CPR (cardio-pulmonary resuscitation), and in the treatment of cardiac arrest and profound hypotension. Norepinephrine is also used for blood pressure control in certain acute hypotensive states, including for example sympathectomy, poliomyelitis, pheochromocytomectomy, spinal anesthesia, myocardial infarction, blood transfusion, and septicemia.

Currently, norepinephrine is marketed as Levophed®, which is a concentrated 4 mg per 4 mL norepinephrine bitartrate formulation to be administered by intravenous infusion following dilution with dextrose or dextrose and sodium chloride injection. Norepinephrine is also marketed by Baxter which supplies as a norepinephrine concentrate that is free of sodium metabisulfite and packaged under nitrogen. Unfortunately, most, if not all diluted commercially available norepinephrine formulations lack storage and should therefore be discarded within one day after reconstitution when stored at room temperature. Consequently, risk for microbial contamination and dilution errors is present. In addition, Levophed also contains sodium metabisulphite as an antioxidant, and carries a warning label that sulfite may cause allergic type reactions including anaphylactic shock and life threating or less severe asthmatic episodes in susceptible people. Table ldepicts ingredients of currently marketed norepinephrine compositions.

TABLE 1

Composition of currently marketed Norepinephrine Bitartrate Products.

Ingredient	Levophed ® (Hospira)	Norepinephrine Bitartarate (Baxter)
Norepinephrine Bitartrate equivalent to Norepinephrine Base	1 mg/mL	1 mg/mL
Sodium Chloride	Isotonic	Isotonic
Sodium Metabisulphite	0.2 mg/mL	—
pH	3-4.5	3-4.5
Water for injection	q.s. 1 mL	q.s 1 mL

Stability of Levophed® and Norepinephrine bitartrate injection (Baxter), in normal saline solutions is presented in Table 2 and Table 3 where norepinephrine was diluted to a concentration of 16 μg/ml. Stability was assessed in 250 ml saline at accelerated (i.e., 40±2° C. and 75±5% relative humidity, duration as indicated) and long term stability (i.e., 25±2° C. and 60±5% relative humidity, duration as indicated) storage conditions.

TABLE 2

Stability study of Levophed ® diluted in 0.9% Saline (Hospira) at 16 μg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	97.3	98.9	97.9	91.9	98.8	96.5	80.2	71.9
Total Impurities	0.05	—	0.71	8.08	0.03	1.96	5.29	9.73

TABLE 3

Stability study of Norepinephrine bitartrate injection [Baxter] diluted in 0.9% Saline (Hospira) at 16 µg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	99.9	99.7	97.0	92.2	99.4	91.5	82.9	77.6
Total Impurities	0.08	1.73	2.68	10.17	0.10	2.34	4.46	6.71

As can be seen from the results, the norepinephrine at ready-to-inject concentrations underwent significant degradation. Oxidative degradation could possibly be reduced or even prevented by addition of effective amounts of sodium metabisulphite to the ready-to-inject norepinephrine solution. However, the quantities of sodium metabisulphite that would be administered by injection of 250 ml of the ready-to-inject solution would be substantial and detrimental to the patient. To avoid issues associated with sodium metabisulphite, efforts have been made to provide norepinephrine formulations with a non-sulfite anti-oxidant. For example US 2016/0058715 teaches a ready-to-inject dosage form of norepinephrine that uses butylated hydroxyl anisole as an anti-oxidant. While generally deemed safe for topical and cosmetic use, butylated hydroxyl anisole was shown to produce some renal and hepatic damage (e.g., *Int J Toxicol.* 2002; 21 Suppl 2:19-94).

In other attempts to provide ready-to-administer norepinephrine formulations with increased storage stability and reduced risk of human error, the pH on the injectable solution was reduced to between 3.2 and 3.6 with 40-200 µg/ml norepinephrine as is described in WO 2015/128418. While such formulations exhibited reduced degradation as compared to higher pH formulations, significant discomfort can occur at the injection site. Worse yet, at the pH used, norepinephrine isomerized relatively quickly from the active R (−) isomer to the inactive S (+) isomer. Isomerization is also encountered at exposure of norepinephrine to higher temperatures.

Therefore, there is a need for improved stable, low concentration, ready-to-inject and antioxidant free norepinephrine formulations, and methods of manufacturing and storing the same.

SUMMARY OF THE INVENTION

The inventive subject matter is directed to antioxidant free sterilizable/autoclavable ready-to-inject norepinephrine compositions having improved stability and a physiologically acceptable pH.

In one aspect of the inventive subject matter, the inventors contemplate a ready to ready-to-inject norepinephrine composition that comprises an aqueous acidic buffer having a pH range of between 3.7 and 4.3, wherein the aqueous buffer further comprises a chelating agent and a pharmaceutically acceptable salt. Most typically, the chelating agent is present in an amount of between 1 µg/ml and 100 µg/ml, and the pharmaceutically acceptable salt is present in an amount of between 0.6 wt % and 1.2 wt %. Norepinephrine (typically enantiomerically pure (i.e., at least 98%) R-isomer) is dissolved at a concentration that is suitable for administration to a patient in need thereof. In further preferred aspects, the ready-to-administer norepinephrine composition is substantially free of antioxidants, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As used herein, reference to the term norepinephrine should be interpreted broadly to include pharmaceutically acceptable salts and prodrugs thereof.

Therefore, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes a step of formulating a liquid parenteral composition that contains in an aqueous acidic buffer norepinephrine as an R-isomer such that (a) the formulation exhibits less than 10% of isomerization of the R-isomer to an S-isomer after three months of storage of the liquid composition, and (b) the formulation exhibits equal or less than 5% degradation of total norepinephrine after three months of storage of the liquid composition. The aqueous acidic buffer will typically have a pH range of between 3.7 and 4.3, and the aqueous buffer will further comprise a chelating agent and a pharmaceutically acceptable salt. In such methods, the total norepinephrine is present in the liquid parenteral composition at a concentration of between 10 µg/ml and 100 µg/ml, and the ready-to-inject norepinephrine composition is substantially free of antioxidants.

Viewed form a different perspective, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes the steps of preparing an aqueous acidic buffer at a pH range of between 3.7 and 4.3, wherein the aqueous buffer also includes a chelating agent and a pharmaceutically acceptable salt. Preferably, the chelating agent is present in an amount of between 1 µg/ml and 100 µg/ml, and tonicity is adjusted with the pharmaceutically acceptable salt (e.g., NaCl). In a further step, norepinephrine (preferably enantiomerically pure R-isomer) is dissolved at a concentration suitable for administration to a patient in need thereof, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As before, it is generally preferred that the ready-to-administer norepinephrine composition is substantially free of antioxidants. In yet another step, the composition is autoclaved to sterility.

Most typically, but not necessarily, the aqueous acidic buffer is a citrate buffer and/or preferably has a concentration of between 5 mM and 20 mM. Furthermore, preferred aqueous acidic buffers will have a pH of between 3.8 and 4.2. With respect to the chelating agent it is contemplated

that such agents are a bicarboxylic acid (e.g., optionally hydroxylated, tartrate), a tricarboxylic acid (e.g., aconitic acid, trimesic acid, citric acid), and/or an aminopolycarboxylic acid (e.g., EDTA, EGTA, etc.), and that such chelating agents are present at low concentrations, preferably between 1 μg/ml and 10 μg/ml, or between 10 μg/ml and 100 μg/ml. The norepinephrine is typically present at a concentration of between 10 μg/ml and 100 μg/ml, for example, at a concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), or 64 μg/ml (+/−10%). Contemplated methods may also include a step of autoclaving the compositions.

With respect to stability it is contemplated that the storage condition is over at least three months at 40° C. and 75% (+/−5) relative humidity, that equal or less than 6% of the R-isomer form will isomerize to the S-isomer, and/or that equal or less than 3.5% of the total norepinephrine will degrade to degradation products.

Where desired, contemplated compositions have a dissolved oxygen concentration of equal or less than 1 ppm (e.g., by formulating the liquid parenteral composition using deoxygenated water), and/or or by packaging the composition together with a (preferably metal free) oxygen scavenger. Packaging may further make use of a container that is configured (e.g., aluminized or otherwise treated) to reduce light-mediated oxidation of the norepinephrine.

DETAILED DESCRIPTION OF THE INVENTION

The inventive subject matter is directed to stable aqueous pharmaceutical preparations of norepinephrine (and pharmaceutically acceptable salts thereof) in a ready-to-inject form that are sterile and preferably substantially free of antioxidants. Most preferably, stability of such compositions is characterized by low (oxidative and photo-induced) degradation as well as low isomerization.

More specifically, the inventors have discovered that formulations can be prepared that will exhibit less than 8%, more typically less than 6%, even more typically less than 4%, and most typically less than 3% of degradation as determined by HPLC-UV, and that will exhibit less than 10%, more typically less than 8%, even more typically less than 6%, and most typically less than 4% of isomerization from R- to S-configuration as determined by HPLC-UV. Most notably, such formulations were found to be stable over extended periods without antioxidants (e.g., at least 1 month, or at least two months, or at least three months), even at elevated storage temperatures (e.g., accelerated storage conditions such as 40° C. and 75% relative humidity (+/−5%)). Even more remarkable, such formulations could also be subjected to thermal sterilization, and particularly sterilizing to sterility (e.g., over at least 5 min, or at least 10 min, or at least 15 min at 121° C.), without substantial increase (i.e., >1.5%, or >1.0%, or >0.7%) of the S-isomer of norepinephrine.

Additionally, it should be appreciated that contemplated formulations can be filled in a polymer bag (e.g., polypropylene) or other container that may subsequently be placed into a secondary container together with an oxygen scavenger, and especially a metal-free oxygen scavenger. Most typically, at least one of the polymer bag and the secondary container may be impervious to light in general or light of a wavelength that promotes photo-initiated degradation. For example, containers may be metalized (e.g., aluminized) or combined or coated with carbonaceous materials or other dye(s). If desired, contemplated formulations are sufficiently stable to also allow filling into containers using a blow-fill-seal (BFS) process.

Therefore, contemplated norepinephrine formulations of the inventive subject matter can advantageously be provided in a ready-to-inject form to thereby avoid the inconvenience associated with diluting concentrated small volume norepinephrine parenteral formulations into diluents prior to infusion. Thus, the ready-to-inject formulations also eliminate microbial contamination risks and calculation errors associated with dilution. Most typically, contemplated formulations will be available in a range of concentrations commonly required by medical practitioners for emergency restoration of blood pressure, for example in cases of acute hypotension. Consequently, norepinephrine will typically be present in formulations at a concentration of between 10 μg/ml and 100 μg/ml, including concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), and 64 μg/ml (+/−10%).

As will be readily appreciated, the norepinephrine for preparation of contemplated formulations is preferably (R)-Norepinephrine, or enantiomerically pure (i.e., at least 98% R-isomer) norepinephrine. However, in less preferred aspects, isomeric purity can also be between 95-98%, or even between 90-95%. Of course, it should also be appreciated that the norepinephrine may be a salt of any suitable and pharmaceutically acceptable form, including mineral salts (e.g., HCl salt) and organic salts (e.g., bitartrate). Similarly, where desired, the norepinephrine may also be used in any suitable prodrug form (e.g., β,3-dihydroxytyrosine, L-dihydroxyphenylserine, etc.).

Suitable buffers are generally buffers that stabilize the pH of the contemplated liquid formulations in an acidic pH range and will therefore include glycine buffers, citrate buffers, citrate/phosphate buffers, acetate buffers, etc. However, the inventors have further discovered that where the norepinephrine is provided as the norepinephrine bitartrate salt, a buffer can advantageously be omitted and the pH can be adjusted with suitable acid and/or base as is well known in the art. Notably, the bitartrate appeared to act as a weak buffer in the stability range for the norepinephrine as is shown in more detail below. Most typically the pH of the formulation will be less than 5.0 and more typically less than 4.5, and most typically less than 4.3, but higher than 3.0, more typically higher than 3.5, and most typically higher than 3.7. For example, suitable buffers will have a pH in the range of between 3.7 and 4.3, or between 3.7 and 4.0, or between 3.8 and 4.1, or between 3.9 and 4.2, or between 4.0 and 4.2. Notably, such pH range provided remarkable stability for low concentrations of norepinephrine, especially when in combination with a chelator and a salt. While not limiting to the inventive subject matter, the buffer strength is typically relatively low, for example, equal or less than 100 mM, and more typically equal or less than 50 mM, and most typically between 5 mM and 20 mM (e.g., 10 mM).

Moreover, in further contemplated aspects, the formulation will also include one or more chelating agents, and particularly metal ion chelators. For example, suitable chelators include various bicarboxylic acids, tricarboxylic acids, and aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and penta(carboxymethyl)diethylenetriamine (DTPA), and salts and hydrates thereof. While not limiting to the inventive subject matter, it is contemplated that the metal ion chelators will slow down both the baseline and metal ion-stimulated autoxidation of norepinephrine. Remarkably, the inventors unexpectedly observed that the desirable effect of the chela-

tors was observable at relatively low concentrations of the chelators. For example, reduction of the baseline and metal ion-stimulated autoxidation of norepinephrine was observed at chelator concentrations of between 1 μg/ml and 10 μg/ml, and between 10 μg/ml and 100 μg/ml. Interestingly, the chelators, and especially the aminopolycarboxylic acids retained stabilizing effect despite the relatively low pH favoring protonated forms of the chelators.

With respect to suitable salts it is contemplated that the salt is a pharmaceutically acceptable salt that can be used to increase tonicity. Therefore, pharmaceutically acceptable salts are contemplated, and especially NaCl, at a concentration of at least 0.6 wt %, or at least 0.7 wt %, or at least 0.8 wt %, or at least 0.9 wt %. For example, suitable salt concentrations are between 0.6 wt % and 1.2 wt %. Depending on the particular salt concentration, additional tonicity agents may be added and suitable tonicity agents include glycerol, thioglycerol, mannitol, lactose, and dextrose. The amount of tonicity adjusting agent used can be adjusted to obtain osmolality of the formulations in the range of 260 to 340 mOsm/kg. An osmometer can be used to check and adjust the amount of tonicity adjusting agent to be added to obtain the desired osmolality.

It should further be appreciated that contemplated compositions are substantially free of antioxidants (i.e., do not include antioxidants in an amount effective to reduce degradation of total norepinephrine by at least 1% when stored over a period of at least three months at 25° C. Indeed, the inventors unexpectedly discovered that some formulations with antioxidants (particularly with ascorbic acid) had decreased stability. Notably, contemplated formulations were stable as described in more detail below, even in the absence of effective quantities of antioxidants, especially where deoxygenated solvents (e.g., typically water and/or buffer) were employed. Deoxygenation (i.e., reduction of molecular dissolved oxygen) can be achieved in numerous manners, including sparging with inert gases (e.g., helium, various freons, argon, xenon), agitation under vacuum, and/or using enzymatic systems that deplete a solution of dissolved oxygen (see e.g., U.S. Pat. No. 9,187,779). Additionally, or alternatively, ingress of molecular oxygen into the formulation can also be reduced by co-packaging a container with the formulation in a secondary container that includes an oxygen scavenger, and especially a metal-free oxygen scavenger (e.g., GLS100, Ageless®, Pharmakeep®, all commercially available from Mitsubishi Gas Chemical America).

With respect to the sterilization of contemplated formulations it should be appreciated that contemplated formulations may be sterilized using all known manners of sterilization, including filtration through 0.22 micron filters, heat sterilization, autoclaving, radiation (e.g., gamma, electron beam, microwave). Unexpectedly, and as shown in more detail below, the inventors have also discovered that contemplated formulations were heat stable and did not undergo significant isomerization, even under conditions of sterilization (exposure to high-pressure saturated steam) at 121° C. for at least 5, or at least 10, or at least 15 minutes.

Based on the unexpected heat stability, the formulations contemplated herein can also be filtered through a 0.22 micron filter, and filled in to a polyethylene, polypropylene or low-density polyethylene containers in a blow-fill-seal (BFS) process. BFS is a form of advanced aseptic manufacturing wherein the container is formed, filled, and sealed in one continuous, automated system not requiring human intervention. The process begins with the extrusion of plastic granules in the form of a hot hollow pipe of molten plastic called a parison. The next step is the blow molding of the container with an open top through which the container is filled, all while the plastic remains hot and in a molten state. Once filled, the container is hermetically sealed and cooled. The blow-fill seal process can take several seconds, and contemplated ready-to-inject compositions advantageously are formulated to withstand the temperature and pressure requirements without substantial degradation of norepinephrine (e.g., less than 5 wt %, less than 3 wt %, less than 2 wt %, less than 1 wt % degradation).

Once the norepinephrine formulations are filled in large volume polymeric, semi-permeable infusion containers (e.g., BFS container or flexible IV bags), the containers can optionally be layered or covered with a secondary packaging system including an aluminum pouch or other oxygen scavenger. For example, the BFS containers can further be sealed in an oxygen and moisture barrier blister packaging. The blister packaging can comprise one or more layers, and the one or more layers can include aluminum foil or other oxygen absorber having an Oxygen Transmission Rate (OTR) between 0.0005 to 5.00 cc/100 in^2/24 hrs. Additionally or alternatively, one or more oxygen absorbers (metal or metal free, organic material) can be incorporated into any portion of the BFS container, the secondary packaging system, or between the two (e.g., between the BFS container and the multi-layer packaging) such that the oxygen absorber removes at least a portion of oxygen from the air surrounding said oxygen-sensitive drug. A beneficial feature of the oxygen absorber is the absorbance and removal of oxygen present in the primary packaging and in the liquid drug itself. Notably, it was found that the oxygen absorber also removed residual headspace oxygen in the primary packaging and also dissolved oxygen in the liquid over time, thereby further improving stability of norepinephrine.

The following examples are provided for illustrative purposes only and should not be interpreted as limiting the present invention.

EXAMPLES

The following examples illustrate some of the experiments leading to the formulations according to the inventive subject matter, however, should not be construed to limit the scope of the claims in any way.

Stability and Isomerization: The ionization behavior of norepinephrine in aqueous solution is complex. Common with other o-hydroquinone systems, norepinephrine in aqueous solution is susceptible to oxidation to form the corresponding o-quinone, which can then also undergo various secondary reactions, which also becomes more prevalent as the pH becomes more alkaline. Norepinephrine may further isomerize to the pharmacologically less active S-enantiomer at low pH values, corresponding to protonation of the hydroxyl group at the benzylic chiral center. Therefore, to prevent norepinephrine cyclization reactions pH values less

than 6.0 are desired. A pH range of 3.0 to 6.2 was screened to determine pH of optimum stability. Composition of norepinephrine bitartrate equivalent to 16 μg/mL norepinephrine base at various pH values were prepared are described below, with Table 4 listing compositions of norepinephrine bitartrate in citrate buffer (10 mM),

For preparation of the solutions, about 90% of the final quantity of water was collected in a glass media bottle. Nitrogen (N_2) gas was purged for about thirty minutes to reduce the dissolved oxygen levels. Sodium chloride was added and the solution was stirred until a homogenous solution was obtained. Citric acid was added and the solution was stirred until a homogenous solution was obtained. The pH of the bulk solutions was adjusted to pH 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, and 6.2, respectively for each formulation composition using sufficient quantity of 10% w/v sodium hydroxide or 10% w/v hydrochloric acid. Norepinephrine bitartrate was added and the solution was stirred for approximately 10 minutes until a clear solution was formed. Solutions were made up to volume with water. The solutions were filled into 10 mL glass vials, overlaid with nitrogen, stoppered, and sealed. The stability was studied at 4° C., 25° C., and 60° C. by assay. Samples were observed visually for precipitation and change in color for a period of 7 days. Data are presented in Table 5.

TABLE 4

Compositions of Norepinephrine Bitartrate for pH dependent stability in Citrate Buffer (10 mM).

	Concentration (mg/mL)								
Ingredients	I	II	III	IV	V	VI	VII	VIII	IX
Norepinephrine Bitartarate equivalent to Norepinephrine base	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Sodium Chloride	9	9	9	9	9	9	9	9	9
Citric acid	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Sodium Citrate	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
HCl/NaOH (q.s. pH)	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2
Water for Injection (q.s. mL)	1	1	1	1	1	1	1	1	1

TABLE 5

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
4° C.	I	96.4	96.5	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	98.5	3.8	No	No
	IV	99.1	98.4	4.2	No	No
	V	98.1	98.6	4.6	No	No
	VI	98.4	98.1	5.0	No	No
	VII	97.1	96.6	5.4	No	No
	VIII	97.8	97.5	5.8	No	No
	IX	91.5	91.2	6.2	No	No
25° C.	I	96.4	96.4	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	97.9	3.8	No	No
	IV	99.1	97.7	4.2	No	No
	V	98.1	97.3	4.6	No	No
	VI	98.4	97.3	5.0	No	No
	VII	97.1	95.9	5.4	No	No
	VIII	97.8	94.5	5.8	No	No
	IX	91.5	80.4	6.2	Yes	No
60° C.	I	96.4	95.2	3.0	No	No
	II	98.0	95.0	3.4	No	No

TABLE 5-continued

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
	III	99.0	95.2	3.8	No	No
	IV	99.1	93.2	4.2	No	No
	V	98.1	88.9	4.6	No	No
	VI	98.4	77.4	5.0	Yes	No
	VII	97.1	46.8	5.4	Yes	No
	VIII	97.8	NT	5.8	Yes	No
	IX	91.5	NT	6.2	Yes	No

No change in physical appearance was observed in the solutions stored at 4° C. In the solutions stored at 25° C., a change in color was observed at pH 6.2. Red brown color was observed in solutions stored at or above pH 5.0 at 60° C. Oxidation and color formation are very common with norepinephrine in unfavorable conditions and the speed of the reaction and the nature of the final products are dependent on the catalysts (e.g., metal ion impurities) and buffers employed. A pH range from 3.0 to 4.5 was selected for further testing.

Stability of Norepinephrine in selected pH ranges and formulations: The formulations for the next experiments are shown in Table 6 below, involving three different compositions of norepinephrine bitartrate at three different pH (3.5, 4.0, 4.5, and 5.0) values. Lab scale batches were prepared and subjected to lab scale stability tests at accelerated (40° C./75% RH) and long term stability (25° C./60% RH) storage conditions. The test results from the stability studies are presented in Table 7-Table 10, with CCS indicating Clear colorless solution; ND indicating Not Detected; NR indicating Not Reported (<0.05%); and NT indicating Not Tested.

TABLE 6

Formulation composition selected for further development activities and optimization

	Quantity (mg/mL) Formulation			
Ingredient	X	XI	XII	XIII
Norepinephrine Bitartrate	0.016	0.016	0.016	0.016
Edetate Sodium	0.10	0.10	0.10	0.10
Sodium chloride	9	9	9	9

TABLE 6-continued

Formulation composition selected for further development activities and optimization				
	Quantity (mg/mL) Formulation			
Ingredient	X	XI	XII	XIII
HCl/NaOH	q.s. pH 3.5	q.s. pH 4.0	q.s. pH 4.5	q.s. pH 5.0

TABLE 6-continued

Formulation composition selected for further development activities and optimization				
	Quantity (mg/mL) Formulation			
Ingredient	X	XI	XII	XIII
Water for Injection Q.S.	1 mL	1 mL	1 mL	1 mL
Dissolved Oxygen (ppm)	<1	<1	<1	<1

TABLE 7

Stability study of Formulation X - Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 3.5).

	Storage Condition												
	25 ± 2° C./60 ± 5% RH Time Point							40 ± 2° C./75 ± 5% RH					
	Initial	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.50	3.65	3.59	3.56	3.58	3.54	3.48	3.66	3.61	3.59	3.64	3.60	3.59
Assay	101.4	99.6	97.1	97.1	101.0	102.3	102.2	99.5	97.0	98.7	100.4	101.7	101.4
S-form	NT	NT	NT	NT	1.8	2.2	2.2	NT	NT	NT	7.6	8.1	9.8
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 8

Stability study of Formulation XI- Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 4.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH Time Point					40 ± 2° C./75 ± 5% RH			
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.02	3.96	3.98	3.97	3.91	4.01	3.99	4.02	4.03
Assay	101.3	98.7	95.5	99.2	100.5	98.6	95.3	97.1	97.5
S-form	NT	NT	NT	NT	1.7	NT	NT	NT	7.8
Total Impurities	0.1	ND	0.06	ND	0.80	ND	0.06	0.1	0.79

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TABLE 9

Stability study of Formulation XII - Norepinephrine Bitartrate Injection (16 µg/ml) filled in glass vial (pH 4.5).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH Time Point					40 ± 2° C./75 ± 5% RH			
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.50	4.35	4.36	4.32	4.33	4.33	4.40	4.39	4.29
Assay	100.1	98.9	95.5	98.2	97.9	97.1	92.5	93.7	77.2
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	2.9
Total Impurities	ND	0.32	0.79	0.52	3.41	1.18	0.38	5.59	10.38

TABLE 10

Stability study of Formulation XIII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 5.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	4 Month	1 Month	2 Month	3 Month	4 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.99	4.62	4.51	4.57	4.51	4.87	4.81	4.83	4.53
Assay	102.7	100.5	95.6	99.2	100.4	98.3	89.8	87.0	72.3
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	3.0
Total Impurities	ND	0.75	0.81	0.48	1.29	0.94	2.4	5.39	14.91

Based on the above considerations, the effect of different levels of EDTA on stability of norepinephrine was determined. Three batches at concentrations of 16 μg/mL, 32 μg/mL, and 64 μg/mL were made with EDTA concentrations of 100 μg/mL: Formulation XIV (16 μg/mL), Formulation XV (32 μg/mL), Formulation XVI (64 μg/mL). Two additional batches were made at 10 μg/mL EDTA Formulation XVII and 1 μg/mL EDTA (Formulation XVIII) at 64 μg/mL Norepinephrine. The composition of the batches is specified in Table 11. The drug product was compounded as described earlier and packaged in 250 mL in polypropylene bags. This was further packed into aluminum overwrap with an oxygen scavenger (GLS 100, Mitsubishi Gas Chemicals). The batches were then stored at room temperature and accelerated temperature conditions.

TABLE 11

Formulation composition selected with different level of EDTA concentrations.

	Quantity (mg/mL) Formulation Number				
Ingredient	XIV	XV	XVI	XVII	XVIII
Norepinephrine Bitartrate	0.016	0.032	0.064	0.064	0.064

TABLE 11-continued

Formulation composition selected with different level of EDTA concentrations.

	Quantity (mg/mL) Formulation Number				
Ingredient	XIV	XV	XVI	XVII	XVIII
Edetate Sodium	0.10	0.10	0.10	0.010	0.0010
Sodium chloride	9	9	9	9	9
Hydrochloric Acid/ Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for Injection	q.s. 1 mL	q.s. 1 mL	q.s.1 mL	q.s. 1 mL	q.s. 1 mL

The resultant stability data on these formulations are presented in Table 12-Table 16 (CCS— Clear colorless solution; ND—Not Detected). The results of the stability studies at different amounts of EDTA at pH 4.0 indicates that both 0.01%, 0.001% of EDTA significantly prevented the degradation rate of norepinephrine in terms of known and unknown impurities. Moreover, with respect to isomerization from the R-isomer to the S-isomer it was notably observed that the amount of EDTA had substantially no influence on racemization or enantiomer formation during stability and after autoclaving.

TABLE 12

Stability study of Formulation XIV - Norepinephrine bitartrate injection (16 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.99	3.96	4.08	4.08	4.02	4.08	4.08
Assay	98.5	100.4	100.1	99.7	100.3	100.0	99.5
S-form	0.9	1.1	1.4	1.3	1.9	2.9	4.2
Total Impurities	0.05	ND	ND	ND	ND	0.10	0.38

TABLE 13

Stability study of Formulation XV - Norepinephrine bitartrate injection (32 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.01	3.99	4.08	4..08	4.02	4.08	4.08
Assay	101.0	102.9	97.1	100.7	102.9	99.4	100.6
S-form	0.9	1.1	1.3	1.4	1.9	3.0	4.1
Total Impurities	0.06	ND	ND	ND	ND	ND	0.14

TABLE 14

Stability study of Formulation XVI - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.99	4.08	4.08	3.98	4.07	4.07
Assay	98.4	103.2	98.7	100.2	104.6	99.3	99.8
S-form	0.9	1.1	1.3	1.3	2.0	3.2	4.2
Total Impurities	0.06	ND	0.12	ND	ND	ND	ND

TABLE 15

Stability study of Formulation XVII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 10 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.06	4.06	3.99	4.05	4.05
Assay	102.7	105.7	103.4	104.3	107.8	103.6	103.9
S-form	0.9	1.1	1.2	1.5	2.0	3.3	4.3
Total	0.06	ND	ND	ND	ND	0.26	ND

TABLE 16

Stability study of Formulation XVIII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 1 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.07	4.07	4.02	4.06	4.06
Assay	98.7	102.6	100.4	100.4	105.0	99.9	99.2

TABLE 16-continued

Stability study of Formulation XVIII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 1 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
S-form	0.9	1.1	1.3	1.4	2.0	3.2	4.3
Total	0.06	ND	ND	ND	ND	ND	ND

Sterilization and Stability: The volume for ready-to-inject formulations is 250 mL and as such classifies as a large volume parenteral (LVP). To achieve a desired or required sterility assurance level of 10^{-6} for a LVP terminal sterilization via heat it is typically required. The inventors therefore investigated whether or not contemplated formulations could be terminally sterilized via autoclaving.

Formulations at a concentration 16 μg/mL and 64 μg/mL (Formulation XVII) Norepinephrine base were prepared substantially as shown in Table 11 above and packaged in secondary packaging of aluminum overwrap with an oxygen scavenger and shipped for terminal sterilization. The secondary packaging was removed and the bags were terminally sterilized using steam sterilizer (Fedegari, Model # FOB3) with an air over-pressure (AOP) sterilization cycle. The terminal sterilization was performed at 121° C. for 5, 10, and 15 min. Post completion of sterilization temperature, the bags underwent spontaneous cooling to 95° C. and forced cooling to 70° C. The total exposure time and calculated F_0 values were 11.09, 17.04, and 22.42 for 5 min, 10 min, and 15 min cycles respectively. The bags were then analyzed for assay, impurities, and S-isoform, and the results are shown in Table 17 and Table 18.

TABLE 17

Stability study of Norepinephrine bitartrate injection (16 μg/mL) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA; terminally sterilized.

	Time Point									
	Initial	5 Min			10 Min			15 Min		
		Bag Number								
		1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.76	3.85	3.78	3.77	3.76	3.76	3.78	3.76	3.75	3.76
Dissolved Oxygen	0.63	4.93	4.86	4.89	0.75	0.48	0.55	0.65	0.78	0.77
Assay	103.1	103.1	103.1	103.1	103.1	103.0	103.1	103.1	103.2	103.1
S-Form	1.0	3.0	3.0	3.0	3.8	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 18

Stability study of Norepinephrine bitartrate injection (64 μg/ml) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA terminally sterilized.

	Time Point									
	Initial	5 Min			10 Min			15 Min		
		Bag Number								
		1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.74	3.74	3.75	3.73	3.74	3.74	3.76	3.74	3.73	3.74
Dissolved Oxygen	0.69	5.15	5.03	5.00	0.52	0.59	0.75	0.69	0.80	0.74
Assay	101.2	102.2	101.2	101.5	101.7	101.2	101.3	101.2	101.3	102.2
S-Form	1.0	3.0	3.0	3.0	3.7	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND

As can be seen from the data, the S-isoform appears to increase proportionally to time during the terminal sterilization cycle. No increase in reportable impurities was observed.

Test method—Determination of norepinephrine and degradation products: Separation of Norepinephrine and related compounds was performed using a gradient HPLC method with UV detection. Pentofluorophenylpropyl terminated silica was used as a stationary phase for chromatographic analysis. The mobile phase was prepared by mixing water and methanol, with both solvents containing formic acid. Related compounds were defined by their relative retention times (RRT) based on the NE peak retention time. Quantitation of related compounds was accomplished by comparing the corresponding peak area from a sample solution chromatogram to that of the NE peak from a Reference Standard (RS) solution of a known concentration. Relative Response Factors (RRF) were used to correct for chemical structure effects on the responses of the identified impurities. Chromatography was performed using parameters and methods as shown in Table 19.

TABLE 19

HPLC	Waters Alliance e2695		
Column	Supelco Discovery HS F-5 Column, 3 µm, 4.6 × 150 mm		
Column Temperature	35° C.		
Sample Temperature	Ambient		
Injection volume	85.0 µL		
Flow Rate	0.8 mL/min		
Detection	Spectrum: 200-600 nm, resolution 1.2 nm Single channel: 280 nm, resolution 4.8 nm PDA Filter Time Constant: Normal Sampling rate: 5 points/sec		
Solution A	0.1% Formic acid in Water		
Solution B	0.1% Formic acid in Methanol		
	Time (mins)	% Solution A	% Solution B
Mobile Phase	0	100	0
	3	100	0
	6	93	7
	8	93	7
	15	88	12
	30	2	98
	35	2	98
	36	100	0
	40	100	0

Test Method—Identification, Assay and Enantiomeric Purity of Norepinephrine: Identification and quantification of S-norepinephrine and R-norepinephrine was performed using an HPLC method with UV detection. HPLC-UV was used to separate and quantitate the amount of (R)- and (S)-enantiomers of norepinephrine (NE) present in the drug product with the NE concentrations of 16, 32 and 64 µg/ml. The comparison of the sum of (R)- and (9-peak responses in a sample chromatogram versus a reference standard chromatogram gives the total amount of NE. The (S)-enantiomer was quantitated based on its peak response as the percentage of the total peak response of both enantiomers.

More specifically, determination of R- and S-enantiomers of norepinephrine in the drug product solution was performed using an isocratic reverse-phase HPLC method with UV detection. Separation was achieved by using a protein-based column with functional chiral selectors. The chiral selector is cellobiohydrolase (CBH), a stable enzyme that has been immobilized onto spherical silica particles. This enzyme preferentially separates compounds containing one or more basic nitrogen groups together with one or more hydrogen-accepting or hydrogen-donating groups. Chromatography was performed using parameters and methods as shown in Table 20.

TABLE 20

HPLC	Agilent 1260 Infinity
Column	Daicel Chiralpak CBH ™ column, 5 µm, 4.0 × 100 mm
Column Temperature	27° C. ± 2° C.
Sample Temperature	Ambient
Injection volume	20.0 µL for 16 mcg/mL, 10.0 µL for 32 mcg/mL, 5.0 µL for 64 mcg/mL
Flow Rate	0.9 mL/min
Detection	Single channel: 280 nm, resolution 4.8 nm Spectrum: 200-600 nm, resolution 1.2 nm
Mobile Phase:	Buffer/IPA 95:5 v/v Buffer: Sodium Phosphate, Disodium Edetate, pH 6.0
Run Time	8 min

While contemplated formulations can be administered following various protocols, the inventors contemplate that administration of the formulations, especially administration for treatment of hypotension, will follow a protocol that comprises at least two distinct steps, with an accelerated administration followed by a maintenance administration as exemplarily described in Table 21 below.

TABLE 21

		Initial Dose		Maintenance Dose	
Presentation (mg/mL)	Concentration (µg/mL)	Dose per Minute (µg/min)	Flow Rate (mL/min)	Dose per Minute (µg/min)	Flow Rate (mL/min)
16 µg/mL (4 mg in 250 mL)	16	8-12	0.500-0.750	2-4	0.125-0.250
32 µg/mL (8 mg in 250 mL)	32		0.250-0.375		0.062-0.125
64 µg/mL (16 mg in 250 mL)	64		0.125-0.187		0.031-0.062

As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set

forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

Moreover, in interpreting the disclosure all terms should be interpreted in the broadest possible manner consistent with the context. In particular the terms "comprises" and "comprising" should be interpreted as referring to the elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps can be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

What is claimed is:

1. A method of treating hypotension, comprising:

administering a ready-to-administer norepinephrine composition at an initial dose per minute;

administering the norepinephrine composition at a maintenance dose per minute, wherein the initial dose per minute is greater than the maintenance dose per minute;

wherein the initial dose per minute is a dose of between 8 and 12 μg/min, and wherein the maintenance dose per minute is a dose of between 2 and 4 μg/min;

wherein the norepinephrine composition comprises norepinephrine or a salt thereof at a concentration of between 10 μg/ml and 100 μg/ml in an aqueous acidic solution having a pH range of between 3.7 and 4.3, wherein the aqueous acidic solution further comprises a chelating agent at a concentration of between 1 μg/ml and 100 μg/ml and a tonicity agent;

wherein the norepinephrine composition is substantially free of antioxidants; and

wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

2. The method of claim **1** wherein the aqueous acidic solution has a pH range of between 4.0 and 4.2.

3. The method of claim **1** wherein the aqueous acidic solution has a pH range of between 3.7 and 4.0.

4. The method of claim **1** wherein the norepinephrine is present in the composition at a concentration of about 16 μg/ml, about 32 μg/ml, or about 64 μg/ml.

5. The method of claim **1** wherein the norepinephrine in the composition is a salt of norepinephrine.

6. The method of claim **5** wherein the salt of norepinephrine in the composition is norepinephrine bitartrate.

7. The method of claim **1** wherein the chelating agent in the composition is selected from the group consisting of a bicarboxylic acid, a tricarboxylic acid, and an aminopolycarboxylic acid.

8. The method of claim **1**, wherein the chelating agent is present in the composition at a concentration of between 10 μg/ml and 100 μg/ml.

9. The method of claim **1**, wherein the tonicity agent is present in the composition in an amount of between 0.6 wt % and 1.2 wt %.

10. The method of claim **1**, wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises at least about 95% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

11. The method of claim **1**, wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises equal or less than about 5% S-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

12. The method of claim **1**, wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises equal or less than about 10% S-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least six months as determined by HPLC.

13. The method of claim **1**, wherein the norepinephrine composition comprises equal or less than about 5% of total degradation of norepinephrine or salt thereof excluding S-norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

14. A method of administering a ready-to-administer norepinephrine composition to an individual in need thereof, comprising:

administering the norepinephrine composition at an initial rate of between 8 and 12 μg/min;

adjusting administration of norepinephrine composition to a maintenance rate of between 2 and 4 μg/min;

wherein the norepinephrine composition comprises norepinephrine or a salt thereof at a concentration of between 10 μg/ml and 100 μg/ml as a base and further comprises a chelating agent in an amount of between 1 μg/ml and 100 μg/ml;

wherein the norepinephrine composition is substantially free of antioxidants; and

wherein the norepinephrine composition comprises norepinephrine or a salt thereof in an aqueous acidic solution having a pH range of between 3.7 and 4.3, wherein the aqueous acidic solution further comprises a tonicity agent; and

wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises at least about 90% R-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

15. The method of claim **14**, wherein the norepinephrine is present in the composition at a concentration of about 16 μg/ml, about 32 μg/ml, or about 64 μg/ml.

16. The method of claim **14**, wherein the norepinephrine in the norepinephrine in the composition is a salt of norepinephrine, and wherein the salt of norepinephrine is norepinephrine bitartrate.

17. The method of claim **14** wherein the aqueous acidic solution has a pH range of between 4.0 and 4.2.

18. The method of claim **14** wherein the aqueous acidic solution has a pH range of between 3.7 and 4.0.

19. The method of claim **14**, wherein the tonicity agent is present in an amount of between 0.6 wt % and 1.2 wt %.

20. The method of claim **14**, wherein the norepinephrine or a salt thereof in the norepinephrine composition comprises at least about 95% R-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

21. The method of claim **14**, wherein the norepinephrine or a salt thereof in the ready-to-administer norepinephrine composition comprises equal or less than about 10% S-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

22. The method of claim **14**, wherein the ready-to-administer norepinephrine composition comprises equal or less than about 5% of total degradation of norpepinephrine or salt thereof excluding S-norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

* * * * *

EXHIBIT C

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(12) **United States Patent**
Hingorani et al.

(10) **Patent No.: US 10,471,026 B2**
(45) **Date of Patent: *Nov. 12, 2019**

(54) **NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR**

(71) Applicant: **Nevakar, Inc.**, Bridgewater, NJ (US)

(72) Inventors: **Tushar Hingorani**, Bridegwater, NJ (US); **Prem Sagar Akasapu**, Edison, NJ (US); **Kumaresh Soppimath**, Skillman, NJ (US)

(73) Assignee: **Nevakar Inc.**, Bridgewater, NJ (US)

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(58) **Field of Classification Search**

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See application file for complete search history.

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Primary Examiner — Theodore R. West

(74) *Attorney, Agent, or Firm* — Umberg Zipser LLP

(57) **ABSTRACT**

The inventive subject matter is directed to compositions and methods for ready-to-inject norepinephrine compositions with improved stability. Most preferably, compositions presented herein are substantially antioxidant free and exhibit less than 10% isomerization of R-norepinephrine and exhibit less than 5% degradation of total norepinephrine.

18 Claims, No Drawings

NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR

This application is a divisional application of copending US non-provisional application with Ser. No. 15/883,798, which was filed Jan. 30, 2018, which claims priority to US provisional application with Ser. No. 62/452,220, which was filed Jan. 30, 2017.

FIELD OF THE INVENTION

The field of the invention is pharmaceutical compositions comprising norepinephrine, especially as it relates to storage stable, ready-to-inject, antioxidant free compositions, and method of manufacturing such compositions.

BACKGROUND

The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

Norepinephrine is often used during CPR (cardio-pulmonary resuscitation), and in the treatment of cardiac arrest and profound hypotension. Norepinephrine is also used for blood pressure control in certain acute hypotensive states, including for example sympathectomy, poliomyelitis, pheochromocytomectomy, spinal anesthesia, myocardial infarction, blood transfusion, and septicemia.

Currently, norepinephrine is marketed as Levophed®, which is a concentrated 4 mg per 4 mL norepinephrine bitartrate formulation to be administered by intravenous infusion following dilution with dextrose or dextrose and sodium chloride injection. Norepinephrine is also marketed by Baxter which supplies as a norepinephrine concentrate that is free of sodium metabisulfite and packaged under nitrogen. Unfortunately, most, if not all diluted commercially available norepinephrine formulations lack storage and should therefore be discarded within one day after reconstitution when stored at room temperature. Consequently, risk for microbial contamination and dilution errors is present. In addition, Levophed also contains sodium metabisulphite as an antioxidant, and carries a warning label that sulfite may cause allergic type reactions including anaphylactic shock and life threating or less severe asthmatic episodes in susceptible people. Table 1 depicts ingredients of currently marketed norepinephrine compositions.

TABLE 1

Composition of currently marketed Norepinephrine Bitartrate Products.

Ingredient	Levophed ® (Hospira)	Norepinephrine Bitartarate (Baxter)
Norepinephrine Bitartrate equivalent to Norepinephrine Base	1 mg/mL	1 mg/mL
Sodium Chloride	Isotonic	Isotonic
Sodium Metabisulphite	0.2 mg/mL	—
pH	3-4.5	3-4.5
Water for injection	q.s. 1 mL	q.s 1 mL

Stability of Levophed® and Norepinephrine bitartrate injection (Baxter), in normal saline solutions is presented in Table 2 and Table 3 where norepinephrine was diluted to a concentration of 16 μg/ml. Stability was assessed in 250 ml saline at accelerated (i.e., 40±2° C. and 75±5% relative humidity, duration as indicated) and long term stability (i.e., 25±2° C. and 60±5% relative humidity, duration as indicated) storage conditions.

TABLE 2

Stability study of Levophed ® diluted in 0.9% Saline (Hospira) at 16 μg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	97.3	98.9	97.9	91.9	98.8	96.5	80.2	71.9
Total Impurities	0.05	—	0.71	8.08	0.03	1.96	5.29	9.73

TABLE 3

Stability study of Norepinephrine bitartrate injection [Baxter] diluted in 0.9% Saline (Hospira) at 16 µg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	99.9	99.7	97.0	92.2	99.4	91.5	82.9	77.6
Total Impurities	0.08	1.73	2.68	10.17	0.10	2.34	4.46	6.71

As can be seen from the results, the norepinephrine at ready-to-inject concentrations underwent significant degradation. Oxidative degradation could possibly be reduced or even prevented by addition of effective amounts of sodium metabisulphite to the ready-to-inject norepinephrine solution. However, the quantities of sodium metabisulphite that would be administered by injection of 250 ml of the ready-to-inject solution would be substantial and detrimental to the patient. To avoid issues associated with sodium metabisulphite, efforts have been made to provide norepinephrine formulations with a non-sulfite anti-oxidant. For example US 2016/0058715 teaches a ready-to-inject dosage form of norepinephrine that uses butylated hydroxyl anisole as an anti-oxidant. While generally deemed safe for topical and cosmetic use, butylated hydroxyl anisole was shown to produce some renal and hepatic damage (e.g., *Int J Toxicol.* 2002; 21 Suppl 2:19-94).

In other attempts to provide ready-to-administer norepinephrine formulations with increased storage stability and reduced risk of human error, the pH on the injectable solution was reduced to between 3.2 and 3.6 with 40-200 µg/ml norepinephrine as is described in WO 2015/128418. While such formulations exhibited reduced degradation as compared to higher pH formulations, significant discomfort can occur at the injection site. Worse yet, at the pH used, norepinephrine isomerized relatively quickly from the active R (−) isomer to the inactive S (+) isomer. Isomerization is also encountered at exposure of norepinephrine to higher temperatures.

Therefore, there is a need for improved stable, low concentration, ready-to-inject and antioxidant free norepinephrine formulations, and methods of manufacturing and storing the same.

SUMMARY OF THE INVENTION

The inventive subject matter is directed to antioxidant free sterilizable/autoclavable ready-to-inject norepinephrine compositions having improved stability and a physiologically acceptable pH.

In one aspect of the inventive subject matter, the inventors contemplate a ready to ready-to-inject norepinephrine composition that comprises an aqueous acidic buffer having a pH range of between 3.7 and 4.3, wherein the aqueous buffer further comprises a chelating agent and a pharmaceutically acceptable salt. Most typically, the chelating agent is present in an amount of between 1 µg/ml and 100 µg/ml, and the pharmaceutically acceptable salt is present in an amount of between 0.6 wt % and 1.2 wt %. Norepinephrine (typically enantiomerically pure (i.e., at least 98%) R-isomer) is dissolved at a concentration that is suitable for administration to a patient in need thereof. In further preferred aspects, the ready-to-administer norepinephrine composition is substantially free of antioxidants, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As used herein, reference to the term norepinephrine should be interpreted broadly to include pharmaceutically acceptable salts and prodrugs thereof.

Therefore, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes a step of formulating a liquid parenteral composition that contains in an aqueous acidic buffer norepinephrine as an R-isomer such that (a) the formulation exhibits less than 10% of isomerization of the R-isomer to an S-isomer after three months of storage of the liquid composition, and (b) the formulation exhibits equal or less than 5% degradation of total norepinephrine after three months of storage of the liquid composition. The aqueous acidic buffer will typically have a pH range of between 3.7 and 4.3, and the aqueous buffer will further comprise a chelating agent and a pharmaceutically acceptable salt. In such methods, the total norepinephrine is present in the liquid parenteral composition at a concentration of between 10 µg/ml and 100 µg/ml, and the ready-to-inject norepinephrine composition is substantially free of antioxidants.

Viewed form a different perspective, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes the steps of preparing an aqueous acidic buffer at a pH range of between 3.7 and 4.3, wherein the aqueous buffer also includes a chelating agent and a pharmaceutically acceptable salt. Preferably, the chelating agent is present in an amount of between 1 µg/ml and 100 µg/ml, and tonicity is adjusted with the pharmaceutically acceptable salt (e.g., NaCl). In a further step, norepinephrine (preferably enantiomerically pure R-isomer) is dissolved at a concentration suitable for administration to a patient in need thereof, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As before, it is generally preferred that the ready-to-administer norepinephrine composition is substantially free of antioxidants. In yet another step, the composition is autoclaved to sterility.

Most typically, but not necessarily, the aqueous acidic buffer is a citrate buffer and/or preferably has a concentration of between 5 mM and 20 mM. Furthermore, preferred aqueous acidic buffers will have a pH of between 3.8 and 4.2. With respect to the chelating agent it is contemplated

that such agents are a bicarboxylic acid (e.g., optionally hydroxylated, tartrate), a tricarboxylic acid (e.g., aconitic acid, trimesic acid, citric acid), and/or an aminopolycarboxylic acid (e.g., EDTA, EGTA, etc.), and that such chelating agents are present at low concentrations, preferably between 1 μg/ml and 10 μg/ml, or between 10 μg/ml and 100 μg/ml. The norepinephrine is typically present at a concentration of between 10 μg/ml and 100 μg/ml, for example, at a concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), or 64 μg/ml (+/−10%). Contemplated methods may also include a step of autoclaving the compositions.

With respect to stability it is contemplated that the storage condition is over at least three months at 40° C. and 75% (+/−5) relative humidity, that equal or less than 6% of the R-isomer form will isomerize to the S-isomer, and/or that equal or less than 3.5% of the total norepinephrine will degrade to degradation products.

Where desired, contemplated compositions have a dissolved oxygen concentration of equal or less than 1 ppm (e.g., by formulating the liquid parenteral composition using deoxygenated water), and/or or by packaging the composition together with a (preferably metal free) oxygen scavenger. Packaging may further make use of a container that is configured (e.g., aluminized or otherwise treated) to reduce light-mediated oxidation of the norepinephrine.

DETAILED DESCRIPTION OF THE INVENTION

The inventive subject matter is directed to stable aqueous pharmaceutical preparations of norepinephrine (and pharmaceutically acceptable salts thereof) in a ready-to-inject form that are sterile and preferably substantially free of antioxidants. Most preferably, stability of such compositions is characterized by low (oxidative and photo-induced) degradation as well as low isomerization.

More specifically, the inventors have discovered that formulations can be prepared that will exhibit less than 8%, more typically less than 6%, even more typically less than 4%, and most typically less than 3% of degradation as determined by HPLC-UV, and that will exhibit less than 10%, more typically less than 8%, even more typically less than 6%, and most typically less than 4% of isomerization from R- to S-configuration as determined by HPLC-UV. Most notably, such formulations were found to be stable over extended periods without antioxidants (e.g., at least 1 month, or at least two months, or at least three months), even at elevated storage temperatures (e.g., accelerated storage conditions such as 40° C. and 75% relative humidity (+/−5%)). Even more remarkable, such formulations could also be subjected to thermal sterilization, and particularly sterilizing to sterility (e.g., over at least 5 min, or at least 10 min, or at least 15 min at 121° C.), without substantial increase (i.e., >1.5%, or >1.0%, or >0.7%) of the S-isomer of norepinephrine.

Additionally, it should be appreciated that contemplated formulations can be filled in a polymer bag (e.g., polypropylene) or other container that may subsequently be placed into a secondary container together with an oxygen scavenger, and especially a metal-free oxygen scavenger. Most typically, at least one of the polymer bag and the secondary container may be impervious to light in general or light of a wavelength that promotes photo-initiated degradation. For example, containers may be metalized (e.g., aluminized) or combined or coated with carbonaceous materials or other dye(s). If desired, contemplated formulations are sufficiently stable to also allow filling into containers using a blow-fill-seal (BFS) process.

Therefore, contemplated norepinephrine formulations of the inventive subject matter can advantageously be provided in a ready-to-inject form to thereby avoid the inconvenience associated with diluting concentrated small volume norepinephrine parenteral formulations into diluents prior to infusion. Thus, the ready-to-inject formulations also eliminate microbial contamination risks and calculation errors associated with dilution. Most typically, contemplated formulations will be available in a range of concentrations commonly required by medical practitioners for emergency restoration of blood pressure, for example in cases of acute hypotension. Consequently, norepinephrine will typically be present in formulations at a concentration of between 10 μg/ml and 100 μg/ml, including concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), and 64 μg/ml (+/−10%).

As will be readily appreciated, the norepinephrine for preparation of contemplated formulations is preferably (R)-Norepinephrine, or enantiomerically pure (i.e., at least 98% R-isomer) norepinephrine. However, in less preferred aspects, isomeric purity can also be between 95-98%, or even between 90-95%. Of course, it should also be appreciated that the norepinephrine may be a salt of any suitable and pharmaceutically acceptable form, including mineral salts (e.g., HCl salt) and organic salts (e.g., bitartrate). Similarly, where desired, the norepinephrine may also be used in any suitable prodrug form (e.g., β,3-dihydroxytyrosine, L-dihydroxyphenylserine, etc.).

Suitable buffers are generally buffers that stabilize the pH of the contemplated liquid formulations in an acidic pH range and will therefore include glycine buffers, citrate buffers, citrate/phosphate buffers, acetate buffers, etc. However, the inventors have further discovered that where the norepinephrine is provided as the norepinephrine bitartrate salt, a buffer can advantageously be omitted and the pH can be adjusted with suitable acid and/or base as is well known in the art. Notably, the bitartrate appeared to act as a weak buffer in the stability range for the norepinephrine as is shown in more detail below. Most typically the pH of the formulation will be less than 5.0 and more typically less than 4.5, and most typically less than 4.3, but higher than 3.0, more typically higher than 3.5, and most typically higher than 3.7. For example, suitable buffers will have a pH in the range of between 3.7 and 4.3, or between 3.7 and 4.0, or between 3.8 and 4.1, or between 3.9 and 4.2, or between 4.0 and 4.2. Notably, such pH range provided remarkable stability for low concentrations of norepinephrine, especially when in combination with a chelator and a salt. While not limiting to the inventive subject matter, the buffer strength is typically relatively low, for example, equal or less than 100 mM, and more typically equal or less than 50 mM, and most typically between 5 mM and 20 mM (e.g., 10 mM).

Moreover, in further contemplated aspects, the formulation will also include one or more chelating agents, and particularly metal ion chelators. For example, suitable chelators include various bicarboxylic acids, tricarboxylic acids, and aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and penta(carboxymethyl)diethylenetriamine (DTPA), and salts and hydrates thereof. While not limiting to the inventive subject matter, it is contemplated that the metal ion chelators will slow down both the baseline and metal ion-stimulated autoxidation of norepinephrine. Remarkably, the inventors unexpectedly observed that the desirable effect of the chela-

tors was observable at relatively low concentrations of the chelators. For example, reduction of the baseline and metal ion-stimulated autoxidation of norepinephrine was observed at chelator concentrations of between 1 μg/ml and 10 μg/ml, and between 10 μg/ml and 100 μg/ml. Interestingly, the chelators, and especially the aminopolycarboxylic acids retained stabilizing effect despite the relatively low pH favoring protonated forms of the chelators.

With respect to suitable salts it is contemplated that the salt is a pharmaceutically acceptable salt that can be used to increase tonicity. Therefore, pharmaceutically acceptable salts are contemplated, and especially NaCl, at a concentration of at least 0.6 wt %, or at least 0.7 wt %, or at least 0.8 wt %, or at least 0.9 wt %. For example, suitable salt concentrations are between 0.6 wt % and 1.2 wt %. Depending on the particular salt concentration, additional tonicity agents may be added and suitable tonicity agents include glycerol, thioglycerol, mannitol, lactose, and dextrose. The amount of tonicity adjusting agent used can be adjusted to obtain osmolality of the formulations in the range of 260 to 340 mOsm/kg. An osmometer can be used to check and adjust the amount of tonicity adjusting agent to be added to obtain the desired osmolality.

It should further be appreciated that contemplated compositions are substantially free of antioxidants (i.e., do not include antioxidants in an amount effective to reduce degradation of total norepinephrine by at least 1% when stored over a period of at least three months at 25° C. Indeed, the inventors unexpectedly discovered that some formulations with antioxidants (particularly with ascorbic acid) had decreased stability. Notably, contemplated formulations were stable as described in more detail below, even in the absence of effective quantities of antioxidants, especially where deoxygenated solvents (e.g., typically water and/or buffer) were employed. Deoxygenation (i.e., reduction of molecular dissolved oxygen) can be achieved in numerous manners, including sparging with inert gases (e.g., helium, various freons, argon, xenon), agitation under vacuum, and/or using enzymatic systems that deplete a solution of dissolved oxygen (see e.g., U.S. Pat. No. 9,187,779). Additionally, or alternatively, ingress of molecular oxygen into the formulation can also be reduced by co-packaging a container with the formulation in a secondary container that includes an oxygen scavenger, and especially a metal-free oxygen scavenger (e.g., GLS100, Ageless®, Pharmakeep®, all commercially available from Mitsubishi Gas Chemical America).

With respect to the sterilization of contemplated formulations it should be appreciated that contemplated formulations may be sterilized using all known manners of sterilization, including filtration through 0.22 micron filters, heat sterilization, autoclaving, radiation (e.g., gamma, electron beam, microwave). Unexpectedly, and as shown in more detail below, the inventors have also discovered that contemplated formulations were heat stable and did not undergo significant isomerization, even under conditions of sterilization (exposure to high-pressure saturated steam) at 121° C. for at least 5, or at least 10, or at least 15 minutes.

Based on the unexpected heat stability, the formulations contemplated herein can also be filtered through a 0.22 micron filter, and filled in to a polyethylene, polypropylene or low-density polyethylene containers in a blow-fill-seal (BFS) process. BFS is a form of advanced aseptic manufacturing wherein the container is formed, filled, and sealed in one continuous, automated system not requiring human intervention. The process begins with the extrusion of plastic granules in the form of a hot hollow pipe of molten plastic called a parison. The next step is the blow molding of the container with an open top through which the container is filled, all while the plastic remains hot and in a molten state. Once filled, the container is hermetically sealed and cooled. The blow-fill seal process can take several seconds, and contemplated ready-to-inject compositions advantageously are formulated to withstand the temperature and pressure requirements without substantial degradation of norepinephrine (e.g., less than 5 wt %, less than 3 wt %, less than 2 wt %, less than 1 wt % degradation).

Once the norepinephrine formulations are filled in large volume polymeric, semi-permeable infusion containers (e.g., BFS container or flexible IV bags), the containers can optionally be layered or covered with a secondary packaging system including an aluminum pouch or other oxygen scavenger. For example, the BFS containers can further be sealed in an oxygen and moisture barrier blister packaging. The blister packaging can comprise one or more layers, and the one or more layers can include aluminum foil or other oxygen absorber having an Oxygen Transmission Rate (OTR) between 0.0005 to 5.00 cc/100 in^2/24 hrs. Additionally or alternatively, one or more oxygen absorbers (metal or metal free, organic material) can be incorporated into any portion of the BFS container, the secondary packaging system, or between the two (e.g., between the BFS container and the multi-layer packaging) such that the oxygen absorber removes at least a portion of oxygen from the air surrounding said oxygen-sensitive drug. A beneficial feature of the oxygen absorber is the absorbance and removal of oxygen present in the primary packaging and in the liquid drug itself. Notably, it was found that the oxygen absorber also removed residual headspace oxygen in the primary packaging and also dissolved oxygen in the liquid over time, thereby further improving stability of norepinephrine.

The following examples are provided for illustrative purposes only and should not be interpreted as limiting the present invention.

EXAMPLES

The following examples illustrate some of the experiments leading to the formulations according to the inventive subject matter, however, should not be construed to limit the scope of the claims in any way.

Stability and Isomerization: The ionization behavior of norepinephrine in aqueous solution is complex. Common with other o-hydroquinone systems, norepinephrine in aqueous solution is susceptible to oxidation to form the corresponding o-quinone, which can then also undergo various secondary reactions, which also becomes more prevalent as the pH becomes more alkaline. Norepinephrine may further isomerize to the pharmacologically less active S-enantiomer at low pH values, corresponding to protonation of the hydroxyl group at the benzylic chiral center. Therefore, to prevent norepinephrine cyclization reactions pH values less than 6.0 are desired. A pH range of 3.0 to 6.2 was screened to determine pH of optimum stability. Composition of norepinephrine bitartrate equivalent to 16 μg/mL norepinephrine base at various pH values were prepared are described below, with Table 4 listing compositions of norepinephrine bitartrate in citrate buffer (10 mM),

For preparation of the solutions, about 90% of the final quantity of water was collected in a glass media bottle. Nitrogen (N_2) gas was purged for about thirty minutes to reduce the dissolved oxygen levels. Sodium chloride was added and the solution was stirred until a homogenous solution was obtained. Citric acid was added and the solution was stirred until a homogenous solution was obtained. The pH of the bulk solutions was adjusted to pH 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, and 6.2, respectively for each formulation composition using sufficient quantity of 10% w/v sodium hydroxide or 10% w/v hydrochloric acid. Norepinephrine bitartrate was added and the solution was stirred for approximately 10 minutes until a clear solution was formed. Solutions were made up to volume with water. The solutions were filled into 10 mL glass vials, overlaid with nitrogen, stoppered, and sealed. The stability was studied at 4° C., 25° C., and 60° C. by assay. Samples were observed visually for precipitation and change in color for a period of 7 days. Data are presented in Table 5.

No change in physical appearance was observed in the solutions stored at 4° C. In the solutions stored at 25° C., a change in color was observed at pH 6.2. Red brown color was observed in solutions stored at or above pH 5.0 at 60° C. Oxidation and color formation are very common with norepinephrine in unfavorable conditions and the speed of the reaction and the nature of the final products are dependent on the catalysts (e.g., metal ion impurities) and buffers employed. A pH range from 3.0 to 4.5 was selected for further testing.

Stability of Norepinephrine in selected pH ranges and formulations: The formulations for the next experiments are shown in Table 6 below, involving three different compositions of norepinephrine bitartrate at three different pH (3.5, 4.0, 4.5, and 5.0) values. Lab scale batches were prepared and subjected to lab scale stability tests at accelerated (40° C./75% RH) and long term stability (25° C./60% RH) storage conditions. The test results from the stability studies are presented in Table 7-Table 10, with CCS indicating Clear colorless solution; ND indicating Not Detected; NR indicating Not Reported (<0.05%); and NT indicating Not Tested.

TABLE 4

Compositions of Norepinephrine Bitartrate for pH dependent stability in Citrate Buffer (10 mM).

	Concentration (mg/mL)								
Ingredients	I	II	III	IV	V	VI	VII	VIII	IX
Norepinephrine Bitartarate equivalent to Norepinephrine base	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Sodium Chloride	9	9	9	9	9	9	9	9	9
Citric acid	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Sodium Citrate	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
HCl/NaOH (q.s. pH)	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2
Water for Injection (q.s. mL)	1	1	1	1	1	1	1	1	1

TABLE 5

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
4° C.	I	96.4	96.5	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	98.5	3.8	No	No
	IV	99.1	98.4	4.2	No	No
	V	98.1	98.6	4.6	No	No
	VI	98.4	98.1	5.0	No	No
	VII	97.1	96.6	5.4	No	No
	VIII	97.8	97.5	5.8	No	No
	IX	91.5	91.2	6.2	No	No
25° C.	I	96.4	96.4	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	97.9	3.8	No	No
	IV	99.1	97.7	4.2	No	No
	V	98.1	97.3	4.6	No	No
	VI	98.4	97.3	5.0	No	No
	VII	97.1	95.9	5.4	No	No
	VIII	97.8	94.5	5.8	No	No
	IX	91.5	80.4	6.2	Yes	No
60° C.	I	96.4	95.2	3.0	No	No
	II	98.0	95.0	3.4	No	No
	III	99.0	95.2	3.8	No	No
	IV	99.1	93.2	4.2	No	No
	V	98.1	88.9	4.6	No	No
	VI	98.4	77.4	5.0	Yes	No
	VII	97.1	46.8	5.4	Yes	No
	VIII	97.8	NT	5.8	Yes	No
	IX	91.5	NT	6.2	Yes	No

TABLE 6

Formulation composition selected for further development activities and optimization

	Quantity (mg/mL) Formulation			
Ingredient	X	XI	XII	XIII
Norepinephrine Bitartrate	0.016	0.016	0.016	0.016
Edetate Sodium	0.10	0.10	0.10	0.10
Sodium chloride	9	9	9	9
HCl/NaOH	q.s. pH 3.5	q.s. pH 4.0	q.s. pH 4.5	q.s. pH 5.0
Water for Injection Q.S.	1 mL	1 mL	1 mL	1 mL
Dissolved Oxygen (ppm)	<1	<1	<1	<1

TABLE 7

Stability study of Formulation X - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 3.5).

	Storage Condition												
	25 ± 2° C./60 ± 5% RH							40 ± 2° C./75 ± 5% RH					
	Time Point												
	Initial	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.50	3.65	3.59	3.56	3.58	3.54	3.48	3.66	3.61	3.59	3.64	3.60	3.59
Assay	101.4	99.6	97.1	97.1	101.0	102.3	102.2	99.5	97.0	98.7	100.4	101.7	101.4
S-form	NT	NT	NT	NT	1.8	2.2	2.2	NT	NT	NT	7.6	8.1	9.8
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 8

Stability study of Formulation XI- Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.02	3.96	3.98	3.97	3.91	4.01	3.99	4.02	4.03
Assay	101.3	98.7	95.5	99.2	100.5	98.6	95.3	97.1	97.5
S-form	NT	NT	NT	NT	1.7	NT	NT	NT	7.8
Total Impurities	0.1	ND	0.06	ND	0.80	ND	0.06	0.1	0.79

TABLE 9

Stability study of Formulation XII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.5).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.50	4.35	4.36	4.32	4.33	4.33	4.40	4.39	4.29
Assay	100.1	98.9	95.5	98.2	97.9	97.1	92.5	93.7	77.2
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	2.9
Total Impurities	ND	0.32	0.79	0.52	3.41	1.18	0.38	5.59	10.38

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TABLE 10

Stability study of Formulation XIII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 5.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	4 Month	1 Month	2 Month	3 Month	4 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.99	4.62	4.51	4.57	4.51	4.87	4.81	4.83	4.53
Assay	102.7	100.5	95.6	99.2	100.4	98.3	89.8	87.0	72.3
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	3.0
Total Impurities	ND	0.75	0.81	0.48	1.29	0.94	2.4	5.39	14.91

Based on the above considerations, the effect of different levels of EDTA on stability of norepinephrine was determined. Three batches at concentrations of 16 μg/mL, 32 μg/mL, and 64 μg/mL were made with EDTA concentrations of 100 μg/mL: Formulation XIV (16 μg/mL), Formulation XV (32 μg/mL), Formulation XVI (64 μg/mL). Two additional batches were made at 10 μg/mL EDTA Formulation XVII and 1 μg/mL EDTA (Formulation XVIII) at 64 μg/mL Norepinephrine. The composition of the batches is specified in Table 11. The drug product was compounded as described earlier and packaged in 250 mL in polypropylene bags. This was further packed into aluminum overwrap with an oxygen scavenger (GLS 100, Mitsubishi Gas Chemicals). The batches were then stored at room temperature and accelerated temperature conditions.

TABLE 11

Formulation composition selected with different level of EDTA concentrations.

	Quantity (mg/mL) Formulation Number				
Ingredient	XIV	XV	XVI	XVII	XVIII
Norepinephrine Bitartrate	0.016	0.032	0.064	0.064	0.064
Edetate Sodium	0.10	0.10	0.10	0.010	0.0010
Sodium chloride	9	9	9	9	9
Hydrochloric Acid/ Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for Injection	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL

The resultant stability data on these formulations are presented in Table 12-Table 16 (CCS— Clear colorless solution; ND—Not Detected). The results of the stability studies at different amounts of EDTA at pH 4.0 indicates that both 0.01%, 0.001% of EDTA significantly prevented the degradation rate of norepinephrine in terms of known and unknown impurities. Moreover, with respect to isomerization from the R-isomer to the S-isomer it was notably observed that the amount of EDTA had substantially no influence on racemization or enantiomer formation during stability and after autoclaving.

TABLE 12

Stability study of Formulation XIV - Norepinephrine bitartrate injection (16 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.99	3.96	4.08	4.08	4.02	4.08	4.08
Assay	98.5	100.4	100.1	99.7	100.3	100.0	99.5
S-form	0.9	1.1	1.4	1.3	1.9	2.9	4.2
Total Impurities	0.05	ND	ND	ND	ND	0.10	0.38

TABLE 13

Stability study of Formulation XV - Norepinephrine bitartrate injection (32 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.01	3.99	4.08	4..08	4.02	4.08	4.08
Assay	101.0	102.9	97.1	100.7	102.9	99.4	100.6
S-form	0.9	1.1	1.3	1.4	1.9	3.0	4.1
Total Impurities	0.06	ND	ND	ND	ND	ND	0.14

TABLE 14

Stability study of Formulation XVI - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.99	4.08	4.08	3.98	4.07	4.07
Assay	98.4	103.2	98.7	100.2	104.6	99.3	99.8
S-form	0.9	1.1	1.3	1.3	2.0	3.2	4.2
Total Impurities	0.06	ND	0.12	ND	ND	ND	ND

TABLE 15

Stability study of Formulation XVII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 10 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.06	4.06	3.99	4.05	4.05
Assay	102.7	105.7	103.4	104.3	107.8	103.6	103.9
S-form	0.9	1.1	1.2	1.5	2.0	3.3	4.3
Total	0.06	ND	ND	ND	ND	0.26	ND

TABLE 16

Stability study of Formulation XVIII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 1 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH Time Point				40 ± 2° C./75 ± 5% RH		
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.07	4.07	4.02	4.06	4.06
Assay	98.7	102.6	100.4	100.4	105.0	99.9	99.2
S-form	0.9	1.1	1.3	1.4	2.0	3.2	4.3
Total	0.06	ND	ND	ND	ND	ND	ND

Sterilization and Stability: The volume for ready-to-inject formulations is 250 mL and as such classifies as a large volume parenteral (LVP). To achieve a desired or required sterility assurance level of 10^{-6} for a LVP terminal sterilization via heat it is typically required. The inventors therefore investigated whether or not contemplated formulations could be terminally sterilized via autoclaving.

Formulations at a concentration 16 μg/mL and 64 μg/mL (Formulation XVII) Norepinephrine base were prepared substantially as shown in Table 11 above and packaged in secondary packaging of aluminum overwrap with an oxygen scavenger and shipped for terminal sterilization. The secondary packaging was removed and the bags were terminally sterilized using steam sterilizer (Fedegari, Model # FOB3) with an air over-pressure (AOP) sterilization cycle. The terminal sterilization was performed at 121° C. for 5, 10, and 15 min. Post completion of sterilization temperature, the bags underwent spontaneous cooling to 95° C. and forced cooling to 70° C. The total exposure time and calculated F_0 values were 11.09, 17.04, and 22.42 for 5 min, 10 min, and 15 min cycles respectively. The bags were then analyzed for assay, impurities, and S-isoform, and the results are shown in Table 17 and Table 18.

TABLE 17

Stability study of Norepinephrine bitartrate injection (16 μg/mL) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA; terminally sterilized.

	Time Point									
	Initial	5 Min			10 Min			15 Min		
		Bag Number								
		1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.76	3.85	3.78	3.77	3.76	3.76	3.78	3.76	3.75	3.76
Dissolved Oxygen	0.63	4.93	4.86	4.89	0.75	0.48	0.55	0.65	0.78	0.77
Assay	103.1	103.1	103.1	103.1	103.1	103.0	103.1	103.1	103.2	103.1
S-Form	1.0	3.0	3.0	3.0	3.8	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 18

Stability study of Norepinephrine bitartrate injection (64 μg/ml) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA terminally sterilized.

	Time Point									
	Initial	5 Min			10 Min			15 Min		
		Bag Number								
		1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.74	3.74	3.75	3.73	3.74	3.74	3.76	3.74	3.73	3.74
Dissolved Oxygen	0.69	5.15	5.03	5.00	0.52	0.59	0.75	0.69	0.80	0.74
Assay	101.2	102.2	101.2	101.5	101.7	101.2	101.3	101.2	101.3	102.2
S-Form	1.0	3.0	3.0	3.0	3.7	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND

As can be seen from the data, the S-isoform appears to increase proportionally to time during the terminal sterilization cycle. No increase in reportable impurities was observed.

Test method—Determination of norepinephrine and degradation products: Separation of Norepinephrine and related compounds was performed using a gradient HPLC method with UV detection. Pentofluorophenylpropyl terminated silica was used as a stationary phase for chromatographic analysis. The mobile phase was prepared by mixing water and methanol, with both solvents containing formic acid. Related compounds were defined by their relative retention times (RRT) based on the NE peak retention time. Quantitation of related compounds was accomplished by comparing the corresponding peak area from a sample solution chromatogram to that of the NE peak from a Reference Standard (RS) solution of a known concentration. Relative Response Factors (RRF) were used to correct for chemical structure effects on the responses of the identified impurities. Chromatography was performed using parameters and methods as shown in Table 19.

TABLE 19

HPLC	Waters Alliance e2695
Column	Supelco Discovery HS F-5 Column, 3 μm, 4.6×150 mm
Column Temperature	35° C.
Sample Temperature	Ambient
Injection volume	85.0 μL
Flow Rate	0.8 mL/min
Detection	Spectrum: 200-600 nm, resolution 1.2 nm Single channel: 280 nm, resolution 4.8 nm PDA Filter Time Constant: Normal Sampling rate: 5 points/sec
Solution A	0.1% Formic acid in Water
Solution B	0.1% Formic acid in Methanol

	Time (mins)	% Solution A	% Solution B
Mobile Phase	0	100	0
	3	100	0
	6	93	7
	8	93	7
	15	88	12
	30	2	98
	35	2	98
	36	100	0
	40	100	0

Test Method—Identification, Assay and Enantiomeric Purity of Norepinephrine: Identification and quantification of S-norepinephrine and R-norepinephrine was performed using an HPLC method with UV detection. HPLC-UV was used to separate and quantitate the amount of (R)- and (S)-enantiomers of norepinephrine (NE) present in the drug product with the NE concentrations of 16, 32 and 64 μg/ml. The comparison of the sum of (R)- and (S)-peak responses in a sample chromatogram versus a reference standard chromatogram gives the total amount of NE. The (S)-enantiomer was quantitated based on its peak response as the percentage of the total peak response of both enantiomers.

More specifically, determination of R- and S-enantiomers of norepinephrine in the drug product solution was per-

formed using an isocratic reverse-phase HPLC method with UV detection. Separation was achieved by using a protein-based column with functional chiral selectors. The chiral selector is cellobiohydrolase (CBH), a stable enzyme that has been immobilized onto spherical silica particles. This enzyme preferentially separates compounds containing one or more basic nitrogen groups together with one or more hydrogen-accepting or hydrogen-donating groups. Chromatography was performed using parameters and methods as shown in Table 20.

TABLE 20

HPLC	Agilent 1260 Infinity
Column	Daicel Chiralpak CBH ™ column, 5 μm, 4.0×100 mm
Column Temperature	27° C. ± 2° C.
Sample Temperature	Ambient
Injection volume	20.0 μL for 16 mcg/mL, 10.0 μL for 32 mcg/mL, 5.0 μL for 64 mcg/mL
Flow Rate	0.9 mL/min
Detection	Single channel: 280 nm, resolution 4.8 nm Spectrum: 200-600 nm, resolution 1.2 nm
Mobile Phase:	Buffer/IPA 95:5 v/v Buffer: Sodium Phosphate, Disodium Edetate, pH 6.0
Run Time	8 min

While contemplated formulations can be administered following various protocols, the inventors contemplate that administration of the formulations, especially administration for treatment of hypotension, will follow a protocol that comprises at least two distinct steps, with an accelerated administration followed by a maintenance administration as exemplarily described in Table 21 below.

TABLE 21

		Initial Dose		Maintenance Dose	
Presentation (mg/mL)	Concentration (μg/mL)	Dose per Minute (μg/min)	Flow Rate (mL/min)	Dose per Minute (μg/min)	Flow Rate (mL/min)
16 μg/mL (4 mg in 250 mL)	16	8-12	0.500-0.750	2-4	0.125-0.250
32 μg/mL (8 mg in 250 mL)	32		0.250-0.375		0.062-0.125
64 μg/mL (16 mg in 250 mL)	64		0.125-0.187		0.031-0.062

As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

Moreover, in interpreting the disclosure all terms should be interpreted in the broadest possible manner consistent with the context. In particular the terms "comprises" and "comprising" should be interpreted as referring to the elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps can be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

What is claimed is:

1. A method of controlling S-isomer content in a ready-to-administer norepinephrine composition comprising:

admixing an R-isomer of norepinephrine or salt thereof, a chelating agent and a tonicity agent into an aqueous acidic solution having a pH between 3.7 and 4.3;

wherein the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and wherein the tonicity agent is present in an amount of between 0.6 wt % and 1.2 wt %; and

wherein the concentration of norepinephrine or salt thereof is between 10 μg/ml and 100 μg/ml, and wherein the composition is substantially free of anti-oxidants.

2. The method of claim **1** wherein, the norepinephrine or a salt thereof in the composition comprises at least about 95% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

3. The method of claim **1** wherein, the norepinephrine or a salt thereof in the composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

4. The method of claim **1** wherein, the norepinephrine or a salt thereof in the composition comprises equal or less than

about 10% S-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

5. The method of claim **1**, wherein the norepinephrine or a salt thereof in the composition comprises equal or less than about 5% S-isomer of norepinephrine or a salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

6. The method of claim **2** wherein the composition comprises equal or less than about 5% of total degradation of norepinephrine or salt thereof excluding s-isomer after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

7. The method of claim **3** wherein the composition comprises equal or less than about 3% of total degradation of norepinephrine or salt thereof excluding s-norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

8. The method of claim **4** wherein the composition comprises equal or less than about 1% of total degradation of norepinephrine or salt thereof excluding S-norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

9. The method of claim **1**, wherein the norepinephrine salt is a bitartrate salt of norepinephrine.

10. The method of claim **1**, wherein the R-isomer of norepinephrine or salt thereof is present in the aqueous acidic solution at a concentration of about 16 μg/ml, about 32 μg/ml, or about 64 μg/ml.

11. The method of claim **1**, wherein the chelating agent is selected from the group consisting of a bicarboxylic acid, a tricarboxylic acid, and an aminopolycarboxylic acid.

12. The method of claim **11**, wherein the chelating agent is an aminopolycarboxylic acid.

13. The method of claim **12**, wherein the aminopolycarboxylic acid is present in aqueous acidic solution at a concentration of about 10 μg/mL.

14. The method of claim **1**, wherein the chelating agent is present in the aqueous acidic solution at a concentration of between 1 μg/ml and 10 μg/ml.

15. The method of claim **1**, wherein the chelating agent is present in the aqueous acidic solution at a concentration of between 10 μg/ml and 100 μg/ml.

16. The method of claim **1**, wherein the aqueous acidic solution is prepared from deoxygenated water having dissolved oxygen at a concentration of equal or less than 1 ppm.

17. The method of claim **1**, further comprising a step of packaging the composition in a primary container, and placing the primary container in a secondary container that includes a metal-free oxygen scavenger.

18. The method of claim **17** wherein the primary container and/or the secondary container is configured to reduce light-mediated oxidation of the norepinephrine.

* * * * *

EXHIBIT D

US010568850B2

(12) **United States Patent**
Hingorani et al.

(10) **Patent No.: US 10,568,850 B2**
(45) **Date of Patent: *Feb. 25, 2020**

(54) **NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR**

(71) Applicant: **Nevakar, Inc.**, Bridgewater, NJ (US)

(72) Inventors: **Tushar Hingorani**, Bridegwater, NJ (US); **Prem Sagar Akasapu**, Edison, NJ (US); **Kumaresh Soppimath**, Skillman, NJ (US)

(73) Assignee: **Nevakar Inc.**, Bridgewater, NJ (US)

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See application file for complete search history.

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Primary Examiner — Theodore R. West
(74) *Attorney, Agent, or Firm* — Umberg Zipser LLP

(57) **ABSTRACT**

The inventive subject matter is directed to compositions and methods for ready-to-inject norepinephrine compositions with improved stability. Most preferably, compositions presented herein are substantially antioxidant free and exhibit less than 10% isomerization of R-norepinephrine and exhibit less than 5% degradation of total norepinephrine.

19 Claims, No Drawings

NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR

This application is a divisional application of allowed US non-provisional application with Ser. No. 15/883,798, which was filed Jan. 30, 2018, which claims priority to US provisional application with Ser. No. 62/452,220, which was filed Jan. 30, 2017.

FIELD OF THE INVENTION

The field of the invention is pharmaceutical compositions comprising norepinephrine, especially as it relates to storage stable, ready-to-inject, antioxidant free compositions, and method of manufacturing such compositions.

BACKGROUND

The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

Norepinephrine is often used during CPR (cardio-pulmonary resuscitation), and in the treatment of cardiac arrest and profound hypotension. Norepinephrine is also used for blood pressure control in certain acute hypotensive states, including for example sympathectomy, poliomyelitis, pheochromocytomectomy, spinal anesthesia, myocardial infarction, blood transfusion, and septicemia.

Currently, norepinephrine is marketed as Levophed®, which is a concentrated 4 mg per 4 mL norepinephrine bitartrate formulation to be administered by intravenous infusion following dilution with dextrose or dextrose and sodium chloride injection. Norepinephrine is also marketed by Baxter which supplies as a norepinephrine concentrate that is free of sodium metabisulfite and packaged under nitrogen. Unfortunately, most, if not all diluted commercially available norepinephrine formulations lack storage and should therefore be discarded within one day after reconstitution when stored at room temperature. Consequently, risk for microbial contamination and dilution errors is present. In addition, Levophed also contains sodium metabisulphite as an antioxidant, and carries a warning label that sulfite may cause allergic type reactions including anaphylactic shock and life threating or less severe asthmatic episodes in susceptible people. Table 1 depicts ingredients of currently marketed norepinephrine compositions.

TABLE 1

Composition of currently marketed Norepinephrine Bitartrate Products.

Ingredient	Levophed ® (Hospira)	Norepinephrine Bitartarate (Baxter)
Norepinephrine Bitartrate equivalent to Norepinephrine Base	1 mg/mL	1 mg/mL
Sodium Chloride	Isotonic	Isotonic
Sodium Metabisulphite	0.2 mg/mL	—
pH	3-4.5	3-4.5
Water for injection	q.s. 1 mL	q.s 1 mL

Stability of Levophed® and Norepinephrine bitartrate injection (Baxter), in normal saline solutions is presented in Table 2 and Table 3 where norepinephrine was diluted to a concentration of 16 μg/ml. Stability was assessed in 250 ml saline at accelerated (i.e., 40±2° C. and 75±5% relative humidity, duration as indicated) and long term stability (i.e., 25±2° C. and 60±5% relative humidity, duration as indicated) storage conditions.

TABLE 2

Stability study of Levophed ® diluted in 0.9% Saline (Hospira) at 16 μg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	97.3	98.9	97.9	91.9	98.8	96.5	80.2	71.9
Total Impurities	0.05	—	0.71	8.08	0.03	1.96	5.29	9.73

TABLE 3

Stability study of Norepinephrine bitartrate injection [Baxter] diluted in 0.9% Saline (Hospira) at 16 μg/mL

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	99.9	99.7	97.0	92.2	99.4	91.5	82.9	77.6
Total Impurities	0.08	1.73	2.68	10.17	0.10	2.34	4.46	6.71

As can be seen from the results, the norepinephrine at ready-to-inject concentrations underwent significant degradation. Oxidative degradation could possibly be reduced or even prevented by addition of effective amounts of sodium metabisulphite to the ready-to-inject norepinephrine solution. However, the quantities of sodium metabisulphite that would be administered by injection of 250 ml of the ready-to-inject solution would be substantial and detrimental to the patient. To avoid issues associated with sodium metabisulphite, efforts have been made to provide norepinephrine formulations with a non-sulfite anti-oxidant. For example US 2016/0058715 teaches a ready-to-inject dosage form of norepinephrine that uses butylated hydroxyl anisole as an anti-oxidant. While generally deemed safe for topical and cosmetic use, butylated hydroxyl anisole was shown to produce some renal and hepatic damage (e.g., *Int J Toxicol.* 2002; 21 Suppl 2:19-94).

In other attempts to provide ready-to-administer norepinephrine formulations with increased storage stability and reduced risk of human error, the pH on the injectable solution was reduced to between 3.2 and 3.6 with 40-200 μg/ml norepinephrine as is described in WO 2015/128418. While such formulations exhibited reduced degradation as compared to higher pH formulations, significant discomfort can occur at the injection site. Worse yet, at the pH used, norepinephrine isomerized relatively quickly from the active R (−) isomer to the inactive S (+) isomer. Isomerization is also encountered at exposure of norepinephrine to higher temperatures.

Therefore, there is a need for improved stable, low concentration, ready-to-inject and antioxidant free norepinephrine formulations, and methods of manufacturing and storing the same.

SUMMARY OF THE INVENTION

The inventive subject matter is directed to antioxidant free sterilizable/autoclavable ready-to-inject norepinephrine compositions having improved stability and a physiologically acceptable pH.

In one aspect of the inventive subject matter, the inventors contemplate a ready to ready-to-inject norepinephrine composition that comprises an aqueous acidic buffer having a pH range of between 3.7 and 4.3, wherein the aqueous buffer further comprises a chelating agent and a pharmaceutically acceptable salt. Most typically, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and the pharmaceutically acceptable salt is present in an amount of between 0.6 wt % and 1.2 wt %. Norepinephrine (typically enantiomerically pure (i.e., at least 98%) R-isomer) is dissolved at a concentration that is suitable for administration to a patient in need thereof. In further preferred aspects, the ready-to-administer norepinephrine composition is substantially free of antioxidants, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As used herein, reference to the term norepinephrine should be interpreted broadly to include pharmaceutically acceptable salts and prodrugs thereof.

Therefore, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes a step of formulating a liquid parenteral composition that contains in an aqueous acidic buffer norepinephrine as an R-isomer such that (a) the formulation exhibits less than 10% of isomerization of the R-isomer to an S-isomer after three months of storage of the liquid composition, and (b) the formulation exhibits equal or less than 5% degradation of total norepinephrine after three months of storage of the liquid composition. The aqueous acidic buffer will typically have a pH range of between 3.7 and 4.3, and the aqueous buffer will further comprise a chelating agent and a pharmaceutically acceptable salt. In such methods, the total norepinephrine is present in the liquid parenteral composition at a concentration of between 10 μg/ml and 100 μg/ml, and the ready-to-inject norepinephrine composition is substantially free of antioxidants.

Viewed form a different perspective, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes the steps of preparing an aqueous acidic buffer at a pH range of between 3.7 and 4.3, wherein the aqueous buffer also includes a chelating agent and a pharmaceutically acceptable salt. Preferably, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and tonicity is adjusted with the pharmaceutically acceptable salt (e.g., NaCl). In a further step, norepinephrine (preferably enantiomerically pure R-isomer) is dissolved at a concentration suitable for administration to a patient in need thereof, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As before, it is generally preferred that the ready-to-administer norepinephrine composition is substantially free of antioxidants. In yet another step, the composition is autoclaved to sterility.

Most typically, but not necessarily, the aqueous acidic buffer is a citrate buffer and/or preferably has a concentration of between 5 mM and 20 mM. Furthermore, preferred aqueous acidic buffers will have a pH of between 3.8 and 4.2. With respect to the chelating agent it is contemplated

that such agents are a bicarboxylic acid (e.g., optionally hydroxylated, tartrate), a tricarboxylic acid (e.g., aconitic acid, trimesic acid, citric acid), and/or an aminopolycarboxylic acid (e.g., EDTA, EGTA, etc.), and that such chelating agents are present at low concentrations, preferably between 1 μg/ml and 10 μg/ml, or between 10 μg/ml and 100 μg/ml. The norepinephrine is typically present at a concentration of between 10 μg/ml and 100 μg/ml, for example, at a concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), or 64 μg/ml (+/−10%). Contemplated methods may also include a step of autoclaving the compositions.

With respect to stability it is contemplated that the storage condition is over at least three months at 40° C. and 75% (+/−5) relative humidity, that equal or less than 6% of the R-isomer form will isomerize to the S-isomer, and/or that equal or less than 3.5% of the total norepinephrine will degrade to degradation products.

Where desired, contemplated compositions have a dissolved oxygen concentration of equal or less than 1 ppm (e.g., by formulating the liquid parenteral composition using deoxygenated water), and/or or by packaging the composition together with a (preferably metal free) oxygen scavenger. Packaging may further make use of a container that is configured (e.g., aluminized or otherwise treated) to reduce light-mediated oxidation of the norepinephrine.

DETAILED DESCRIPTION OF THE INVENTION

The inventive subject matter is directed to stable aqueous pharmaceutical preparations of norepinephrine (and pharmaceutically acceptable salts thereof) in a ready-to-inject form that are sterile and preferably substantially free of antioxidants. Most preferably, stability of such compositions is characterized by low (oxidative and photo-induced) degradation as well as low isomerization.

More specifically, the inventors have discovered that formulations can be prepared that will exhibit less than 8%, more typically less than 6%, even more typically less than 4%, and most typically less than 3% of degradation as determined by HPLC-UV, and that will exhibit less than 10%, more typically less than 8%, even more typically less than 6%, and most typically less than 4% of isomerization from R- to S-configuration as determined by HPLC-UV. Most notably, such formulations were found to be stable over extended periods without antioxidants (e.g., at least 1 month, or at least two months, or at least three months), even at elevated storage temperatures (e.g., accelerated storage conditions such as 40° C. and 75% relative humidity (+/−5%)). Even more remarkable, such formulations could also be subjected to thermal sterilization, and particularly sterilizing to sterility (e.g., over at least 5 min, or at least 10 min, or at least 15 min at 121° C.), without substantial increase (i.e., >1.5%, or >1.0%, or >0.7%) of the S-isomer of norepinephrine.

Additionally, it should be appreciated that contemplated formulations can be filled in a polymer bag (e.g., polypropylene) or other container that may subsequently be placed into a secondary container together with an oxygen scavenger, and especially a metal-free oxygen scavenger. Most typically, at least one of the polymer bag and the secondary container may be impervious to light in general or light of a wavelength that promotes photo-initiated degradation. For example, containers may be metalized (e.g., aluminized) or combined or coated with carbonaceous materials or other dye(s). If desired, contemplated formulations are sufficiently stable to also allow filling into containers using a blow-fill-seal (BFS) process.

Therefore, contemplated norepinephrine formulations of the inventive subject matter can advantageously be provided in a ready-to-inject form to thereby avoid the inconvenience associated with diluting concentrated small volume norepinephrine parenteral formulations into diluents prior to infusion. Thus, the ready-to-inject formulations also eliminate microbial contamination risks and calculation errors associated with dilution. Most typically, contemplated formulations will be available in a range of concentrations commonly required by medical practitioners for emergency restoration of blood pressure, for example in cases of acute hypotension. Consequently, norepinephrine will typically be present in formulations at a concentration of between 10 μg/ml and 100 μg/ml, including concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), and 64 μg/ml (+/−10%).

As will be readily appreciated, the norepinephrine for preparation of contemplated formulations is preferably (R)-Norepinephrine, or enantiomerically pure (i.e., at least 98% R-isomer) norepinephrine. However, in less preferred aspects, isomeric purity can also be between 95-98%, or even between 90-95%. Of course, it should also be appreciated that the norepinephrine may be a salt of any suitable and pharmaceutically acceptable form, including mineral salts (e.g., HCl salt) and organic salts (e.g., bitartrate). Similarly, where desired, the norepinephrine may also be used in any suitable prodrug form (e.g., β,3-dihydroxytyrosine, L-dihydroxyphenylserine, etc.).

Suitable buffers are generally buffers that stabilize the pH of the contemplated liquid formulations in an acidic pH range and will therefore include glycine buffers, citrate buffers, citrate/phosphate buffers, acetate buffers, etc. However, the inventors have further discovered that where the norepinephrine is provided as the norepinephrine bitartrate salt, a buffer can advantageously be omitted and the pH can be adjusted with suitable acid and/or base as is well known in the art. Notably, the bitartrate appeared to act as a weak buffer in the stability range for the norepinephrine as is shown in more detail below. Most typically the pH of the formulation will be less than 5.0 and more typically less than 4.5, and most typically less than 4.3, but higher than 3.0, more typically higher than 3.5, and most typically higher than 3.7. For example, suitable buffers will have a pH in the range of between 3.7 and 4.3, or between 3.7 and 4.0, or between 3.8 and 4.1, or between 3.9 and 4.2, or between 4.0 and 4.2. Notably, such pH range provided remarkable stability for low concentrations of norepinephrine, especially when in combination with a chelator and a salt. While not limiting to the inventive subject matter, the buffer strength is typically relatively low, for example, equal or less than 100 mM, and more typically equal or less than 50 mM, and most typically between 5 mM and 20 mM (e.g., 10 mM).

Moreover, in further contemplated aspects, the formulation will also include one or more chelating agents, and particularly metal ion chelators. For example, suitable chelators include various bicarboxylic acids, tricarboxylic acids, and aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and penta(carboxymethyl)diethylenetriamine (DTPA), and salts and hydrates thereof. While not limiting to the inventive subject matter, it is contemplated that the metal ion chelators will slow down both the baseline and metal ion-stimulated autoxidation of norepinephrine. Remarkably, the inventors unexpectedly observed that the desirable effect of the chela-

tors was observable at relatively low concentrations of the chelators. For example, reduction of the baseline and metal ion-stimulated autoxidation of norepinephrine was observed at chelator concentrations of between 1 μg/ml and 10 μg/ml, and between 10 μg/ml and 100 μg/ml. Interestingly, the chelators, and especially the aminopolycarboxylic acids retained stabilizing effect despite the relatively low pH favoring protonated forms of the chelators.

With respect to suitable salts it is contemplated that the salt is a pharmaceutically acceptable salt that can be used to increase tonicity. Therefore, pharmaceutically acceptable salts are contemplated, and especially NaCl, at a concentration of at least 0.6 wt %, or at least 0.7 wt %, or at least 0.8 wt %, or at least 0.9 wt %. For example, suitable salt concentrations are between 0.6 wt % and 1.2 wt %. Depending on the particular salt concentration, additional tonicity agents may be added and suitable tonicity agents include glycerol, thioglycerol, mannitol, lactose, and dextrose. The amount of tonicity adjusting agent used can be adjusted to obtain osmolality of the formulations in the range of 260 to 340 mOsm/kg. An osmometer can be used to check and adjust the amount of tonicity adjusting agent to be added to obtain the desired osmolality.

It should further be appreciated that contemplated compositions are substantially free of antioxidants (i.e., do not include antioxidants in an amount effective to reduce degradation of total norepinephrine by at least 1% when stored over a period of at least three months at 25° C. Indeed, the inventors unexpectedly discovered that some formulations with antioxidants (particularly with ascorbic acid) had decreased stability. Notably, contemplated formulations were stable as described in more detail below, even in the absence of effective quantities of antioxidants, especially where deoxygenated solvents (e.g., typically water and/or buffer) were employed. Deoxygenation (i.e., reduction of molecular dissolved oxygen) can be achieved in numerous manners, including sparging with inert gases (e.g., helium, various freons, argon, xenon), agitation under vacuum, and/or using enzymatic systems that deplete a solution of dissolved oxygen (see e.g., U.S. Pat. No. 9,187,779). Additionally, or alternatively, ingress of molecular oxygen into the formulation can also be reduced by co-packaging a container with the formulation in a secondary container that includes an oxygen scavenger, and especially a metal-free oxygen scavenger (e.g., GLS100, Ageless®, Pharmakeep®, all commercially available from Mitsubishi Gas Chemical America).

With respect to the sterilization of contemplated formulations it should be appreciated that contemplated formulations may be sterilized using all known manners of sterilization, including filtration through 0.22 micron filters, heat sterilization, autoclaving, radiation (e.g., gamma, electron beam, microwave). Unexpectedly, and as shown in more detail below, the inventors have also discovered that contemplated formulations were heat stable and did not undergo significant isomerization, even under conditions of sterilization (exposure to high-pressure saturated steam) at 121° C. for at least 5, or at least 10, or at least 15 minutes.

Based on the unexpected heat stability, the formulations contemplated herein can also be filtered through a 0.22 micron filter, and filled in to a polyethylene, polypropylene or low-density polyethylene containers in a blow-fill-seal (BFS) process. BFS is a form of advanced aseptic manufacturing wherein the container is formed, filled, and sealed in one continuous, automated system not requiring human intervention. The process begins with the extrusion of plastic granules in the form of a hot hollow pipe of molten plastic called a parison. The next step is the blow molding of the container with an open top through which the container is filled, all while the plastic remains hot and in a molten state. Once filled, the container is hermetically sealed and cooled. The blow-fill seal process can take several seconds, and contemplated ready-to-inject compositions advantageously are formulated to withstand the temperature and pressure requirements without substantial degradation of norepinephrine (e.g., less than 5 wt %, less than 3 wt %, less than 2 wt %, less than 1 wt % degradation).

Once the norepinephrine formulations are filled in large volume polymeric, semi-permeable infusion containers (e.g., BFS container or flexible IV bags), the containers can optionally be layered or covered with a secondary packaging system including an aluminum pouch or other oxygen scavenger. For example, the BFS containers can further be sealed in an oxygen and moisture barrier blister packaging. The blister packaging can comprise one or more layers, and the one or more layers can include aluminum foil or other oxygen absorber having an Oxygen Transmission Rate (OTR) between 0.0005 to 5.00 cc/100 in^2/24 hrs. Additionally or alternatively, one or more oxygen absorbers (metal or metal free, organic material) can be incorporated into any portion of the BFS container, the secondary packaging system, or between the two (e.g., between the BFS container and the multi-layer packaging) such that the oxygen absorber removes at least a portion of oxygen from the air surrounding said oxygen-sensitive drug. A beneficial feature of the oxygen absorber is the absorbance and removal of oxygen present in the primary packaging and in the liquid drug itself. Notably, it was found that the oxygen absorber also removed residual headspace oxygen in the primary packaging and also dissolved oxygen in the liquid over time, thereby further improving stability of norepinephrine.

The following examples are provided for illustrative purposes only and should not be interpreted as limiting the present invention.

EXAMPLES

The following examples illustrate some of the experiments leading to the formulations according to the inventive subject matter, however, should not be construed to limit the scope of the claims in any way.

Stability and Isomerization: The ionization behavior of norepinephrine in aqueous solution is complex. Common with other o-hydroquinone systems, norepinephrine in aqueous solution is susceptible to oxidation to form the corresponding o-quinone, which can then also undergo various secondary reactions, which also becomes more prevalent as the pH becomes more alkaline. Norepinephrine may further isomerize to the pharmacologically less active S-enantiomer at low pH values, corresponding to protonation of the hydroxyl group at the benzylic chiral center. Therefore, to prevent norepinephrine cyclization reactions pH values less than 6.0 are desired. A pH range of 3.0 to 6.2 was screened

to determine pH of optimum stability. Composition of norepinephrine bitartrate equivalent to 16 μg/mL norepinephrine base at various pH values were prepared are described below, with Table 4 listing compositions of norepinephrine bitartrate in citrate buffer (10 mM),

For preparation of the solutions, about 90% of the final quantity of water was collected in a glass media bottle. Nitrogen (N_2) gas was purged for about thirty minutes to reduce the dissolved oxygen levels. Sodium chloride was added and the solution was stirred until a homogenous solution was obtained. Citric acid was added and the solution was stirred until a homogenous solution was obtained. The pH of the bulk solutions was adjusted to pH 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, and 6.2, respectively for each formulation composition using sufficient quantity of 10% w/v sodium hydroxide or 10% w/v hydrochloric acid. Norepinephrine bitartrate was added and the solution was stirred for approximately 10 minutes until a clear solution was formed. Solutions were made up to volume with water. The solutions were filled into 10 mL glass vials, overlaid with nitrogen, stoppered, and sealed. The stability was studied at 4° C., 25° C., and 60° C. by assay. Samples were observed visually for precipitation and change in color for a period of 7 days. Data are presented in Table 5.

TABLE 4

Compositions of Norepinephrine Bitartrate for pH dependent stability in Citrate Buffer (10 mM).

	Concentration (mg/mL)								
Ingredients	I	II	III	IV	V	VI	VII	VIII	IX
Norepinephrine Bitartarate equivalent to Norepinephrine base	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Sodium Chloride	9	9	9	9	9	9	9	9	9
Citric acid	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Sodium Citrate	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
HCl/NaOH (q.s. pH)	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2
Water for Injection (q.s. mL)	1	1	1	1	1	1	1	1	1

TABLE 5

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
4° C.	I	96.4	96.5	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	98.5	3.8	No	No
	IV	99.1	98.4	4.2	No	No
	V	98.1	98.6	4.6	No	No
	VI	98.4	98.1	5.0	No	No
	VII	97.1	96.6	5.4	No	No
	VIII	97.8	97.5	5.8	No	No
	IX	91.5	91.2	6.2	No	No
25° C.	I	96.4	96.4	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	97.9	3.8	No	No
	IV	99.1	97.7	4.2	No	No
	V	98.1	97.3	4.6	No	No
	VI	98.4	97.3	5.0	No	No
	VII	97.1	95.9	5.4	No	No
	VIII	97.8	94.5	5.8	No	No
	IX	91.5	80.4	6.2	Yes	No
60° C.	I	96.4	95.2	3.0	No	No
	II	98.0	95.0	3.4	No	No
	III	99.0	95.2	3.8	No	No
	IV	99.1	93.2	4.2	No	No
	V	98.1	88.9	4.6	No	No
	VI	98.4	77.4	5.0	Yes	No
	VII	97.1	46.8	5.4	Yes	No
	VIII	97.8	NT	5.8	Yes	No
	IX	91.5	NT	6.2	Yes	No

No change in physical appearance was observed in the solutions stored at 4° C. In the solutions stored at 25° C., a change in color was observed at pH 6.2. Red brown color was observed in solutions stored at or above pH 5.0 at 60° C. Oxidation and color formation are very common with norepinephrine in unfavorable conditions and the speed of the reaction and the nature of the final products are dependent on the catalysts (e.g., metal ion impurities) and buffers employed. A pH range from 3.0 to 4.5 was selected for further testing.

Stability of Norepinephrine in selected pH ranges and formulations: The formulations for the next experiments are shown in Table 6 below, involving three different compositions of norepinephrine bitartrate at three different pH (3.5, 4.0, 4.5, and 5.0) values. Lab scale batches were prepared and subjected to lab scale stability tests at accelerated (40° C./75% RH) and long term stability (25° C./60% RH) storage conditions. The test results from the stability studies are presented in Table 7-Table 10, with CCS indicating Clear colorless solution; ND indicating Not Detected; NR indicating Not Reported (<0.05%); and NT indicating Not Tested.

TABLE 6

Formulation composition selected for further development activities and optimization

	Quantity (mg/mL) Formulation			
Ingredient	X	XI	XII	XIII
Norepinephrine Bitartrate	0.016	0.016	0.016	0.016
Edetate Sodium	0.10	0.10	0.10	0.10
Sodium chloride	9	9	9	9
HCl/NaOH	q.s. pH 3.5	q.s. pH 4.0	q.s. pH 4.5	q.s. pH 5.0
Water for Injection Q.S.	1 mL	1 mL	1 mL	1 mL
Dissolved Oxygen (ppm)	<1	<1	<1	<1

TABLE 7

Stability study of Formulation X - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 3.5).

	Storage Condition												
	25 ± 2° C./60 ± 5% RH							40 ± 2° C./75 ± 5% RH					
	Time Point												
	Initial	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.50	3.65	3.59	3.56	3.58	3.54	3.48	3.66	3.61	3.59	3.64	3.60	3.59
Assay	101.4	99.6	97.1	97.1	101.0	102.3	102.2	99.5	97.0	98.7	100.4	101.7	101.4
S-form	NT	NT	NT	NT	1.8	2.2	2.2	NT	NT	NT	7.6	8.1	9.8
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 8

Stability study of Formulation XI - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.02	3.96	3.98	3.97	3.91	4.01	3.99	4.02	4.03
Assay	101.3	98.7	95.5	99.2	100.5	98.6	95.3	97.1	97.5
S-form	NT	NT	NT	NT	1.7	NT	NT	NT	7.8
Total Impurities	0.1	ND	0.06	ND	0.80	ND	0.06	0.1	0.79

TABLE 9

Stability study of Formulation XII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.5).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.50	4.35	4.36	4.32	4.33	4.33	4.40	4.39	4.29
Assay	100.1	98.9	95.5	98.2	97.9	97.1	92.5	93.7	77.2
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	2.9
Total Impurities	ND	0.32	0.79	0.52	3.41	1.18	0.38	5.59	10.38

TABLE 10

Stability study of Formulation XIII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 5.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	4 Month	1 Month	2 Month	3 Month	4 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.99	4.62	4.51	4.57	4.51	4.87	4.81	4.83	4.53
Assay	102.7	100.5	95.6	99.2	100.4	98.3	89.8	87.0	72.3
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	3.0
Total Impurities	ND	0.75	0.81	0.48	1.29	0.94	2.4	5.39	14.91

Based on the above considerations, the effect of different levels of EDTA on stability of norepinephrine was determined. Three batches at concentrations of 16 μg/mL, 32 μg/mL, and 64 μg/mL were made with EDTA concentrations of 100 μg/mL: Formulation XIV (16 μg/mL), Formulation XV (32 μg/mL), Formulation XVI (64 μg/mL). Two additional batches were made at 10 μg/mL EDTA Formulation XVII and 1 μg/mL EDTA (Formulation XVIII) at 64 μg/mL Norepinephrine. The composition of the batches is specified in Table 11. The drug product was compounded as described earlier and packaged in 250 mL in polypropylene bags. This was further packed into aluminum overwrap with an oxygen scavenger (GLS 100, Mitsubishi Gas Chemicals). The batches were then stored at room temperature and accelerated temperature conditions.

TABLE 11

Formulation composition selected with different level of EDTA concentrations.

	Quantity (mg/mL) Formulation Number				
Ingredient	XIV	XV	XVI	XVII	XVIII
Norepinephrine Bitartrate	0.016	0.032	0.064	0.064	0.064

TABLE 11-continued

Formulation composition selected with different level of EDTA concentrations.

	Quantity (mg/mL) Formulation Number				
Ingredient	XIV	XV	XVI	XVII	XVIII
Edetate Sodium	0.10	0.10	0.10	0.010	0.0010
Sodium chloride	9	9	9	9	9
Hydrochloric Acid/Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for Injection	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL

The resultant stability data on these formulations are presented in Table 12-Table 16 (CCS-Clear colorless solution; ND—Not Detected). The results of the stability studies at different amounts of EDTA at pH 4.0 indicates that both 0.01%, 0.001% of EDTA significantly prevented the degradation rate of norepinephrine in terms of known and unknown impurities. Moreover, with respect to isomerization from the R-isomer to the S-isomer it was notably observed that the amount of EDTA had substantially no influence on racemization or enantiomer formation during stability and after autoclaving.

TABLE 12

Stability study of Formulation XIV - Norepinephrine bitartrate injection (16 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.99	3.96	4.08	4.08	4.02	4.08	4.08
Assay	98.5	100.4	100.1	99.7	100.3	100.0	99.5
S-form	0.9	1.1	1.4	1.3	1.9	2.9	4.2
Total Impurities	0.05	ND	ND	ND	ND	0.10	0.38

TABLE 13

Stability study of Formulation XV - Norepinephrine bitartrate injection (32 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.01	3.99	4.08	4..08	4.02	4.08	4.08
Assay	101.0	102.9	97.1	100.7	102.9	99.4	100.6
S-form	0.9	1.1	1.3	1.4	1.9	3.0	4.1
Total Impurities	0.06	ND	ND	ND	ND	ND	0.14

TABLE 14

Stability study of Formulation XVI - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 100 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.99	4.08	4.08	3.98	4.07	4.07
Assay	98.4	103.2	98.7	100.2	104.6	99.3	99.8
S-form	0.9	1.1	1.3	1.3	2.0	3.2	4.2
Total Impurities	0.06	ND	0.12	ND	ND	ND	ND

TABLE 15

Stability study of Formulation XVII - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 10 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.06	4.06	3.99	4.05	4.05
Assay	102.7	105.7	103.4	104.3	107.8	103.6	103.9
S-form	0.9	1.1	1.2	1.5	2.0	3.3	4.3
Total	0.06	ND	ND	ND	ND	0.26	ND

TABLE 16

Stability study of Formulation XVIII - Norepinephrine bitartrate injection (64 µg/mL); pH 4.0 at 1 µg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.07	4.07	4.02	4.06	4.06
Assay	98.7	102.6	100.4	100.4	105.0	99.9	99.2
S-form	0.9	1.1	1.3	1.4	2.0	3.2	4.3
Total	0.06	ND	ND	ND	ND	ND	ND

Sterilization and Stability: The volume for ready-to-inject formulations is 250 mL and as such classifies as a large volume parenteral (LVP). To achieve a desired or required sterility assurance level of 10^{-6} for a LVP terminal sterilization via heat it is typically required. The inventors therefore investigated whether or not contemplated formulations could be terminally sterilized via autoclaving.

Formulations at a concentration 16 µg/mL and 64 µg/mL (Formulation XVII) Norepinephrine base were prepared substantially as shown in Table 11 above and packaged in secondary packaging of aluminum overwrap with an oxygen scavenger and shipped for terminal sterilization. The secondary packaging was removed and the bags were terminally sterilized using steam sterilizer (Fedegari, Model # FOB3) with an air over-pressure (AOP) sterilization cycle. The terminal sterilization was performed at 121° C. for 5, 10, and 15 min. Post completion of sterilization temperature, the bags underwent spontaneous cooling to 95° C. and forced cooling to 70° C. The total exposure time and calculated F_0 values were 11.09, 17.04, and 22.42 for 5 min, 10 min, and 15 min cycles respectively. The bags were then analyzed for assay, impurities, and S-isoform, and the results are shown in Table 17 and Table 18.

TABLE 17

Stability study of Norepinephrine bitartrate injection (16 μg/mL) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA; terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.76	3.85	3.78	3.77	3.76	3.76	3.78	3.76	3.75	3.76
Dissolved Oxygen	0.63	4.93	4.86	4.89	0.75	0.48	0.55	0.65	0.78	0.77
Assay	103.1	103.1	103.1	103.1	103.1	103.0	103.1	103.1	103.2	103.1
S-Form	1.0	3.0	3.0	3.0	3.8	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 18

Stability study of Norepinephrine bitartrate injection (64 μg/ml) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.74	3.74	3.75	3.73	3.74	3.74	3.76	3.74	3.73	3.74
Dissolved Oxygen	0.69	5.15	5.03	5.00	0.52	0.59	0.75	0.69	0.80	0.74
Assay	101.2	102.2	101.2	101.5	101.7	101.2	101.3	101.2	101.3	102.2
S-Form	1.0	3.0	3.0	3.0	3.7	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND

As can be seen from the data, the S-isoform appears to increase proportionally to time during the terminal sterilization cycle. No increase in reportable impurities was observed.

Test method—Determination of norepinephrine and degradation products: Separation of Norepinephrine and related compounds was performed using a gradient HPLC method with UV detection. Pentofluorophenylpropyl terminated silica was used as a stationary phase for chromatographic analysis. The mobile phase was prepared by mixing water and methanol, with both solvents containing formic acid. Related compounds were defined by their relative retention times (RRT) based on the NE peak retention time. Quantitation of related compounds was accomplished by comparing the corresponding peak area from a sample solution chromatogram to that of the NE peak from a Reference Standard (RS) solution of a known concentration. Relative Response Factors (RRF) were used to correct for chemical structure effects on the responses of the identified impurities. Chromatography was performed using parameters and methods as shown in Table 19.

TABLE 19

HPLC	Waters Alliance e2695
Column	Supelco Discovery HS F-5 Column, 3 μm, 4.6 × 150 mm
Column Temperature	35° C.
Sample Temperature	Ambient
Injection volume	85.0 μL
Flow Rate	0.8 mL/min
Detection	Spectrum: 200-600 nm, resolution 1.2 nm Single channel: 280 nm, resolution 4.8 nm PDA Filter Time Constant: Normal Sampling rate: 5 points/sec
Solution A	0.1% Formic acid in Water
Solution B	0.1% Formic acid in Methanol

Mobile Phase	Time (mins)	% Solution A	% Solution B
	0	100	0
	3	100	0
	6	93	7
	8	93	7
	15	88	12
	30	2	98
	35	2	98
	36	100	0
	40	100	0

Test Method—Identification, Assay and Enantiomeric Purity of Norepinephrine: Identification and quantification of S-norepinephrine and R-norepinephrine was performed using an HPLC method with UV detection. HPLC-UV was used to separate and quantitate the amount of (R)- and (S)-enantiomers of norepinephrine (NE) present in the drug product with the NE concentrations of 16, 32 and 64 μg/ml. The comparison of the sum of (R)- and (S)-peak responses in a sample chromatogram versus a reference standard chromatogram gives the total amount of NE. The (S)-enantiomer was quantitated based on its peak response as the percentage of the total peak response of both enantiomers.

More specifically, determination of R- and S-enantiomers of norepinephrine in the drug product solution was per-

formed using an isocratic reverse-phase HPLC method with UV detection. Separation was achieved by using a protein-based column with functional chiral selectors. The chiral selector is cellobiohydrolase (CBH), a stable enzyme that has been immobilized onto spherical silica particles. This enzyme preferentially separates compounds containing one or more basic nitrogen groups together with one or more hydrogen-accepting or hydrogen-donating groups. Chromatography was performed using parameters and methods as shown in Table 20.

TABLE 20

HPLC	Agilent 1260 Infinity
Column	Daicel Chiralpak CBH ™ column, 5 µm, 4.0 × 100 mm
Column Temperature	27° C. ± 2° C.
Sample Temperature	Ambient
Injection volume	20.0 µL for 16 mcg/mL, 10.0 µL for 32 mcg/mL, 5.0 µL for 64 mcg/mL
Flow Rate	0.9 mL/min
Detection	Single channel: 280 nm, resolution 4.8 nm Spectrum: 200-600 nm, resolution 1.2 nm
Mobile Phase:	Buffer/IPA 95:5 v/v Buffer: Sodium Phosphate, Disodium Edetate, pH 6.0
Run Time	8 min

While contemplated formulations can be administered following various protocols, the inventors contemplate that administration of the formulations, especially administration for treatment of hypotension, will follow a protocol that comprises at least two distinct steps, with an accelerated administration followed by a maintenance administration as exemplarily described in Table 21 below.

TABLE 21

		Initial Dose		Maintenance Dose	
Presentation (mg/mL)	Concentration (µg/mL)	Dose per Minute (µg/min)	Flow Rate (mL/min)	Dose per Minute (µg/min)	Flow Rate (mL/min)
16 µg/mL (4 mg in 250 mL)	16	8-12	0.500-0.750	2-4	0.125-0.250
32 µg/mL (8 mg in 250 mL)	32		0.250-0.375		0.062-0.125
64 µg/mL (16 mg in	64		0.125-0.187		0.031-0.062

As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

Moreover, in interpreting the disclosure all terms should be interpreted in the broadest possible manner consistent with the context. In particular the terms "comprises" and "comprising" should be interpreted as referring to the elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps can be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

What is claimed is:

1. A sterile, ready-to-administer, packaged norepinephrine composition, comprising:

a container filled with a sterile, ready-to-administer norepinephrine composition and packaged in a secondary container;

wherein the sterile, ready-to-administer norepinephrine composition comprises norepinephrine or a salt thereof in an amount of between 10 µg/ml and 100 µg/ml, a chelating agent in an amount of between 1 µg/ml and 100 µg/ml, a tonicity adjusting agent in an amount of between 0.6 wt % and 1.2 wt %, and an aqueous acidic solution, wherein the norepinephrine comprises at least 95% of R-isomer of norepinephrine;

wherein the sterile, ready-to-administer norepinephrine composition is substantially free of antioxidants;

wherein the sterile, ready-to-administer norepinephrine composition has a pH of between 3.7 and 4.3; and

wherein the sterile, ready-to-administer, packaged norepinephrine composition comprises at least about 90% R-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

2. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the container is a large volume, polymeric, semi-permeable infusion container.

3. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the container is a polymer bag.

4. The sterile, ready-to-administer, packaged norepinephrine composition of claim **3**, wherein the polymer is polypropylene, polyethylene, or low-density polyethylene.

5. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the secondary container is impervious to light.

6. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the secondary container is impervious to light of a wavelength that promotes

photo-initiated degradation of the norepinephrine or salt thereof in the sterile, ready-to-administer norepinephrine composition.

7. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the container and/or the secondary container includes an oxygen scavenger.

8. The sterile, ready-to-administer, packaged norepinephrine composition of claim **7**, wherein the oxygen scavenger is a metal-free oxygen scavenger.

9. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the secondary container comprises an aluminum overwrap.

10. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the container is a polymer bag and the secondary container comprises an aluminum overwrap.

11. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the sterile, ready-to-administer norepinephrine composition has a pH between 3.7 and 4.0.

12. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the norepinephrine is norepinephrine bitartrate.

13. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the norepinephrine is present in the sterile, ready-to-administer norepinephrine composition in an amount of about 16 μg/ml, about 32 μg/ml, or about 64 μg/ml.

14. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the chelating agent is selected from a group consisting of a bicarboxylic acid, a tricarboxylic acid, and an aminopolycarboxylic acid.

15. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

16. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, where the tonicity adjusting agent is sodium chloride.

17. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the aqueous acidic solution has dissolved oxygen at a concentration of equal or less than 1 ppm.

18. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the norepinephrine or a salt thereof in the sterile, ready-to-administer, packaged norepinephrine composition comprises equal or less than about 10% S-isomer of norepinephrine or salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

19. The sterile, ready-to-administer, packaged norepinephrine composition of claim **1**, wherein the sterile, ready-to-administer norepinephrine composition comprises equal or less than about 5% of total degradation of norepinephrine or salt thereof excluding S-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

* * * * *

EXHIBIT E

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(12) **United States Patent**
Hingorani et al.

(10) **Patent No.: US 10,646,458 B2**
(45) **Date of Patent: *May 12, 2020**

(54) **NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR**

(71) Applicant: **Nevakar, Inc.**, Bridgewater, NJ (US)

(72) Inventors: **Tushar Hingorani**, Bridegwater, NJ (US); **Prem Sagar Akasapu**, Edison, NJ (US); **Kumaresh Soppimath**, Skillman, NJ (US)

(73) Assignee: **Nevakar Inc.**, Bridegwater, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(58) **Field of Classification Search**

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See application file for complete search history.

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Primary Examiner — Theodore R. West
(74) *Attorney, Agent, or Firm* — Umberg Zipser LLP

(57) **ABSTRACT**

The inventive subject matter is directed to compositions and methods for ready-to-inject norepinephrine compositions with improved stability. Most preferably, compositions presented herein are substantially antioxidant free and exhibit less than 10% isomerization of R-norepinephrine and exhibit less than 5% degradation of total norepinephrine.

23 Claims, No Drawings

NOREPINEPHRINE COMPOSITIONS AND METHODS THEREFOR

This application is a divisional application of allowed U.S. non-provisional application with Ser. No. 15/883,798, which was filed Jan. 30, 2018, which claims priority to U.S. provisional application with Ser. No. 62/452,220, which was filed Jan. 30, 2017.

FIELD OF THE INVENTION

The field of the invention is pharmaceutical compositions comprising norepinephrine, especially as it relates to storage stable, ready-to-inject, antioxidant free compositions, and method of manufacturing such compositions.

BACKGROUND

The background description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Where a definition or use of a term in an incorporated reference is inconsistent or contrary to the definition of that term provided herein, the definition of that term provided herein applies and the definition of that term in the reference does not apply.

Norepinephrine is often used during CPR (cardio-pulmonary resuscitation), and in the treatment of cardiac arrest and profound hypotension. Norepinephrine is also used for blood pressure control in certain acute hypotensive states, including for example sympathectomy, poliomyelitis, pheochromocytomectomy, spinal anesthesia, myocardial infarction, blood transfusion, and septicemia.

Currently, norepinephrine is marketed as Levophed®, which is a concentrated 4 mg per 4 mL norepinephrine bitartrate formulation to be administered by intravenous infusion following dilution with dextrose or dextrose and sodium chloride injection. Norepinephrine is also marketed by Baxter which supplies as a norepinephrine concentrate that is free of sodium metabisulfite and packaged under nitrogen. Unfortunately, most, if not all diluted commercially available norepinephrine formulations lack storage and should therefore be discarded within one day after reconstitution when stored at room temperature. Consequently, risk for microbial contamination and dilution errors is present. In addition, Levophed also contains sodium metabisulphite as an antioxidant, and carries a warning label that sulfite may cause allergic type reactions including anaphylactic shock and life threatening or less severe asthmatic episodes in susceptible people. Table 1 depicts ingredients of currently marketed norepinephrine compositions.

TABLE 1

Composition of currently marketed Norepinephrine Bitartrate Products.

Ingredient	Levophed ® (Hospira)	Norepinephrine Bitartarate (Baxter)
Norepinephrine Bitartrate equivalent to Norepinephrine Base	1 mg/mL	1 mg/mL
Sodium Chloride	Isotonic	Isotonic
Sodium Metabisulphite	0.2 mg/mL	—
pH	3-4.5	3-4.5
Water for injection	q.s. 1 mL	q.s 1 mL

Stability of Levophed® and Norepinephrine bitartrate injection (Baxter), in normal saline solutions is presented in Table 2 and Table 3 where norepinephrine was diluted to a concentration of 16 μg/ml. Stability was assessed in 250 ml saline at accelerated (i.e., 40±2° C. and 75±5% relative humidity, duration as indicated) and long term stability (i.e., 25±2° C. and 60±5% relative humidity, duration as indicated) storage conditions.

TABLE 2

Stability study of Levophed ® diluted in 0.9% Saline (Hospira) at 16 μg/mL.

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	97.3	98.9	97.9	91.9	98.8	96.5	80.2	71.9
Total Impurities	0.05	—	0.71	8.08	0.03	1.96	5.29	9.73

TABLE 3

Stability study of Norepinephrine bitartrate injection [Baxter] diluted in 0.9% Saline (Hospira) at 16 μg/mL

	Storage Condition							
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH			
	Time Point							
	Initial	1 Month	2 Month	3 Month	Initial	1 Month	2 Month	3 Month
Assay	99.9	99.7	97.0	92.2	99.4	91.5	82.9	77.6
Total Impurities	0.08	1.73	2.68	10.17	0.10	2.34	4.46	6.71

As can be seen from the results, the norepinephrine at ready-to-inject concentrations underwent significant degradation. Oxidative degradation could possibly be reduced or even prevented by addition of effective amounts of sodium metabisulphite to the ready-to-inject norepinephrine solution. However, the quantities of sodium metabisulphite that would be administered by injection of 250 ml of the ready-to-inject solution would be substantial and detrimental to the patient. To avoid issues associated with sodium metabisulphite, efforts have been made to provide norepinephrine formulations with a non-sulfite anti-oxidant. For example US 2016/0058715 teaches a ready-to-inject dosage form of norepinephrine that uses butylated hydroxyl anisole as an anti-oxidant. While generally deemed safe for topical and cosmetic use, butylated hydroxyl anisole was shown to produce some renal and hepatic damage (e.g., *Int J Toxicol.* 2002; 21 Suppl 2:19-94).

In other attempts to provide ready-to-administer norepinephrine formulations with increased storage stability and reduced risk of human error, the pH on the injectable solution was reduced to between 3.2 and 3.6 with 40-200 μg/ml norepinephrine as is described in WO 2015/128418. While such formulations exhibited reduced degradation as compared to higher pH formulations, significant discomfort can occur at the injection site. Worse yet, at the pH used, norepinephrine isomerized relatively quickly from the active R (−) isomer to the inactive S (+) isomer. Isomerization is also encountered at exposure of norepinephrine to higher temperatures.

Therefore, there is a need for improved stable, low concentration, ready-to-inject and antioxidant free norepinephrine formulations, and methods of manufacturing and storing the same.

SUMMARY OF THE INVENTION

The inventive subject matter is directed to antioxidant free sterilizable/autoclavable ready-to-inject norepinephrine compositions having improved stability and a physiologically acceptable pH.

In one aspect of the inventive subject matter, the inventors contemplate a ready to ready-to-inject norepinephrine composition that comprises an aqueous acidic buffer having a pH range of between 3.7 and 4.3, wherein the aqueous buffer further comprises a chelating agent and a pharmaceutically acceptable salt. Most typically, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and the pharmaceutically acceptable salt is present in an amount of between 0.6 wt % and 1.2 wt %. Norepinephrine (typically enantiomerically pure (i.e., at least 98%) R-isomer) is dissolved at a concentration that is suitable for administration to a patient in need thereof. In further preferred aspects, the ready-to-administer norepinephrine composition is substantially free of antioxidants, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As used herein, reference to the term norepinephrine should be interpreted broadly to include pharmaceutically acceptable salts and prodrugs thereof.

Therefore, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes a step of formulating a liquid parenteral composition that contains in an aqueous acidic buffer norepinephrine as an R-isomer such that (a) the formulation exhibits less than 10% of isomerization of the R-isomer to an S-isomer after three months of storage of the liquid composition, and (b) the formulation exhibits equal or less than 5% degradation of total norepinephrine after three months of storage of the liquid composition. The aqueous acidic buffer will typically have a pH range of between 3.7 and 4.3, and the aqueous buffer will further comprise a chelating agent and a pharmaceutically acceptable salt. In such methods, the total norepinephrine is present in the liquid parenteral composition at a concentration of between 10 μg/ml and 100 μg/ml, and the ready-to-inject norepinephrine composition is substantially free of antioxidants.

Viewed form a different perspective, the inventors also contemplate a method of preparing a ready-to-inject norepinephrine composition that includes the steps of preparing an aqueous acidic buffer at a pH range of between 3.7 and 4.3, wherein the aqueous buffer also includes a chelating agent and a pharmaceutically acceptable salt. Preferably, the chelating agent is present in an amount of between 1 μg/ml and 100 μg/ml, and tonicity is adjusted with the pharmaceutically acceptable salt (e.g., NaCl). In a further step, norepinephrine (preferably enantiomerically pure R-isomer) is dissolved at a concentration suitable for administration to a patient in need thereof, and the ready-to-administer norepinephrine composition is formulated such that after storage over at least three months equal or less than 10% of the R-isomer form will isomerize to the S-isomer and such that equal or less than 5% of the total norepinephrine will degrade to degradation products. As before, it is generally preferred that the ready-to-administer norepinephrine composition is substantially free of antioxidants. In yet another step, the composition is autoclaved to sterility.

Most typically, but not necessarily, the aqueous acidic buffer is a citrate buffer and/or preferably has a concentration of between 5 mM and 20 mM. Furthermore, preferred aqueous acidic buffers will have a pH of between 3.8 and 4.2. With respect to the chelating agent it is contemplated

that such agents are a bicarboxylic acid (e.g., optionally hydroxylated, tartrate), a tricarboxylic acid (e.g., aconitic acid, trimesic acid, citric acid), and/or an aminopolycarboxylic acid (e.g., EDTA, EGTA, etc.), and that such chelating agents are present at low concentrations, preferably between 1 μg/ml and 10 μg/ml, or between 10 μg/ml and 100 μg/ml. The norepinephrine is typically present at a concentration of between 10 μg/ml and 100 μg/ml, for example, at a concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), or 64 μg/ml (+/−10%). Contemplated methods may also include a step of autoclaving the compositions.

With respect to stability it is contemplated that the storage condition is over at least three months at 40° C. and 75% (+/−5) relative humidity, that equal or less than 6% of the R-isomer form will isomerize to the S-isomer, and/or that equal or less than 3.5% of the total norepinephrine will degrade to degradation products.

Where desired, contemplated compositions have a dissolved oxygen concentration of equal or less than 1 ppm (e.g., by formulating the liquid parenteral composition using deoxygenated water), and/or or by packaging the composition together with a (preferably metal free) oxygen scavenger. Packaging may further make use of a container that is configured (e.g., aluminized or otherwise treated) to reduce light-mediated oxidation of the norepinephrine.

DETAILED DESCRIPTION OF THE INVENTION

The inventive subject matter is directed to stable aqueous pharmaceutical preparations of norepinephrine (and pharmaceutically acceptable salts thereof) in a ready-to-inject form that are sterile and preferably substantially free of antioxidants. Most preferably, stability of such compositions is characterized by low (oxidative and photo-induced) degradation as well as low isomerization.

More specifically, the inventors have discovered that formulations can be prepared that will exhibit less than 8%, more typically less than 6%, even more typically less than 4%, and most typically less than 3% of degradation as determined by HPLC-UV, and that will exhibit less than 10%, more typically less than 8%, even more typically less than 6%, and most typically less than 4% of isomerization from R- to S-configuration as determined by HPLC-UV. Most notably, such formulations were found to be stable over extended periods without antioxidants (e.g., at least 1 month, or at least two months, or at least three months), even at elevated storage temperatures (e.g., accelerated storage conditions such as 40° C. and 75% relative humidity (+/−5%)). Even more remarkable, such formulations could also be subjected to thermal sterilization, and particularly sterilizing to sterility (e.g., over at least 5 min, or at least 10 min, or at least 15 min at 121° C.), without substantial increase (i.e., >1.5%, or >1.0%, or >0.7%) of the S-isomer of norepinephrine.

Additionally, it should be appreciated that contemplated formulations can be filled in a polymer bag (e.g., polypropylene) or other container that may subsequently be placed into a secondary container together with an oxygen scavenger, and especially a metal-free oxygen scavenger. Most typically, at least one of the polymer bag and the secondary container may be impervious to light in general or light of a wavelength that promotes photo-initiated degradation. For example, containers may be metallized (e.g., aluminized) or combined or coated with carbonaceous materials or other dye(s). If desired, contemplated formulations are sufficiently stable to also allow filling into containers using a blow-fill-seal (BFS) process.

Therefore, contemplated norepinephrine formulations of the inventive subject matter can advantageously be provided in a ready-to-inject form to thereby avoid the inconvenience associated with diluting concentrated small volume norepinephrine parenteral formulations into diluents prior to infusion. Thus, the ready-to-inject formulations also eliminate microbial contamination risks and calculation errors associated with dilution. Most typically, contemplated formulations will be available in a range of concentrations commonly required by medical practitioners for emergency restoration of blood pressure, for example in cases of acute hypotension. Consequently, norepinephrine will typically be present in formulations at a concentration of between 10 μg/ml and 100 μg/ml, including concentration of 16 μg/ml (+/−10%), 32 μg/ml (+/−10%), and 64 μg/ml (+/−10%).

As will be readily appreciated, the norepinephrine for preparation of contemplated formulations is preferably (R)-Norepinephrine, or enantiomerically pure (i.e., at least 98% R-isomer) norepinephrine. However, in less preferred aspects, isomeric purity can also be between 95-98%, or even between 90-95%. Of course, it should also be appreciated that the norepinephrine may be a salt of any suitable and pharmaceutically acceptable form, including mineral salts (e.g., HCl salt) and organic salts (e.g., bitartrate). Similarly, where desired, the norepinephrine may also be used in any suitable prodrug form (e.g., β,3-dihydroxytyrosine, L-dihydroxyphenylserine, etc.).

Suitable buffers are generally buffers that stabilize the pH of the contemplated liquid formulations in an acidic pH range and will therefore include glycine buffers, citrate buffers, citrate/phosphate buffers, acetate buffers, etc. However, the inventors have further discovered that where the norepinephrine is provided as the norepinephrine bitartrate salt, a buffer can advantageously be omitted and the pH can be adjusted with suitable acid and/or base as is well known in the art. Notably, the bitartrate appeared to act as a weak buffer in the stability range for the norepinephrine as is shown in more detail below. Most typically the pH of the formulation will be less than 5.0 and more typically less than 4.5, and most typically less than 4.3, but higher than 3.0, more typically higher than 3.5, and most typically higher than 3.7. For example, suitable buffers will have a pH in the range of between 3.7 and 4.3, or between 3.7 and 4.0, or between 3.8 and 4.1, or between 3.9 and 4.2, or between 4.0 and 4.2. Notably, such pH range provided remarkable stability for low concentrations of norepinephrine, especially when in combination with a chelator and a salt. While not limiting to the inventive subject matter, the buffer strength is typically relatively low, for example, equal or less than 100 mM, and more typically equal or less than 50 mM, and most typically between 5 mM and 20 mM (e.g., 10 mM).

Moreover, in further contemplated aspects, the formulation will also include one or more chelating agents, and particularly metal ion chelators. For example, suitable chelators include various bicarboxylic acids, tricarboxylic acids, and aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and penta(carboxymethyl)diethylenetriamine (DTPA), and salts and hydrates thereof. While not limiting to the inventive subject matter, it is contemplated that the metal ion chelators will slow down both the baseline and metal ion-stimulated autoxidation of norepinephrine. Remarkably, the inventors unexpectedly observed that the desirable effect of the chela-

tors was observable at relatively low concentrations of the chelators. For example, reduction of the baseline and metal ion-stimulated autoxidation of norepinephrine was observed at chelator concentrations of between 1 μg/ml and 10 μg/ml, and between 10 μg/ml and 100 μg/ml. Interestingly, the chelators, and especially the aminopolycarboxylic acids retained stabilizing effect despite the relatively low pH favoring protonated forms of the chelators.

With respect to suitable salts it is contemplated that the salt is a pharmaceutically acceptable salt that can be used to increase tonicity. Therefore, pharmaceutically acceptable salts are contemplated, and especially NaCl, at a concentration of at least 0.6 wt %, or at least 0.7 wt %, or at least 0.8 wt %, or at least 0.9 wt %. For example, suitable salt concentrations are between 0.6 wt % and 1.2 wt %. Depending on the particular salt concentration, additional tonicity agents may be added and suitable tonicity agents include glycerol, thioglycerol, mannitol, lactose, and dextrose. The amount of tonicity adjusting agent used can be adjusted to obtain osmolality of the formulations in the range of 260 to 340 mOsm/kg. An osmometer can be used to check and adjust the amount of tonicity adjusting agent to be added to obtain the desired osmolality.

It should further be appreciated that contemplated compositions are substantially free of antioxidants (i.e., do not include antioxidants in an amount effective to reduce degradation of total norepinephrine by at least 1% when stored over a period of at least three months at 25° C. Indeed, the inventors unexpectedly discovered that some formulations with antioxidants (particularly with ascorbic acid) had decreased stability. Notably, contemplated formulations were stable as described in more detail below, even in the absence of effective quantities of antioxidants, especially where deoxygenated solvents (e.g., typically water and/or buffer) were employed. Deoxygenation (i.e., reduction of molecular dissolved oxygen) can be achieved in numerous manners, including sparging with inert gases (e.g., helium, various freons, argon, xenon), agitation under vacuum, and/or using enzymatic systems that deplete a solution of dissolved oxygen (see e.g., U.S. Pat. No. 9,187,779). Additionally, or alternatively, ingress of molecular oxygen into the formulation can also be reduced by co-packaging a container with the formulation in a secondary container that includes an oxygen scavenger, and especially a metal-free oxygen scavenger (e.g., GLS100, Ageless®, Pharmakeep®, all commercially available from Mitsubishi Gas Chemical America).

With respect to the sterilization of contemplated formulations it should be appreciated that contemplated formulations may be sterilized using all known manners of sterilization, including filtration through 0.22 micron filters, heat sterilization, autoclaving, radiation (e.g., gamma, electron beam, microwave). Unexpectedly, and as shown in more detail below, the inventors have also discovered that contemplated formulations were heat stable and did not undergo significant isomerization, even under conditions of sterilization (exposure to high-pressure saturated steam) at 121° C. for at least 5, or at least 10, or at least 15 minutes.

Based on the unexpected heat stability, the formulations contemplated herein can also be filtered through a 0.22 micron filter, and filled in to a polyethylene, polypropylene or low-density polyethylene containers in a blow-fill-seal (BFS) process. BFS is a form of advanced aseptic manufacturing wherein the container is formed, filled, and sealed in one continuous, automated system not requiring human intervention. The process begins with the extrusion of plastic granules in the form of a hot hollow pipe of molten plastic called a parison. The next step is the blow molding of the container with an open top through which the container is filled, all while the plastic remains hot and in a molten state. Once filled, the container is hermetically sealed and cooled. The blow-fill seal process can take several seconds, and contemplated ready-to-inject compositions advantageously are formulated to withstand the temperature and pressure requirements without substantial degradation of norepinephrine (e.g., less than 5 wt %, less than 3 wt %, less than 2 wt %, less than 1 wt % degradation).

Once the norepinephrine formulations are filled in large volume polymeric, semi-permeable infusion containers (e.g., BFS container or flexible IV bags), the containers can optionally be layered or covered with a secondary packaging system including an aluminum pouch or other oxygen scavenger. For example, the BFS containers can further be sealed in an oxygen and moisture barrier blister packaging. The blister packaging can comprise one or more layers, and the one or more layers can include aluminum foil or other oxygen absorber having an Oxygen Transmission Rate (OTR) between 0.0005 to 5.00 cc/100 in^2/24 hrs. Additionally or alternatively, one or more oxygen absorbers (metal or metal free, organic material) can be incorporated into any portion of the BFS container, the secondary packaging system, or between the two (e.g., between the BFS container and the multi-layer packaging) such that the oxygen absorber removes at least a portion of oxygen from the air surrounding said oxygen-sensitive drug. A beneficial feature of the oxygen absorber is the absorbance and removal of oxygen present in the primary packaging and in the liquid drug itself. Notably, it was found that the oxygen absorber also removed residual headspace oxygen in the primary packaging and also dissolved oxygen in the liquid over time, thereby further improving stability of norepinephrine.

The following examples are provided for illustrative purposes only and should not be interpreted as limiting the present invention.

EXAMPLES

The following examples illustrate some of the experiments leading to the formulations according to the inventive subject matter, however, should not be construed to limit the scope of the claims in any way.

Stability and Isomerization: The ionization behavior of norepinephrine in aqueous solution is complex. Common with other o-hydroquinone systems, norepinephrine in aqueous solution is susceptible to oxidation to form the corresponding o-quinone, which can then also undergo various secondary reactions, which also becomes more prevalent as the pH becomes more alkaline. Norepinephrine may further isomerize to the pharmacologically less active S-enantiomer at low pH values, corresponding to protonation of the hydroxyl group at the benzylic chiral center. Therefore, to prevent norepinephrine cyclization reactions pH values less than 6.0 are desired. A pH range of 3.0 to 6.2 was screened to determine pH of optimum stability. Composition of norepinephrine bitartrate equivalent to 16 μg/mL norepinephrine base at various pH values were prepared are described below, with Table 4 listing compositions of norepinephrine bitartrate in citrate buffer (10 mM),

For preparation of the solutions, about 90% of the final quantity of water was collected in a glass media bottle. Nitrogen (N_2) gas was purged for about thirty minutes to reduce the dissolved oxygen levels. Sodium chloride was added and the solution was stirred until a homogenous solution was obtained. Citric acid was added and the solution was stirred until a homogenous solution was obtained. The pH of the bulk solutions was adjusted to pH 3.0, 3.4, 3.8, 4.2, 4.6, 5.0, 5.4, 5.8, and 6.2, respectively for each formulation composition using sufficient quantity of 10% w/v sodium hydroxide or 10% w/v hydrochloric acid. Norepinephrine bitartrate was added and the solution was stirred for approximately 10 minutes until a clear solution was formed. Solutions were made up to volume with water. The solutions were filled into 10 mL glass vials, overlaid with nitrogen, stoppered, and sealed. The stability was studied at 4° C., 25° C., and 60° C. by assay. Samples were observed visually for precipitation and change in color for a period of 7 days. Data are presented in Table 5.

TABLE 4

Compositions of Norepinephrine Bitartrate for pH dependent stability in Citrate Buffer (10 mM).

Ingredients	Concentration (mg/mL)								
	I	II	III	IV	V	VI	VII	VIII	IX
Norepinephrine Bitartarate equivalent to Norepinephrine base	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Sodium Chloride	9	9	9	9	9	9	9	9	9
Citric acid	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92	1.92
Sodium Citrate	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94	2.94
HCl/NaOH (q.s. pH)	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2
Water for Injection (q.s. mL)	1	1	1	1	1	1	1	1	1

TABLE 5

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
4° C.	I	96.4	96.5	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	98.5	3.8	No	No
	IV	99.1	98.4	4.2	No	No
	V	98.1	98.6	4.6	No	No
	VI	98.4	98.1	5.0	No	No
	VII	97.1	96.6	5.4	No	No
	VIII	97.8	97.5	5.8	No	No
	IX	91.5	91.2	6.2	No	No
25° C.	I	96.4	96.4	3.0	No	No
	II	98.0	97.5	3.4	No	No
	III	99.0	97.9	3.8	No	No
	IV	99.1	97.7	4.2	No	No
	V	98.1	97.3	4.6	No	No
	VI	98.4	97.3	5.0	No	No
	VII	97.1	95.9	5.4	No	No
	VIII	97.8	94.5	5.8	No	No
	IX	91.5	80.4	6.2	Yes	No
60° C.	I	96.4	95.2	3.0	No	No
	II	98.0	95.0	3.4	No	No
	III	99.0	95.2	3.8	No	No
	IV	99.1	93.2	4.2	No	No
	V	98.1	88.9	4.6	No	No
	VI	98.4	77.4	5.0	Yes	No
	VII	97.1	46.8	5.4	Yes	No

TABLE 5-continued

Effect of pH on stability of Norepinephrine Bitartrate in citrate buffer.

Temperature	Formulation	Assay To	Assay T_7	pH	Color	Precipitation
	VIII	97.8	NT	5.8	Yes	No
	IX	91.5	NT	6.2	Yes	No

No change in physical appearance was observed in the solutions stored at 4° C. In the solutions stored at 25° C., a change in color was observed at pH 6.2. Red brown color was observed in solutions stored at or above pH 5.0 at 60° C. Oxidation and color formation are very common with norepinephrine in unfavorable conditions and the speed of the reaction and the nature of the final products are dependent on the catalysts (e.g., metal ion impurities) and buffers employed. A pH range from 3.0 to 4.5 was selected for further testing.

Stability of Norepinephrine in selected pH ranges and formulations: The formulations for the next experiments are shown in Table 6 below, involving three different compositions of norepinephrine bitartrate at three different pH (3.5, 4.0, 4.5, and 5.0) values. Lab scale batches were prepared and subjected to lab scale stability tests at accelerated (40° C./75% RH) and long term stability (25° C./60% RH) storage conditions. The test results from the stability studies are presented in Table 7-Table 10, with CCS indicating Clear colorless solution; ND indicating Not Detected; NR indicating Not Reported (<0.05%); and NT indicating Not Tested.

TABLE 6

Formulation composition selected for further development activities and optimization

Ingredient	Quantity (mg/mL)			
Formulation	X	XI	XII	XIII
Norepinephrine Bitartrate	0.016	0.016	0.016	0.016
Edetate Sodium	0.10	0.10	0.10	0.10
Sodium chloride	9	9	9	9
HCl/NaOH	q.s. pH 3.5	q.s. pH 4.0	q.s. pH 4.5	q.s. pH 5.0
Water for Injection Q.S.	1 mL	1 mL	1 mL	1 mL
Dissolved Oxygen (ppm)	<1	<1	<1	<1

TABLE 7

Stability study of Formulation X - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 3.5).

	Storage Condition												
	25 ± 2° C./60 ± 5% RH							40 ± 2° C./75 ± 5% RH					
	Time Point												
	Initial	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.50	3.65	3.59	3.56	3.58	3.54	3.48	3.66	3.61	3.59	3.64	3.60	3.59
Assay	101.4	99.6	97.1	97.1	101.0	102.3	102.2	99.5	97.0	98.7	100.4	101.7	101.4
S-form	NT	NT	NT	NT	1.8	2.2	2.2	NT	NT	NT	7.6	8.1	9.8
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 8

Stability study of Formulation XI - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.02	3.96	3.98	3.97	3.91	4.01	3.99	4.02	4.03
Assay	101.3	98.7	95.5	99.2	100.5	98.6	95.3	97.1	97.5
S-form	NT	NT	NT	NT	1.7	NT	NT	NT	7.8
Total Impurities	0.1	ND	0.06	ND	0.80	ND	0.06	0.1	0.79

TABLE 9

Stability study of Formulation XII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 4.5).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	6 Month	1 Month	2 Month	3 Month	6 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.50	4.35	4.36	4.32	4.33	4.33	4.40	4.39	4.29
Assay	100.1	98.9	95.5	98.2	97.9	97.1	92.5	93.7	77.2
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	2.9
Total Impurities	ND	0.32	0.79	0.52	3.41	1.18	0.38	5.59	10.38

TABLE 10

Stability study of Formulation XIII - Norepinephrine Bitartrate Injection (16 μg/ml) filled in glass vial (pH 5.0).

	Storage Condition								
	25 ± 2° C./60 ± 5% RH					40 ± 2° C./75 ± 5% RH			
	Time Point								
	Initial	1 Month	2 Month	3 Month	4 Month	1 Month	2 Month	3 Month	4 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.99	4.62	4.51	4.57	4.51	4.87	4.81	4.83	4.53
Assay	102.7	100.5	95.6	99.2	100.4	98.3	89.8	87.0	72.3
S-form	NT	NT	NT	NT	1.2	NT	NT	NT	3.0
Total Impurities	ND	0.75	0.81	0.48	1.29	0.94	2.4	5.39	14.91

Based on the above considerations, the effect of different levels of EDTA on stability of norepinephrine was determined. Three batches at concentrations of 16 μg/mL, 32 μg/mL, and 64 μg/mL were made with EDTA concentrations of 100 μg/mL: Formulation XIV (16 μg/mL), Formulation XV (32 μg/mL), Formulation XVI (64 μg/mL). Two additional batches were made at 10 μg/mL EDTA Formulation XVII and 1 μg/mL EDTA (Formulation XVIII) at 64 μg/mL Norepinephrine. The composition of the batches is specified in Table 11. The drug product was compounded as described earlier and packaged in 250 mL in polypropylene bags. This was further packed into aluminum overwrap with an oxygen scavenger (GLS 100, Mitsubishi Gas Chemicals). The batches were then stored at room temperature and accelerated temperature conditions.

TABLE 11

Formulation composition selected with different level of EDTA concentrations.

Ingredient	Quantity (mg/mL)				
Formulation Number	XIV	XV	XVI	XVII	XVIII
Norepinephrine Bitartrate	0.016	0.032	0.064	0.064	0.064
Edetate Sodium	0.10	0.10	0.10	0.010	0.0010
Sodium chloride	9	9	9	9	9
Hydrochloric Acid/ Sodium Hydroxide	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for Injection	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL	q.s. 1 mL

The resultant stability data on these formulations are presented in Table 12-Table 16 (CCS—Clear colorless solution; ND—Not Detected). The results of the stability studies at different amounts of EDTA at pH 4.0 indicates that both 0.01%, 0.001% of EDTA significantly prevented the degradation rate of norepinephrine in terms of known and unknown impurities. Moreover, with respect to isomerization from the R-isomer to the S-isomer it was notably observed that the amount of EDTA had substantially no influence on racemization or enantiomer formation during stability and after autoclaving.

TABLE 12

Stability study of Formulation XIV - Norepinephrine bitartrate injection (16 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.99	3.96	4.08	4.08	4.02	4.08	4.08
Assay	98.5	100.4	100.1	99.7	100.3	100.0	99.5
S-form	0.9	1.1	1.4	1.3	1.9	2.9	4.2
Total Impurities	0.05	ND	ND	ND	ND	0.10	0.38

TABLE 13

Stability study of Formulation XV - Norepinephrine bitartrate injection (32 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.01	3.99	4.08	4..08	4.02	4.08	4.08
Assay	101.0	102.9	97.1	100.7	102.9	99.4	100.6
S-form	0.9	1.1	1.3	1.4	1.9	3.0	4.1
Total Impurities	0.06	ND	ND	ND	ND	ND	0.14

TABLE 14

Stability study of Formulation XVI - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 100 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.99	4.08	4.08	3.98	4.07	4.07
Assay	98.4	103.2	98.7	100.2	104.6	99.3	99.8
S-form	0.9	1.1	1.3	1.3	2.0	3.2	4.2
Total Impurities	0.06	ND	0.12	ND	ND	ND	ND

TABLE 15

Stability study of Formulation XVII - Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 10 μg/mL EDTA.

	Storage Condition						
	25 ± 2° C./60 ± 5% RH				40 ± 2° C./75 ± 5% RH		
	Time Point						
	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.06	4.06	3.99	4.05	4.05
Assay	102.7	105.7	103.4	104.3	107.8	103.6	103.9
S-form	0.9	1.1	1.2	1.5	2.0	3.3	4.3
Total	0.06	ND	ND	ND	ND	0.26	ND

TABLE 16

Stability study of Formulation XVIII-Norepinephrine bitartrate injection (64 μg/mL); pH 4.0 at 1 μg/mL EDTA.

Storage Condition	25 ± 2° C./ 60 ±5% RH				40 ± 2° C./ 75 ±5% RH		
Time Point	Initial	1 Month	2 Month	3 Month	1 Month	2 Month	3 Month
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	4.00	3.98	4.07	4.07	4.02	4.06	4.06
Assay	98.7	102.6	100.4	100.4	105.0	99.9	99.2
S-form	0.9	1.1	1.3	1.4	2.0	3.2	4.3
Total	0.06	ND	ND	ND	ND	ND	ND

Sterilization and Stability: The volume for ready-to-inject formulations is 250 mL and as such classifies as a large volume parenteral (LVP). To achieve a desired or required sterility assurance level of 10^{-6} for a LVP terminal sterilization via heat it is typically required. The inventors therefore investigated whether or not contemplated formulations could be terminally sterilized via autoclaving.

Formulations at a concentration 16 μg/mL and 64 μg/mL (Formulation XVII) Norepinephrine base were prepared substantially as shown in Table 11 above and packaged in secondary packaging of aluminum overwrap with an oxygen scavenger and shipped for terminal sterilization. The secondary packaging was removed and the bags were terminally sterilized using steam sterilizer (Fedegari, Model # FOB3) with an air over-pressure (AOP) sterilization cycle. The terminal sterilization was performed at 121° C. for 5, 10, and 15 min. Post completion of sterilization temperature, the bags underwent spontaneous cooling to 95° C. and forced cooling to 70° C. The total exposure time and calculated F_0 values were 11.09, 17.04, and 22.42 for 5 min, 10 min, and 15 min cycles respectively. The bags were then analyzed for assay, impurities, and S-isoform, and the results are shown in Table 17 and Table 18.

TABLE 17

Stability study of Norepinephrine bitartrate injection (16 μg/mL) filled in 100 mL PP bags (pH 4.0); 10 μg/mL EDTA; terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.76	3.85	3.78	3.77	3.76	3.76	3.78	3.76	3.75	3.76
Dissolved Oxygen	0.63	4.93	4.86	4.89	0.75	0.48	0.55	0.65	0.78	0.77
Assay	103.1	103.1	103.1	103.1	103.1	103.0	103.1	103.1	103.2	103.1

TABLE 17-continued

Stability study of Norepinephrine bitartrate injection (16 µg/mL) filled in 100 mL PP bags (pH 4.0); 10 µg/mL EDTA; terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
S-Form	1.0	3.0	3.0	3.0	3.8	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TABLE 18

Stability study of Norepinephrine bitartrate injection (64 µg/ml) filled in 100 mL PP bags (pH 4.0); 10 µg/mL EDTA terminally sterilized.

	Time Point									
		5 Min			10 Min			15 Min		
		Bag Number								
	Initial	1	2	3	1	2	3	1	2	3
Appearance	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS	CCS
pH	3.74	3.74	3.75	3.73	3.74	3.74	3.76	3.74	3.73	3.74
Dissolved Oxygen	0.69	5.15	5.03	5.00	0.52	0.59	0.75	0.69	0.80	0.74
Assay	101.2	102.2	101.2	101.5	101.7	101.2	101.3	101.2	101.3	102.2
S-Form	1.0	3.0	3.0	3.0	3.7	3.7	3.7	4.3	4.3	4.3
Total	ND	ND	ND	ND	ND	ND	ND	0.1	ND	ND

As can be seen from the data, the S-isoform appears to increase proportionally to time during the terminal sterilization cycle. No increase in reportable impurities was observed.

Test method—Determination of norepinephrine and degradation products: Separation of Norepinephrine and related compounds was performed using a gradient HPLC method with UV detection. Pentofluorophenylpropyl terminated silica was used as a stationary phase for chromatographic analysis. The mobile phase was prepared by mixing water and methanol, with both solvents containing formic acid. Related compounds were defined by their relative retention times (RRT) based on the NE peak retention time. Quantitation of related compounds was accomplished by comparing the corresponding peak area from a sample solution chromatogram to that of the NE peak from a Reference Standard (RS) solution of a known concentration. Relative Response Factors (RRF) were used to correct for chemical structure effects on the responses of the identified impurities. Chromatography was performed using parameters and methods as shown in Table 19.

TABLE 19

HPLC	Waters Alliance e2695
Column	Supelco Discovery HS F-5 Column, 3 µm, 4.6 × 150 mm
Column Temperature	35° C.
Sample Temperature	Ambient
Injection volume	85.0 µL
Flow Rate	0.8 mL/min
Detection	Spectrum: 200-600 nm, resolution 1.2 nm
	Single channel: 280 nm, resolution 4.8 nm
	PDA Filter Time Constant Normal
Sampling rate: 5 points/sec	
Solution A	0.1% Formic acid in Water
Solution B	0.1% Formic acid in Methanol

Mobile Phase	Time (mins)	% Solution A	% Solution B
	0	100	0
	3	100	0
	6	93	7
	8	93	7
	15	88	12
	30	2	98
	35	2	98
	36	100	0
	40	100	0

Test Method—Identification, Assay and Enantiomeric Purity of Norepinephrine: Identification and quantification of S-norepinephrine and R-norepinephrine was performed using an HPLC method with UV detection. HPLC-UV was used to separate and quantitate the amount of (R)- and (S)-enantiomers of norepinephrine (NE) present in the drug product with the NE concentrations of 16, 32 and 64 μg/ml. The comparison of the sum of (R)- and (9-peak responses in a sample chromatogram versus a reference standard chromatogram gives the total amount of NE. The (S)-enantiomer was quantitated based on its peak response as the percentage of the total peak response of both enantiomers.

More specifically, determination of R- and S-enantiomers of norepinephrine in the drug product solution was performed using an isocratic reverse-phase HPLC method with UV detection. Separation was achieved by using a protein-based column with functional chiral selectors. The chiral selector is cellobiohydrolase (CBH), a stable enzyme that has been immobilized onto spherical silica particles. This enzyme preferentially separates compounds containing one or more basic nitrogen groups together with one or more hydrogen-accepting or hydrogen-donating groups. Chromatography was performed using parameters and methods as shown in Table 20.

TABLE 20

HPLC	Agilent 1260 Infinity
Column	Daicel Chiralpak CBH ™ column, 5 μm, 4.0 × 100 mm
Column Temperature	27° C. ± 2° C.
Sample Temperature	Ambient
Injection volume	20.0 μL for 16 mcg/mL, 10.0 μL for 32 mcg/mL, 5.0 μL for 64 mcg/mL
Flow Rate	0.9 mL/min
Detection	Single channel: 280 nm, resolution 4.8 nm Spectrum: 200-600 nm, resolution 1.2 nm
Mobile Phase:	Buffer/IPA 95:5 v/v Buffer: Sodium Phosphate, Disodium Edetate, pH 6.0
Run Time	8 min

While contemplated formulations can be administered following various protocols, the inventors contemplate that administration of the formulations, especially administration for treatment of hypotension, will follow a protocol that comprises at least two distinct steps, with an accelerated administration followed by a maintenance administration as exemplarily described in Table 21 below.

TABLE 21

		Initial Dose		Maintenance Dose	
Presentation (mg/mL)	Concentration (μg/mL)	Dose per Minute (μg/min)	Flow Rate (mL/min)	Dose per Minute (μg/min)	Flow Rate (mL/min)
16 μg/mL (4 mg in 250 mL)	16	8-12	0.500-0.750	2-4	0.125-0.250
32 μg/mL (8 mg in 250 mL)	32		0.250-0.375		0.062-0.125
64 μg/mL (16 mg in 250 mL)	64		0.125-0.187		0.031-0.062

As used in the description herein and throughout the claims that follow, the meaning of "a," "an," and "the" includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein, the meaning of "in" includes "in" and "on" unless the context clearly dictates otherwise.

In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

Moreover, in interpreting the disclosure all terms should be interpreted in the broadest possible manner consistent with the context. In particular the terms "comprises" and "comprising" should be interpreted as referring to the elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps can be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

What is claimed is:

1. A method of preparing a sterile, ready-to-administer norepinephrine composition, comprising the steps of:

(a) combining norepinephrine or a salt thereof, a chelating agent, a tonicity adjusting agent, and an aqueous acidic solution to form a liquid parenteral composition, wherein the norepinephrine comprises at least 95% of R-isomer of norepinephrine, wherein the norepinephrine or salt thereof is present in the liquid parenteral composition in an amount of between 10 μg/ml and 100 μm/ml, wherein the chelating agent is present in the liquid parenteral composition in an amount of between 1 μg/ml and 100 μm/ml, and wherein the tonicity adjusting agent is present in the liquid parenteral composition in an amount of between 0.6 wt % and 1.2 wt %;

(b) adjusting the pH of the liquid parenteral composition to a pH range of between 3.7 and 4.3;

(c) filling the liquid parenteral composition into a container; and

(d) heat sterilizing the liquid parenteral composition in the container to sterility to form the sterile, ready-to-administer norepinephrine composition;

wherein the sterile, ready-to-administer norepinephrine composition is substantially free of antioxidants; and

wherein the sterile ready-to-administer norepinephrine composition comprises at least about 90% R-isomer of

norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

2. The method of claim **1**, further comprising a step of placing the container into a secondary container.

3. The method of claim **2**, wherein the container and/or the secondary container include a metal-free oxygen scavenger.

4. The method of claim **2**, wherein the container is a polypropylene bag and the secondary container comprises an aluminum overwrap.

5. The method of claim **4**, wherein the aluminum overwrap comprises an oxygen scavenger.

6. The method of claim **1**, wherein the heat sterilizing step is performed using a steam sterilizer with an air overpressure sterilization cycle.

7. The method of claim **1**, wherein the heat sterilizing is performed at 121° C.

8. The method of claim **7**, wherein the heat sterilizing is performed for at least 5 minutes.

9. The method of claim **7**, wherein the heat sterilizing is performed for at least 10 minutes.

10. The method of claim **7**, wherein the heat sterilizing is performed for at least 15 minutes.

11. The method of claim **1**, further comprising a step of (e) cooling the sterile, ready-to-administer norepinephrine composition.

12. The method of claim **11**, wherein the sterile, ready-to-administer norepinephrine composition is cooled to 70° C.-95° C.

13. The method of claim **11**, wherein the cooling step comprises spontaneous cooling to about 95° C. followed by forced cooling to about 70° C.

14. The method of claim **1**, wherein the liquid parenteral composition has a pH between 3.7 and 4.0.

15. The method of claim **1**, wherein norepinephrine in the sterile ready-to-administer norepinephrine composition is norepinephrine bitartrate.

16. The method of claim **1**, wherein the norepinephrine is present in the liquid parenteral composition at a concentration of about 16 μg/ml, about 32 μg/ml, or about 64 μg/ml.

17. The method of claim **1**, wherein the chelating agent is selected from the group consisting of a bicarboxylic acid, a tricarboxylic acid, and an aminopolycarboxylic acid.

18. The method of claim **1**, wherein the chelating agent is ethylenediaminetetraacetic acid (EDTA).

19. The method of claim **1**, where the tonicity adjusting agent is sodium chloride.

20. The method of claim **1**, wherein the aqueous acidic solution has dissolved oxygen at a concentration of equal or less than 1 ppm.

21. The method of claim **1**, wherein the sterile ready-to-administer norepinephrine composition comprises equal or less than about 10% of S-isomer of norepinephrine or a salt thereof after the heat sterilizing step.

22. The method of claim **1**, wherein the norepinephrine or a salt thereof in the sterile ready-to-administer norepinephrine composition comprises equal or less than about 10% S-isomer of norepinephrine or salt thereof after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

23. The method of claim **1**, wherein the sterile ready-to-administer norepinephrine composition comprises equal or less than about 5% of total degradation of norepinephrine or salt thereof excluding S-isomer of norepinephrine after storage at 25±2° C. and 60±5% relative humidity, over at least three months as determined by HPLC.

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