IN THE UNITED STATES DISTRICT COURT

FOR THE DISTRICT OF DELAWARE

CEPHALON, INC.,	)	
	)	
Plaintiff,	)	
	)	
v.	)	C.A. No. 14-671-GMS
	)	
BRECKENRIDGE PHARMACEUTICAL, INC. and NATCO PHARMA LTD.,	)	
	)	
Defendants.	)	

FIRST AMENDED COMPLAINT

Cephalon, Inc. ("Cephalon" or "Plaintiff") brings this action for patent infringement against Defendants Breckenridge Pharmaceutical, Inc. ("Breckenridge") and Natco Pharma Ltd. ("Natco") (collectively "Defendants").

1. This is an action by Cephalon against Defendants for infringement of United States Patent No. 8,445,524 ("the '524 patent"), United States Patent No. 8,436,190 ("the '190 patent"), U.S. Patent No. 8,609,863 ("the '863 patent") and United States Patent No. 8,791,270 ("the '270 patent"). This action arises out of Breckenridge's filing of an Abbreviated New Drug Application ("ANDA") seeking approval by the United States Food and Drug Administration ("FDA") to sell generic versions of TREANDA®, Cephalon's innovative treatment for chronic lymphocytic leukemia and non-Hodgkin's lymphoma, prior to the expiration of the '524 patent, the '190 patent, the '863 patent and the '270 patent.

THE PARTIES

Cephalon, Inc.

2. Plaintiff Cephalon, Inc. is a corporation operating and existing under the laws of Delaware, with its principal place of business at 41 Moores Road, Frazer, Pennsylvania 19355.

Cephalon is engaged in the business of research, development, manufacture, and sale of innovative pharmaceutical products throughout the world.

Defendants

3. On information and belief, Breckenridge is a corporation organized and existing under the laws of Florida, having a principal place of business at 6111 Broken Sound Parkway, NW, Suite 170, Boca Raton, FL 33487.

4. On information and belief, Breckenridge is in the business of making and selling generic pharmaceutical products, which it distributes, markets, and/or sells in Delaware, and throughout the United States.

5. On information and belief, Natco is an Indian company having a principal place of business at Natco House, Road No.2, Banjara Hills, Hyderabad-500 033, India.

6. On information and belief, Natco is in the business of, among other things, making and selling generic pharmaceutical substances and products, which it distributes, markets, and/or sells in Delaware, and throughout the United States.

7. On information and belief, Natco Ltd. has partnered with Breckenridge to market and distribute Natco Ltd.'s generic drug products complained of herein, including in this District.

JURISDICTION AND VENUE

Subject Matter Jurisdiction

8. This action for patent infringement arises under 35 U.S.C. § 271.

9. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a), and the Declaratory Judgment Act, 28 U.S.C §§ 2201 and 2202.

Personal Jurisdiction Over Defendants

10. On information and belief, this Court has personal jurisdiction over Defendants.

11. On information and belief, this Court has personal jurisdiction over Breckenridge because Breckenridge: (1) conducts business in this Judicial District and (2) has engaged in continuous and systematic contacts with Delaware and/or purposefully availed itself of this forum by, among other things, making, marketing, shipping, using, offering to sell or selling, or causing others to use, offer to sell, or sell, Breckenridge pharmaceutical products in this Judicial District, and deriving substantial revenue from such activities. On information and belief, Breckenridge has also committed, or aided, abetted, contributed to and/or participated in the commission of, the tortious action of patent infringement that has led to foreseeable harm and injury to Cephalon, which manufactures TREANDA® for sale and use throughout the United States, including the State of Delaware.

12. On information and belief, this Court also has personal jurisdiction over Breckenridge because Breckenridge previously has filed a patent litigation in this Judicial District, *see Pamlab LLC, Metabolite Labs, Inc. and Breckenridge Pharmaceutical, Inc. v. Acella Pharmaceuticals, LLC*, 1:12-cv-01403-SLR, and has been sued in this Judicial District and did not challenge this Court's exertion of personal jurisdiction over it. *See USB Inc. v. Breckenridge Pharmaceutical, Inc.*, 13-1211-LPS (D. Del.); *Cephalon, Inc. et al. v. Breckenridge Pharmaceutical, Inc., and Natco Pharma Limited*, C.A. No. 11-1070-GMS (D. Del.).

13. On information and belief, this Court has personal jurisdiction over Natco because Natco has engaged in continuous and systematic contacts with Delaware and/or purposefully availed itself of this forum, including through its wholly owned subsidiary Natco Pharma Inc., a Delaware company, by, among other things, making, marketing, shipping, using, offering to sell or selling, or causing others to use, offer to sell, or sell, Natco pharmaceutical products in this

Judicial District, and deriving substantial revenue from such activities. On information and belief, Natco has also committed, or aided, abetted, contributed to and/or participated in the commission of, the tortious action of patent infringement that has led to foreseeable harm and injury to Cephalon, which manufactures TREANDA® for sale and use throughout the United States, including the State of Delaware.

14. On information and belief, this Court also has personal jurisdiction over Natco because Natco has been sued in this Judicial District and did not challenge this Court's exertion of personal jurisdiction over it. *See Cephalon, Inc. et al. v. Breckenridge Pharmaceutical, Inc., and Natco Pharma Limited*, C.A. No. 11-1070-GMS (D. Del.).

Venue

15. Venue is proper in this Judicial District under 28 U.S.C. §§ 1391 and 1400(b).

BACKGROUND

The '524 Patent

16. The '524 patent, entitled "Solid Forms of Bendamustine Hydrochloride," was duly and lawfully issued on May 21, 2013 to inventors Laurent D. Courvoisier, Robert E. McKean, Hans-Joachim Jänsch, and Veronique Courvoisier.

17. The named inventors of the '524 patent assigned their rights in the '524 patent to Cephalon.

18. Cephalon is the sole owner by assignment of all rights, title and interest in the '524 patent.

19. The '524 patent is listed in FDA publication "Approved Drug Products with Therapeutic Equivalence Evaluations," commonly referred to as "the Orange Book" ("Orange Book"), with respect to TREANDA®.

20. The ’524 patent will expire on March 26, 2029. A true and accurate copy of the ’524 patent is attached hereto as Exhibit A.

The ’190 Patent

21. The ’190 patent, entitled “Bendamustine Pharmaceutical Compositions,” was duly and lawfully issued on May 7, 2013 to inventors Jason Edward Brittain and Joe Craig Franklin.

22. The named inventors of the ’190 patent assigned their rights in the ’190 patent to Cephalon.

23. Cephalon is the sole owner by assignment of all rights, title and interest in the ’190 patent.

24. The ’190 patent is listed in the Orange Book with respect to TREANDA®.

25. The ’190 patent will expire on October 26, 2030. A true and accurate copy of the ’190 patent is attached hereto as Exhibit B.

The ’863 Patent

26. The ’863 patent, entitled “Bendamustine Pharmaceutical Compositions,” was duly and lawfully issued on December 17, 2013 to inventors Jason Edward Brittain and Joe Craig Franklin.

27. The named inventors of the ’863 patent assigned their rights in the ’863 patent to Cephalon.

28. Cephalon is the sole owner by assignment of all rights, title and interest in the ’863 patent.

29. The ’863 patent is listed in the Orange Book with respect to TREANDA®.

30. The ’863 patent will expire on January 12, 2026. A true and accurate copy of the ’863 patent is attached hereto as Exhibit C.

The ’270 Patent

31. The ’270 patent, entitled “Bendamustine Pharmaceutical Compositions,” was duly and lawfully issued on July 29, 2014 to inventors Jason E. Brittain and Joe C. Franklin.

32. The named inventors of the ’270 patent assigned their rights in the ’270 patent to Cephalon.

33. Cephalon is the sole owner by assignment of all rights, title and interest in the ’270 patent.

34. The ’270 patent is listed in the Orange Book with respect to TREANDA®.

35. The ’270 patent will expire on January 12, 2026. A true and accurate copy of the ’270 patent is attached hereto as Exhibit D.

The TREANDA® Drug Product

36. Cephalon researched, developed, applied for and obtained FDA approval to manufacture, sell, promote and/or market bendamustine hydrochloride products known as TREANDA®.

37. Cephalon has been selling, promoting, distributing and marketing TREANDA® in the United States since 2008.

38. TREANDA® is indicated to treat chronic lymphocytic leukemia and non-Hodgkin’s lymphoma.

39. Cephalon holds New Drug Application No. 22249 and No. 22303 under Section 505(a) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 355(a), for multiple TREANDA® products used for treating chronic lymphocytic leukemia and non-Hodgkin’s lymphoma.

The Breckenridge ANDA

40. Breckenridge filed with FDA an Abbreviated New Drug Application under 21 U.S.C. § 355(j) seeking approval to manufacture, use, offer for sale, sell and import into the United States a bendamustine hydrochloride powder for IV (infusion), 25 mg/vial and 100 mg/vial ("Defendants' Bendamustine Product") prior to the expiration of the '524 patent, the '190 patent, the '863 patent and the '270 patent. On information and belief, Breckenridge and Natco partnered in the development and filing of the Breckenridge ANDA.

41. FDA assigned the ANDA for Defendants' Bendamustine Product the number 205447.

42. Breckenridge also filed with FDA, pursuant to 21 U.S.C. § 355(j)(2)(B)(iv), a certification alleging that the claims of the '524 patent, the '190 patent, the '863 patent and the '270 patent are invalid, unenforceable and/or would not be infringed by the manufacture, use, importation, sale or offer for sale of Defendants' Bendamustine Product ("Breckenridge's Paragraph IV Certification").

43. By letter dated April 14, 2014, Breckenridge notified Cephalon that it had filed ANDA No. 205574 seeking approval to market Defendants' Bendamustine Product prior to the expiration of the '524 patent, the '190 patent, and the '863 patent ("Breckenridge's First Notice Letter"). Breckenridge then notified Cephalon by a letter dated August 1, 2014, that it had filed an amendment to ANDA No. 205574 seeking approval to market Defendants' Bendamustine Product prior to the expiration of the '270 patent ("Breckenridge's Second Notice Letter").

44. On May 6, 2014, pursuant to an Offer of Confidential Access, Cephalon received portions of the ANDA filed by Breckenridge, and Cephalon reviewed those portions of the ANDA with respect to the '524 patent, the '190 patent and the '863 patent only.

45. This Action is being commenced before the expiration of forty-five days from the date of receipt of the Breckenridge's First Notice Letter.

COUNT I FOR INFRINGEMENT OF U.S. PATENT NO. 8,445,524 BY DEFENDANTS

46. The allegations of the preceding paragraphs 1–45 are re-alleged and incorporated herein by reference.

47. The use of Defendants' Bendamustine Product is covered by one or more claims of the '524 patent.

48. The commercial manufacture, use, offer for sale, sale, marketing, distribution and/or importation of Defendants' Bendamustine Product would infringe one or more claims of the '524 patent.

49. Under 35 U.S.C. § 271(e)(2)(A), Breckenridge's submission to FDA of the Breckenridge ANDA to obtain approval for Defendants' Bendamustine Product with a Paragraph IV Certification related thereto before the expiration of the '524 patent constitutes an act of infringement, and if approved, the commercial manufacture, use, offer to sell, sale, or importation of Defendants' Bendamustine Product containing bendamustine hydrochloride, would infringe one or more claims of the '524 patent.

50. Defendants were aware of the '524 patent when engaging in these knowing and purposeful activities and were aware that filing the Breckenridge ANDA with Breckenridge's Paragraph IV Certification with respect to the '524 patent constituted an act of infringement of the '524 patent.

51. On information and belief, Defendants' Bendamustine Product contains the same active pharmaceutical ingredient, bendamustine hydrochloride, as that used in Cephalon's TREANDA® products and claimed in the '524 patent.

52. On information and belief, the manufacture of Defendants' Bendamustine Product is made using the solid form of bendamustine hydrochloride described in one or more claims of the '524 patent.

53. Defendants' use of the solid form of bendamustine hydrochloride in the manufacture of Defendants' Bendamustine Product infringes one or more claims of the '524 patent.

54. On information and belief, Defendants plan and intend to, and will, infringe the '524 patent immediately and imminently upon approval of the Breckenridge ANDA.

55. On information and belief, Defendants, under 35 U.S.C. § 271(b), acted in concert, actively supported, participated in, encouraged, and/or induced the infringement of one or more claims of the '524 patent.

56. On information and belief, Defendants plan and intend to, and will, actively induce infringement of the '524 patent when the Breckenridge ANDA is approved, and plan and intend to, and will, do so immediately and imminently upon approval.

57. On information and belief, Defendants know that the solid form of bendamustine hydrochloride used to manufacture Defendants' Bendamustine Product is especially made or adapted for use in infringing the '524 patent and that the solid form of bendamustine hydrochloride used to manufacture Defendants' Bendamustine Product is not suitable for substantial non-infringing uses. On information and belief, Defendants plan and intend to, and will, contribute to the infringement of the '524 patent immediately and imminently upon approval of the Breckenridge ANDA.

58. The foregoing actions by Defendants constitute and/or would constitute infringement of the ’524 patent, active inducement of infringement of the ’524 patent and/or contribution to the infringement by others of the ’524 patent.

59. On information and belief, Defendants acted without a reasonable basis for believing that they would not be liable for infringing the ’524 patent, actively inducing infringement of the ’524 patent and/or contributing to the infringement by others of the ’524 patent.

60. Cephalon will be substantially and irreparably harmed by Defendants’ infringing activities unless the Court enjoins those activities. Cephalon will have no adequate remedy at law if Defendants are not enjoined from the commercial manufacture, use, offer to sell, sale in and importation into the United States of Defendants’ Bendamustine Product.

61. Defendants’ activities render this case an exceptional one, and Cephalon is entitled to an award of their reasonable attorney fees under 35 U.S.C. § 285.

COUNT II FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF U.S. PATENT NO. 8,445,524 BY DEFENDANTS

62. The allegations of the preceding paragraphs 1–61 are re-alleged and incorporated herein by reference.

63. On information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Defendants’ Bendamustine Product soon after FDA approval of the Breckenridge ANDA.

64. Such conduct will constitute direct infringement of one or more claims on the ’524 patent under 35 U.S.C. § 271(a), inducement of infringement of the ’524 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

65. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the Breckenridge ANDA.

66. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Cephalon and Defendants as to liability for the infringement of the '524 patent. Defendants' actions have created in Cephalon a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

67. On information and belief, Defendants will knowingly and willfully infringe the '524 patent.

68. Cephalon will be irreparably harmed if Defendants are not enjoined from infringing the '524 patent.

COUNT III FOR INFRINGEMENT OF U.S. PATENT NO. 8,436,190 BY DEFENDANTS

69. The allegations of the preceding paragraphs 1–68 are re-alleged and incorporated herein by reference.

70. The use of Defendants' Bendamustine Product is covered by one or more claims of the '190 patent.

71. The commercial manufacture, use, offer for sale, sale, marketing, distribution and/or importation of Defendants' Bendamustine Product would infringe one or more claims of the '190 patent.

72. Under 35 U.S.C. § 271(e)(2)(A), Breckenridge's submission to FDA of the Breckenridge ANDA to obtain approval for Defendants' Bendamustine Product with a Paragraph IV Certification related thereto before the expiration of the '190 patent constitutes an act of infringement, and if approved, the commercial manufacture, use, offer to sell, sale, or

importation of Defendants' Bendamustine Product containing bendamustine hydrochloride, would infringe one or more claims of the '190 patent.

73. Defendants were aware of the '190 patent when engaging in these knowing and purposeful activities and were aware that filing the Breckenridge ANDA with Breckenridge's Paragraph IV Certification with respect to the '190 patent constituted an act of infringement of the '190 patent.

74. On information and belief, Defendants' Bendamustine Product contains the same active pharmaceutical ingredient, bendamustine hydrochloride, as that used in Cephalon's TREANDA® products and claimed in the '190 patent.

75. On information and belief, the manufacture of Defendants' Bendamustine Product is made by lyophilizing the bendamustine hydrochloride pharmaceutical compositions described in one or more claims of the '190 patent.

76. Defendants' use of a lyophilized bendamustine hydrochloride pharmaceutical composition in the manufacture of Defendants' Bendamustine Product infringes one or more claims of the '190 patent.

77. On information and belief, Defendants plan and intend to, and will, infringe the '190 patent immediately and imminently upon approval of the Breckenridge ANDA.

78. On information and belief, Defendants, under 35 U.S.C. § 271(b), acted in concert, actively supported, participated in, encouraged, and/or induced the infringement of one or more claims of the '190 patent.

79. On information and belief, Defendants plan and intend to, and will, actively induce infringement of the '190 patent when the Breckenridge ANDA is approved, and plan and intend to, and will, do so immediately and imminently upon approval.

80. On information and belief, Defendants know that the lyophilized bendamustine hydrochloride pharmaceutical composition used to manufacture Defendants' Bendamustine Product is especially made or adapted for use in infringing the '190 patent and that the bendamustine hydrochloride pharmaceutical composition used to manufacture Defendants' Bendamustine Product is not suitable for substantial non-infringing uses. On information and belief, Defendants plan and intend to, and will, contribute to the infringement of the '190 patent immediately and imminently upon approval of the Breckenridge ANDA.

81. The foregoing actions by Defendants constitute and/or would constitute infringement of the '190 patent, active inducement of infringement of the '190 patent and/or contribution to the infringement by others of the '190 patent.

82. On information and belief, Defendants acted without a reasonable basis for believing that they would not be liable for infringing the '190 patent, actively inducing infringement of the '190 patent and/or contributing to the infringement by others of the '190 patent.

83. Cephalon will be substantially and irreparably harmed by Defendants' infringing activities unless the Court enjoins those activities. Cephalon will have no adequate remedy at law if Defendants are not enjoined from the commercial manufacture, use, offer to sell, sale in and importation into the United States of Defendants' Bendamustine Product.

84. Defendants' activities render this case an exceptional one, and Cephalon is entitled to an award of their reasonable attorney fees under 35 U.S.C. § 285.

COUNT IV FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF U.S. PATENT NO. 8,436,190 BY DEFENDANTS

85. The allegations of the preceding paragraphs 1–84 are re-alleged and incorporated herein by reference.

86. On information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Defendants' Bendamustine Product soon after FDA approval of the Breckenridge ANDA.

87. Such conduct will constitute direct infringement of one or more claims on the '190 patent under 35 U.S.C. § 271(a), inducement of infringement of the '190 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

88. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the Breckenridge ANDA.

89. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Cephalon and Defendants as to liability for the infringement of the '190 patent. Defendants' actions have created in Cephalon a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

90. On information and belief, Defendants will knowingly and willfully infringe the '190 patent.

91. Cephalon will be irreparably harmed if Defendants are not enjoined from infringing the '190 patent.

COUNT V FOR INFRINGEMENT OF U.S. PATENT NO. 8,609,863 BY DEFENDANTS

92. The allegations of the preceding paragraphs 1–91 are re-alleged and incorporated herein by reference.

93. The use of Defendants' Bendamustine Product is covered by one or more claims of the '863 patent.

94. The commercial manufacture, use, offer for sale, sale, marketing, distribution and/or importation of Defendants' Bendamustine Product would infringe one or more claims of the '863 patent.

95. Under 35 U.S.C. § 271(e)(2)(A), Breckenridge's submission to FDA of the amendment to the Breckenridge ANDA to obtain approval for Defendants' Bendamustine Product with a Paragraph IV Certification related thereto before the expiration of the '863 patent constitutes an act of infringement, and if approved, the commercial manufacture, use, offer to sell, sale, or importation of Defendants' Bendamustine Product containing bendamustine hydrochloride, would infringe one or more claims of the '863 patent.

96. Defendants were aware of the '863 patent when engaging in these knowing and purposeful activities and were aware that filing the Breckenridge ANDA with Breckenridge's Paragraph IV Certification with respect to the '863 patent constituted an act of infringement of the '863 patent.

97. On information and belief, Defendants' Bendamustine Product contains the same active pharmaceutical ingredient, bendamustine hydrochloride, as that used in Cephalon's TREANDA® products and claimed in the '863 patent.

98. On information and belief, the manufacture of Defendants' Bendamustine Product is made by lyophilizing a bendamustine hydrochloride pharmaceutical composition covered by one or more claims of the '863 patent.

99. Defendants' use of a lyophilized bendamustine hydrochloride pharmaceutical composition in the manufacture of Defendants' Bendamustine Product infringes one or more claims of the '863 patent.

100. On information and belief, Defendants plan and intend to, and will, infringe the ’863 patent immediately and imminently upon approval of the Breckenridge ANDA.

101. On information and belief, Defendants, under 35 U.S.C. § 271(b), acted in concert, actively supported, participated in, encouraged, and/or induced the infringement of one or more claims of the ’863 patent.

102. On information and belief, Defendants plan and intend to, and will, actively induce infringement of the ’863 patent when the Breckenridge ANDA is approved, and plan and intend to, and will, do so immediately and imminently upon approval.

103. On information and belief, Defendants know that the lyophilized bendamustine hydrochloride pharmaceutical composition used to manufacture Defendants’ Bendamustine Product is especially made or adapted for use in infringing the ’863 patent and that the lyophilized bendamustine hydrochloride pharmaceutical composition used to manufacture Defendants’ Bendamustine Product is not suitable for substantial non-infringing uses. On information and belief, Defendants plan and intend to, and will, contribute to the infringement of the ’863 patent immediately and imminently upon approval of the Breckenridge ANDA.

104. The foregoing actions by Defendants constitute and/or would constitute infringement of the ’863 patent, active inducement of infringement of the ’863 patent and/or contribution to the infringement by others of the ’863 patent.

105. On information and belief, Defendants acted without a reasonable basis for believing that they would not be liable for infringing the ’863 patent, actively inducing infringement of the ’863 patent and/or contributing to the infringement by others of the ’863 patent.

106. Cephalon will be substantially and irreparably harmed by Defendants' infringing activities unless the Court enjoins those activities. Cephalon will have no adequate remedy at law if Defendants are not enjoined from the commercial manufacture, use, offer to sell, sale in and importation into the United States of Defendants' Bendamustine Product.

107. Defendants' activities render this case an exceptional one, and Cephalon is entitled to an award of their reasonable attorney fees under 35 U.S.C. § 285.

COUNT VI FOR DECLARATORY JUDGMENT OF INFRINGEMENT OF U.S. PATENT NO. 8,609,863 BY DEFENDANTS

108. The allegations of the preceding paragraphs 1–107 are re-alleged and incorporated herein by reference.

109. On information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Defendants' Bendamustine Product soon after FDA approval of the Breckenridge ANDA.

110. Such conduct will constitute direct infringement of one or more claims of the '863 patent under 35 U.S.C. § 271(a), inducement of infringement of the '863 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

111. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the Breckenridge ANDA.

112. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Cephalon and Defendants as to liability for the infringement of the '863 patent. Defendants' actions have created in Cephalon a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

113. On information and belief, Defendants will knowingly and willfully infringe the '863 patent.

114. Cephalon will be irreparably harmed if Defendants are not enjoined from infringing the ’863 patent.

COUNT VII FOR INFRINGEMENT OF U.S. PATENT NO. 8,791,270 BY DEFENDANTS

115. The allegations of the preceding paragraphs 1–114 are re-alleged and incorporated herein by reference.

116. The use of Defendants’ Bendamustine Product is covered by one or more claims of the ’270 patent.

117. The commercial manufacture, use, offer for sale, sale, marketing, distribution and/or importation of Defendants’ Bendamustine Product would infringe one or more claims of the ’270 patent.

118. Under 35 U.S.C. § 271(e)(2)(A), Defendants’ submission to FDA of the Breckenridge ANDA to obtain approval for Defendants’ Bendamustine Product with a Paragraph IV Certification related thereto before the expiration of the ’270 patent constitutes an act of infringement, and if approved, the commercial manufacture, use, offer to sell, sale, or importation of Defendants’ Bendamustine Product containing bendamustine hydrochloride, would infringe one or more claims of the ’270 patent.

119. Defendants were aware of the ’270 patent when engaging in these knowing and purposeful activities and were aware that filing the Breckenridge ANDA with Breckenridge’s Paragraph IV Certification with respect to the ’270 patent constituted an act of infringement of the ’270 patent.

120. On information and belief, Defendants’ Bendamustine Product contains the same active pharmaceutical ingredient, bendamustine hydrochloride, as that used in Cephalon’s TREANDA® products and claimed in the ’270 patent.

121. On information and belief, Defendants' Bendamustine Product is the pharmaceutical composition of bendamustine hydrochloride, containing less than or equal to 4.0% (area percent of bendamustine) of bendamustine degradants, recited in one or more claims of the '270 patent.

122. On information and belief, Defendants' Bendamustine Product is the pharmaceutical composition of bendamustine hydrochloride, containing not more than the amount of the HP1 degradant, recited in one or more claims of the '270 patent.

123. On information and belief, Defendants' Bendamustine Product infringes one or more claims of the '270 patent.

124. On information and belief, Defendants plan and intend to, and will, infringe the '270 patent immediately and imminently upon approval of the Breckenridge ANDA.

125. On information and belief, Defendants, under 35 U.S.C. § 271(b), acted in concert, actively supported, participated in, encouraged, and/or induced the infringement of one or more claims of the '270 patent.

126. On information and belief, Defendants plan and intend to, and will, actively induce infringement of the '270 patent when the Breckenridge ANDA is approved, and plan and intend to, and will, do so immediately and imminently upon approval.

127. On information and belief, Defendants know that Defendants' Bendamustine Product is especially made or adapted for use in infringing the '270 patent and that Defendants' Bendamustine Product is not suitable for substantial non-infringing uses. On information and belief, Defendants plan and intend to, and will, contribute to the infringement of the '270 patent immediately and imminently upon approval of the Breckenridge ANDA.

128. The foregoing actions by Defendants constitute and/or would constitute infringement of the ’270 patent, active inducement of infringement of the ’270 patent and/or contribution to the infringement by others of the ’270 patent.

129. On information and belief, Defendants acted without a reasonable basis for believing that they would not be liable for infringing the ’270 patent, actively inducing infringement of the ’270 patent and/or contributing to the infringement by others of the ’270 patent.

130. Cephalon will be substantially and irreparably harmed by Defendants’ infringing activities unless the Court enjoins those activities. Cephalon will have no adequate remedy at law if Defendants are not enjoined from the commercial manufacture, use, offer to sell, sale in and importation into the United States of Defendants’ Bendamustine Product.

131. Defendants’ activities render this case an exceptional one, and Cephalon is entitled to an award of its reasonable attorneys’ fees under 35 U.S.C. § 285.

COUNT VIII DECLARATORY JUDGMENT OF INFRINGEMENT OF U.S. PATENT NO. 8,791,270 BY DEFENDANTS

132. The allegations of the preceding paragraphs 1–131 are re-alleged and incorporated herein by reference.

133. On information and belief, Defendants plan to begin manufacturing, marketing, selling, offering to sell and/or importing Defendants’ Bendamustine Product soon after FDA approval of the Breckenridge ANDA.

134. Such conduct will constitute direct infringement of one or more claims on the ’270 patent under 35 U.S.C. § 271(a), inducement of infringement of the ’270 patent under 35 U.S.C. § 271(b), and contributory infringement under 35 U.S.C. § 271(c).

135. Defendants' infringing patent activity complained of herein is imminent and will begin following FDA approval of the Breckenridge ANDA.

136. As a result of the foregoing facts, there is a real, substantial, and continuing justiciable controversy between Cephalon and Defendants as to liability for the infringement of the '270 patent. Defendants' actions have created in Cephalon a reasonable apprehension of irreparable harm and loss resulting from Defendants' threatened imminent actions.

137. On information and belief, Defendants will knowingly and willfully infringe the '270 patent.

138. Cephalon will be irreparably harmed if Defendants are not enjoined from infringing the '270 patent.

PRAYER FOR RELIEF

WHEREFORE, Cephalon respectfully request the following relief:

a. a judgment that the '524 patent, the '190 patent, the '863 and the '270 patent are valid and enforceable;

b. a judgment that Breckenridge's submission of the Breckenridge ANDA No. 205574, including any amendments, was an act of infringement of one or more claims of the '524 patent, the '190 patent, the '863 patent and the '270 patent, and that the making, using, offering to sell, selling, marketing, distributing, or importing of Defendants' Bendamustine Products prior to the expiration of the '524 patent, the '190 patent, the '863 patent, and the '270 patent will infringe, actively induce infringement and/or contribute to the infringement of one or more claims of the'524 patent, the '190 patent, the '863 patent, and the '270 patent;

c. an Order pursuant to 35 U.S.C. § 271(e)(4)(A) providing that the effective date of any FDA approval of the Breckenridge ANDA No. 205574 or any product or compound the use

of which infringes the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent, shall be a date that is not earlier than the expiration of the’524 patent, the ’190 patent, the ’863 patent or the ’270 patent;

d. an Order pursuant to 35 U.S.C. § 271(e)(4)(B) permanently enjoining Defendants and all persons acting in concert with Defendants from commercially manufacturing, using, offering for sale, selling, marketing, distributing, or importing Defendants’ Bendamustine Products, or any product or compound the use of which infringes the ’524 patent, the ’190 patent, the ’863 patent, or the ’270 patent, or inducing or contributing to the infringement of the ’524 patent, the ’190 patent, the ’863 patent, or the ’270 patent until after the expiration of the ’524 patent, the ’190 patent, the ’863 patent, or the ’270 patent;

e. an Order pursuant to 35 U.S.C. § 283 permanently enjoining Defendants and all persons acting in concert with Defendants from commercially manufacturing, using, offering for sale, selling, marketing, distributing, or importing Defendants’ Bendamustine Products, or any product or compound the use of which infringes the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent, or inducing or contributing to the infringement of the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent until after the expiration of the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent;

f. an Order enjoining Defendants and all persons acting in concert with Defendants from seeking, obtaining, or maintaining approval of the Breckenridge ANDA No. 205447 before the expiration of the ’524 patent, the ’190 patent, the ’863 patent or ’270 patent;

g. an award of Cephalon’s damages or other monetary relief to compensate Cephalon if Breckenridge engages in the commercial manufacture, use, offer to sell, sale or marketing or distribution in, or importation into the United States of Defendants’ Bendamustine

Products, or any product or compound the use of which infringes the ’524 patent, the ’190 patent, the ’863 patent, or the ’270 patent, or the inducement or contribution of the foregoing, prior to the expiration of the ’524 patent, the ’190 patent, the ’863 patent, or the ’270 patent in accordance with 35 U.S.C. § 271(e)(4)(C);

h. an award of Cephalon’s damages or other monetary relief to compensate Cephalon if Breckenridge engages in the commercial manufacture, use, offer to sell, sale or marketing or distribution in, or importation into the United States of Defendants’ Bendamustine Products, or any product or compound the use of which infringes the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent, or the inducement or contribution of the foregoing, prior to the expiration of the ’524 patent, the ’190 patent, the ’863 patent or the ’270 patent;

i. a judgment that this is an exceptional case and awarding Cephalon its attorneys’ fees under 35 U.S.C. § 285;

j. an award of Cephalon’s reasonable costs and expenses in this action; and

k. an award of any further and additional relief to Cephalon as this Court deems just and proper.

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Dated: September 3, 2014

Exhibit A

US008445524B2

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

(10) **Patent No.: US 8,445,524 B2**
(45) **Date of Patent: May 21, 2013**

(54) **SOLID FORMS OF BENDAMUSTINE HYDROCHLORIDE**

(75) Inventors: **Laurent D. Courvoisier**, Thorndale, PA (US); **Robert E. McKean**, Chester Springs, PA (US); **Hans-Joachim Jänsch**, Radebeul (DE); **Veronique Courvoisier**, legal representative, Thorndale, PA (US)

(73) Assignee: **Cephalon, Inc.**, Frazer, PA (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.

(21) Appl. No.: **13/301,979**

(22) Filed: **Nov. 22, 2011**

(65) **Prior Publication Data**

US 2012/0071532 A1 Mar. 22, 2012

Related U.S. Application Data

(63) Continuation of application No. 12/411,929, filed on Mar. 26, 2009, now abandoned.

(60) Provisional application No. 61/039,752, filed on Mar. 26, 2008.

(51) **Int. Cl.**

A61K 31/4184	(2006.01)
C07D 235/16	(2006.01)
A61P 35/02	(2006.01)
A61P 35/00	(2006.01)

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

USPC **514/394**; 548/310.1

(58) **Field of Classification Search**

None

See application file for complete search history.

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Assistant Examiner — Dennis Heyer
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(57) **ABSTRACT**

Novel solid forms of bendamustine hydrochloride are described, as well as methods of their preparation and use.

19 Claims, 15 Drawing Sheets

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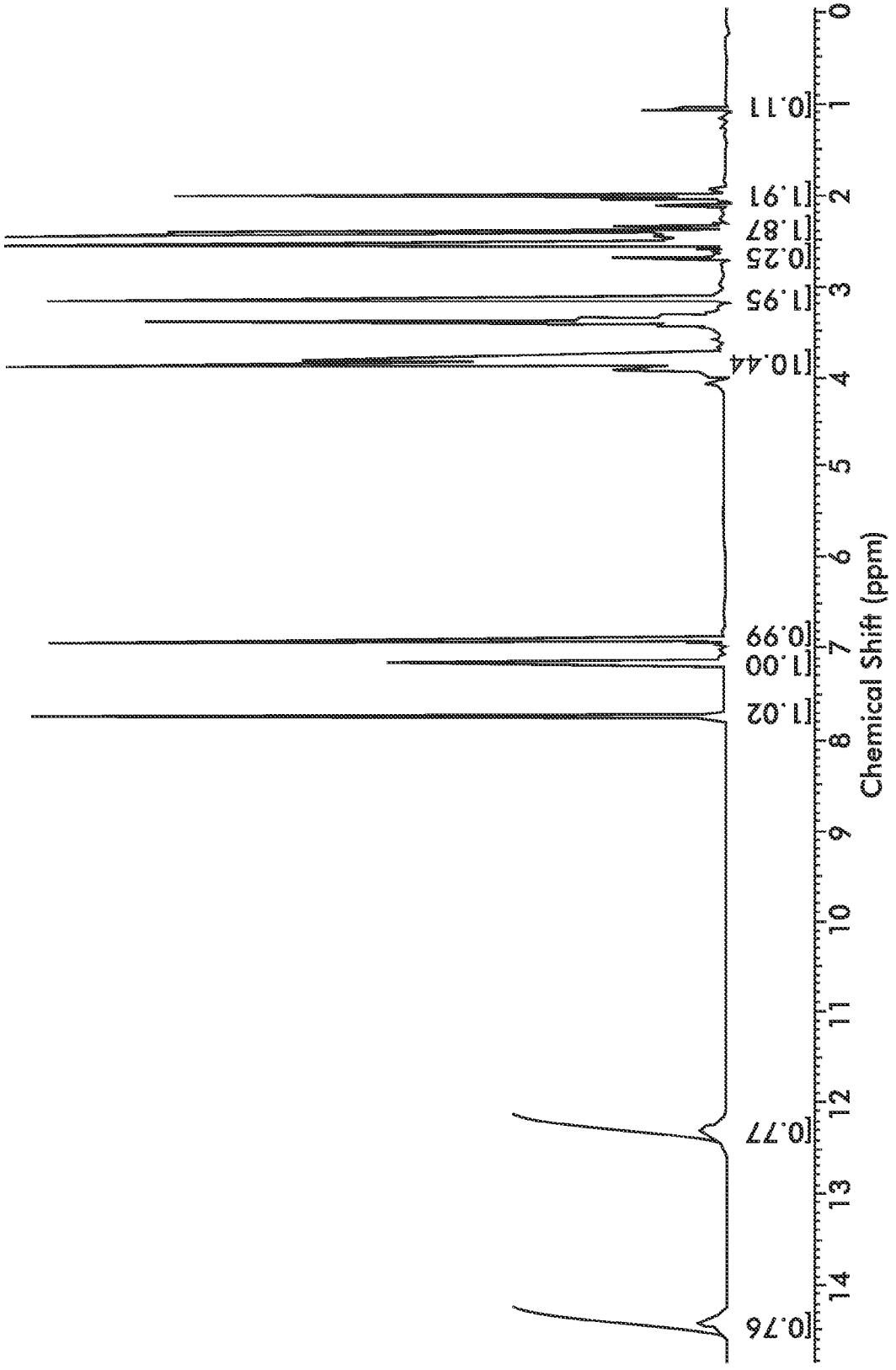
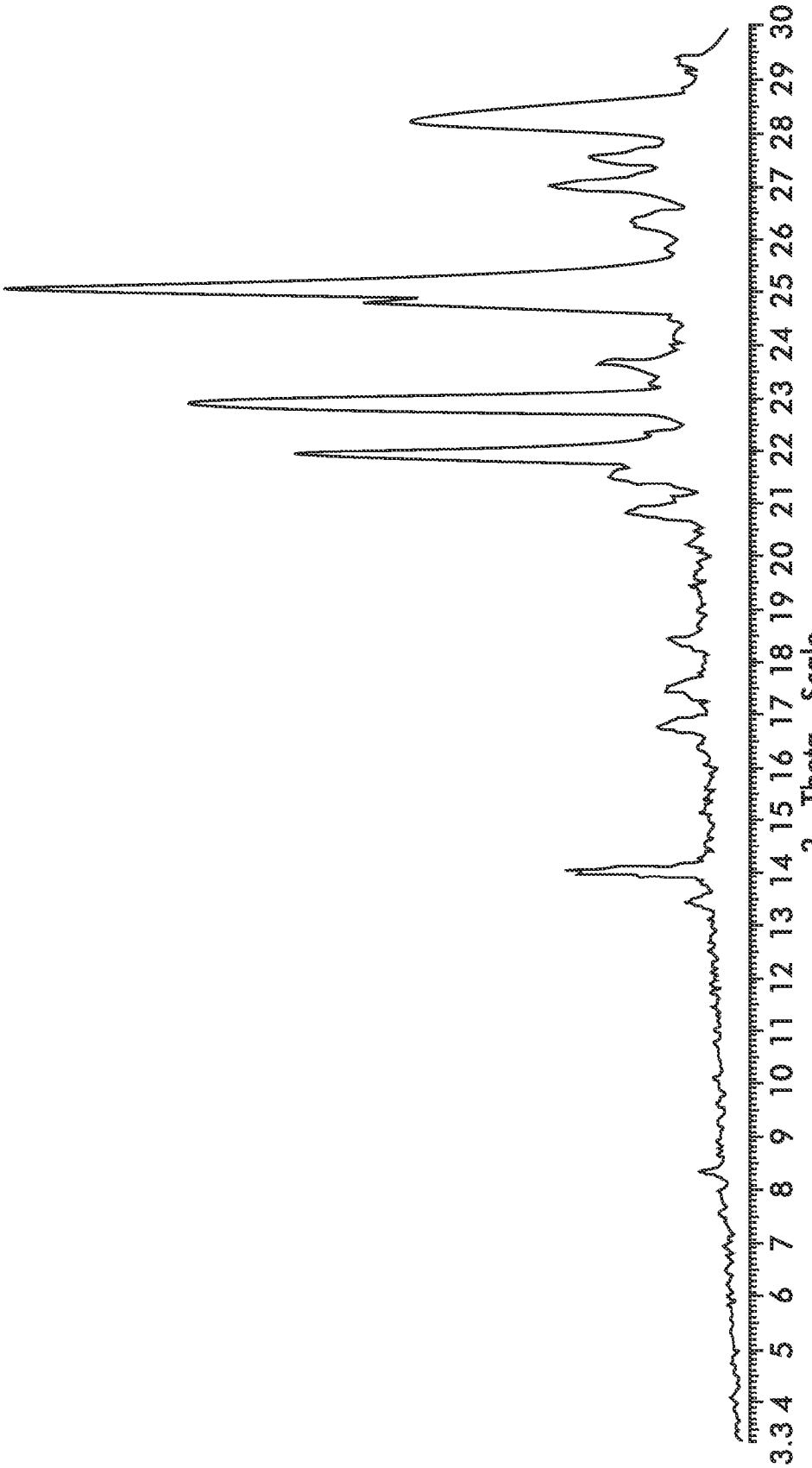

0.11
1.91
1.87
0.25
1.95
10.44
0.99
1.00
1.02
0.77
0.76
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Chemical Shift (ppm)

FIG. 1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2 - Theta - Scale

FIG. 2

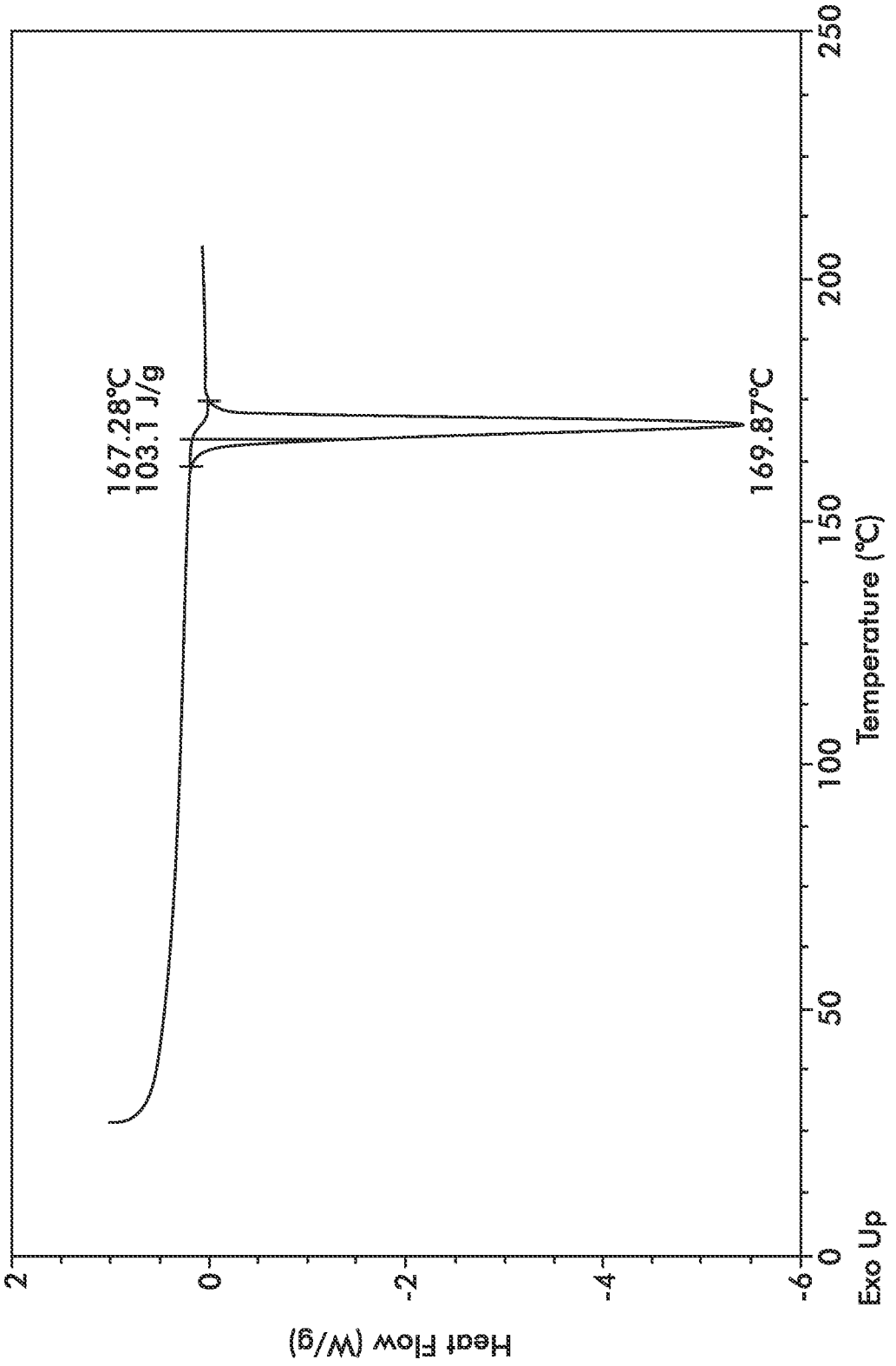

167.28°C
103.1 J/g
169.87°C
Heat Flow (W/g)
2
0
-2
-4
-6
Exo Up
0
50
100
150
200
250
Temperature (°C)

FIG. 3

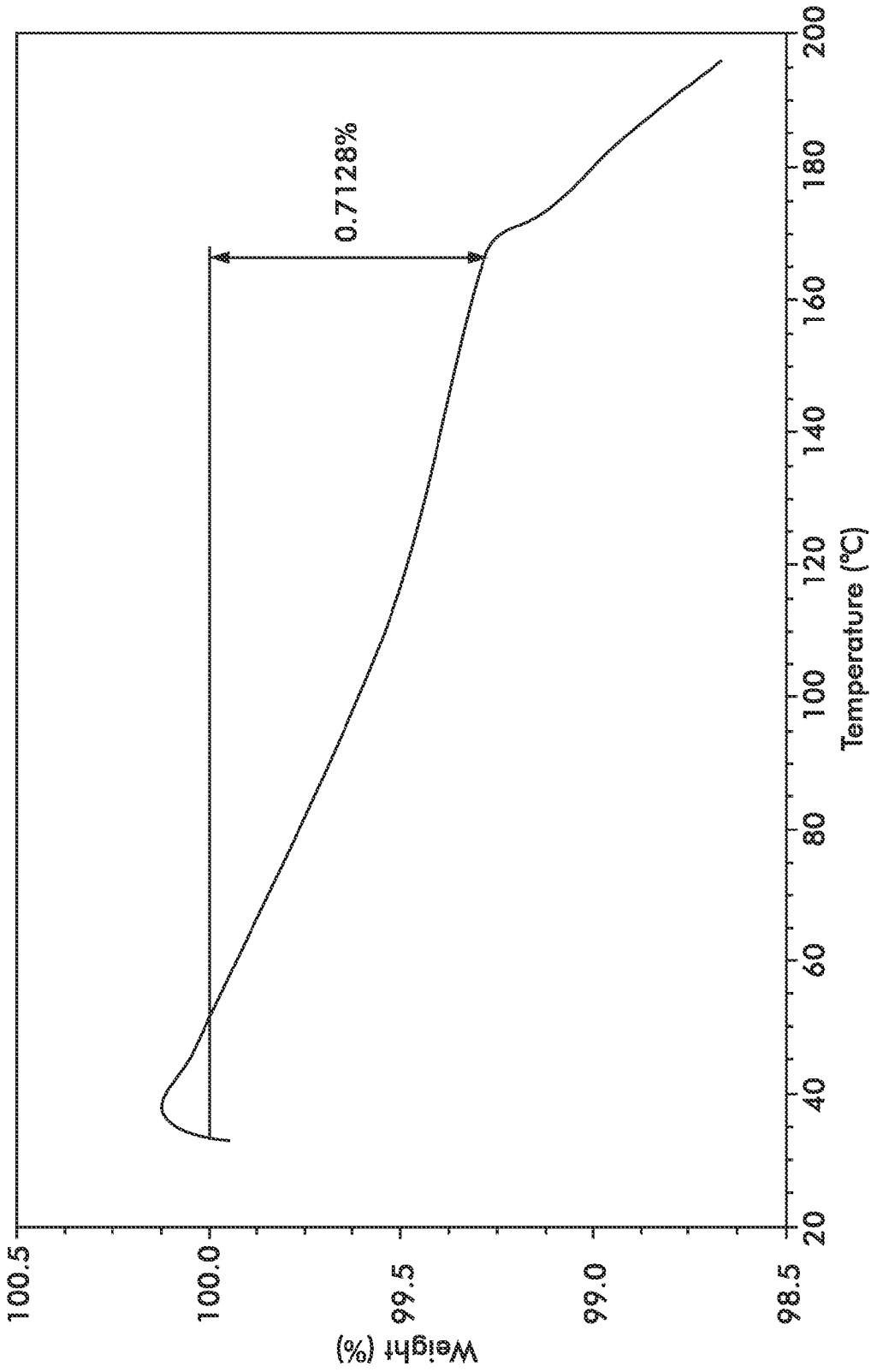

0.7128%
Weight (%)
100.5
100.0
99.5
99.0
98.5
20
40
60
80
100
120
140
160
180
200
Temperature (°C)

FIG. 4

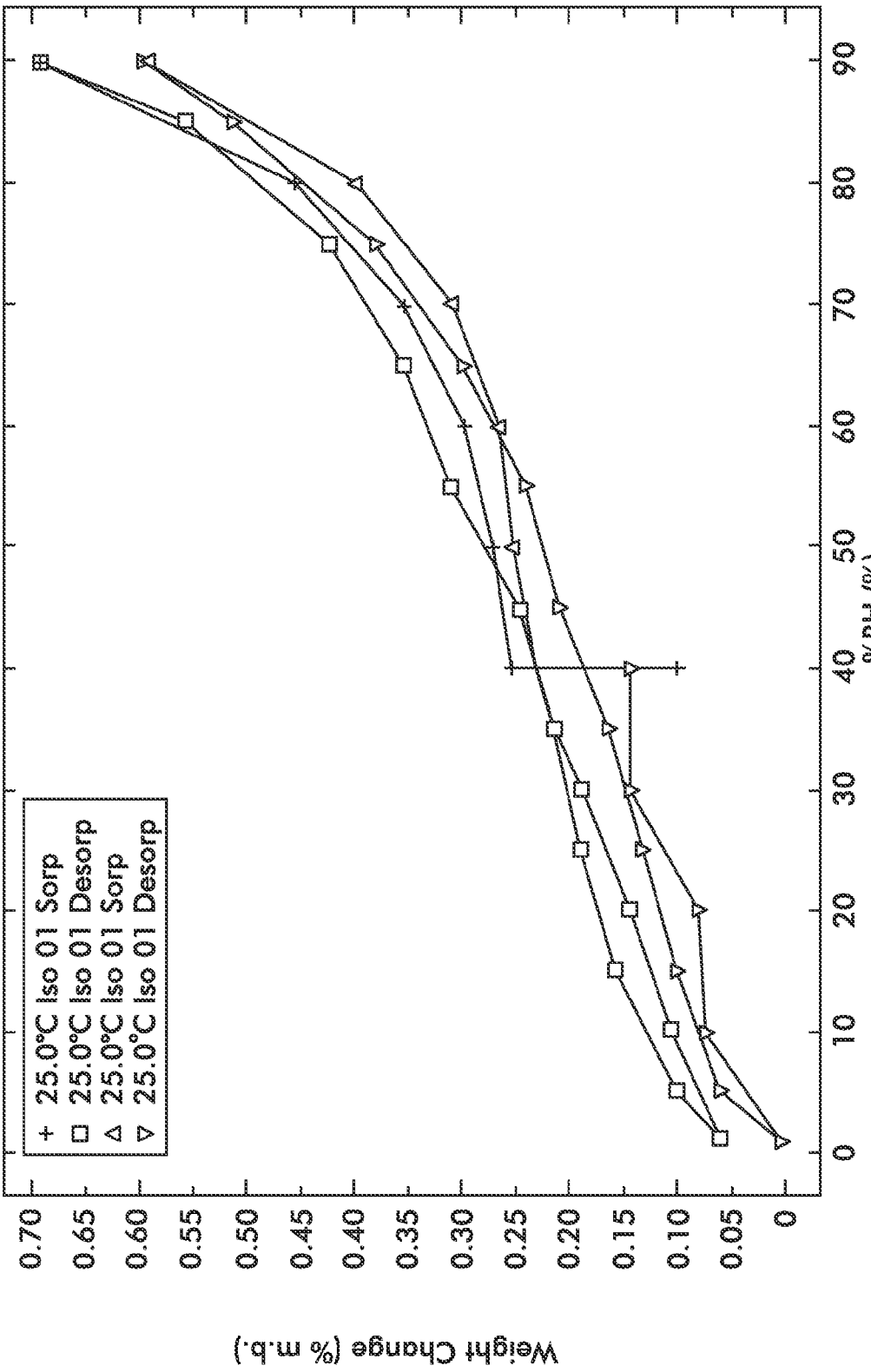

Weight Change (% m.b.)
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0
%RH (%)
0
10
20
30
40
50
60
70
80
90
+ 25.0°C Iso 01 Sorp
□ 25.0°C Iso 01 Desorp
△ 25.0°C Iso 01 Sorp
▽ 25.0°C Iso 01 Desorp

FIG.5

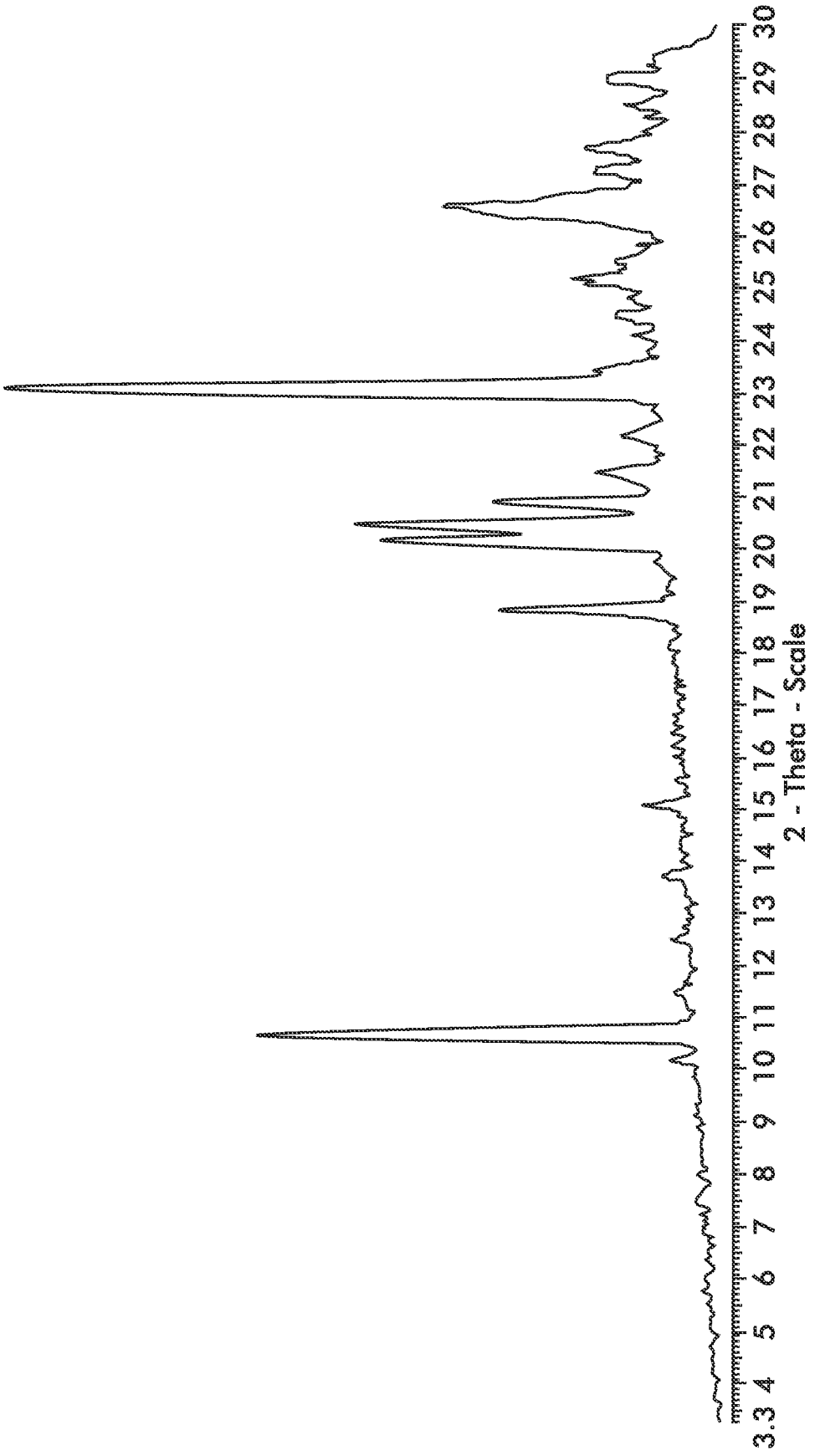

3.3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2 - Theta - Scale

FIG. 6

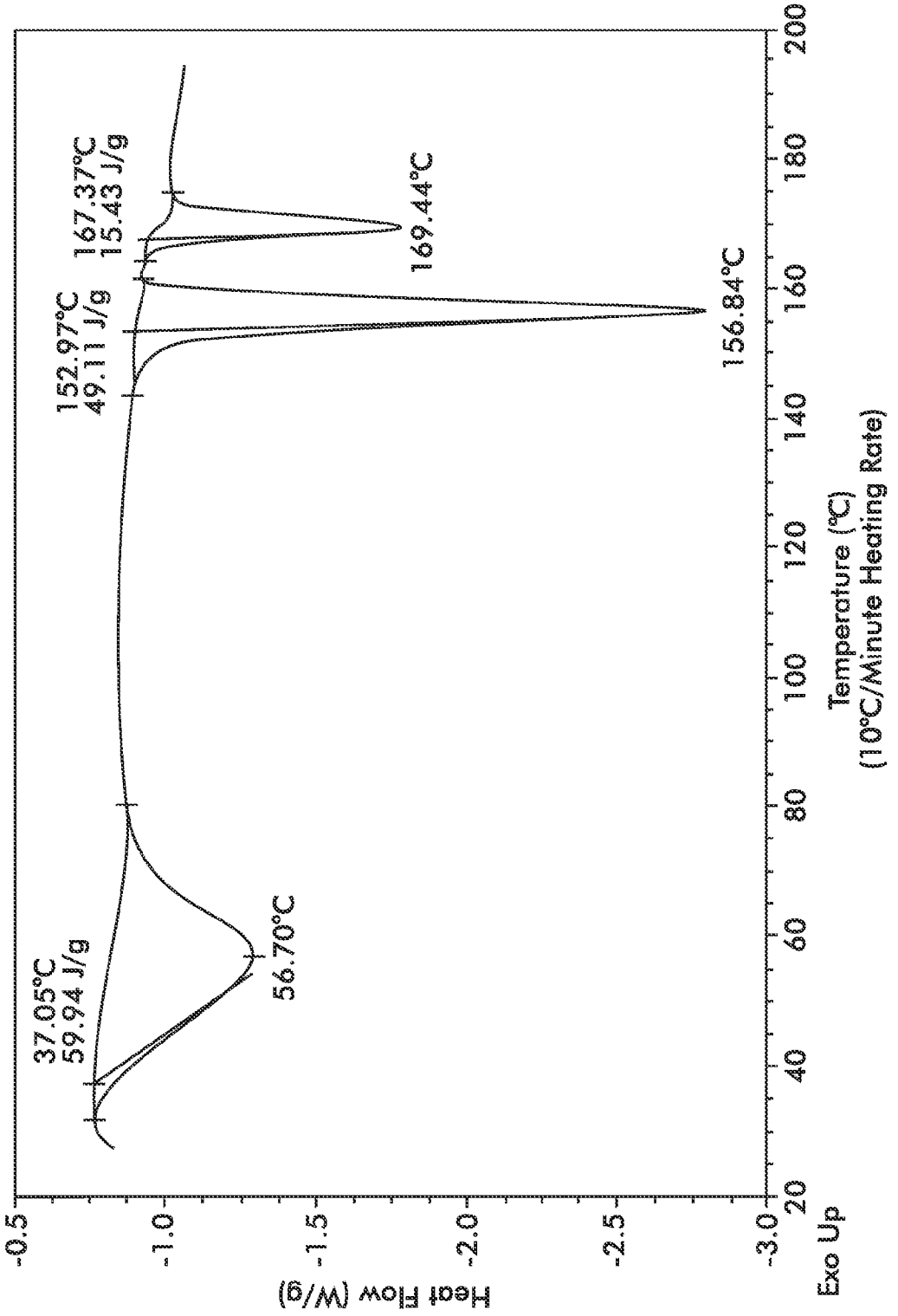

37.05°C
59.94 J/g
56.70°C
152.97°C
49.11 J/g
167.37°C
15.43 J/g
169.44°C
156.84°C
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Heat Flow (W/g)
Exo Up
20
40
60
80
100
120
140
160
180
200
Temperature (°C)
(10°C/Minute Heating Rate)

FIG. 7A

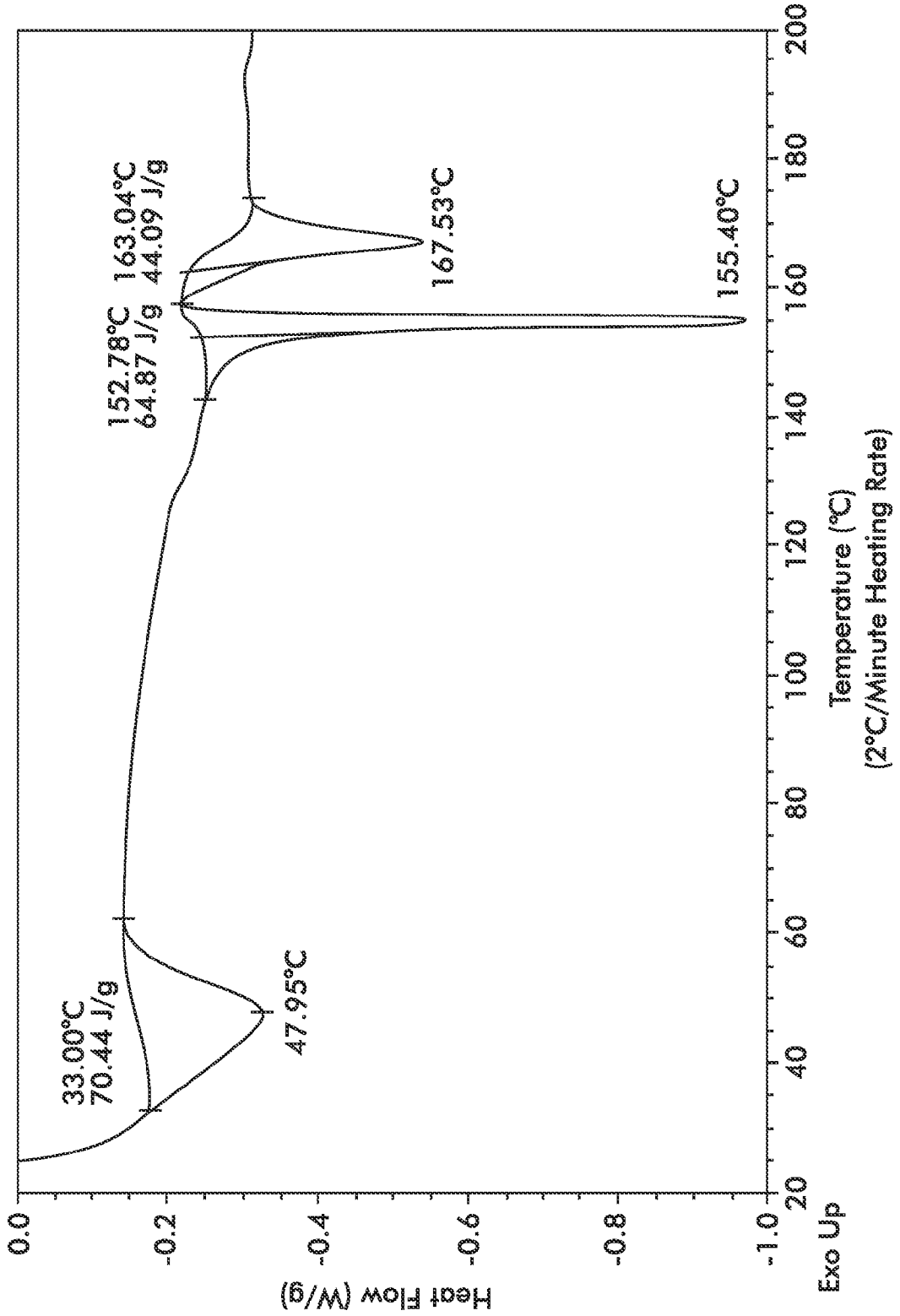

33.00°C
70.44 J/g
47.95°C
152.78°C
64.87 J/g
163.04°C
44.09 J/g
167.53°C
155.40°C
Heat Flow (W/g)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Exo Up
20
40
60
80
100
120
140
160
180
200
Temperature (°C)
(2°C/Minute Heating Rate)

FIG. 7B

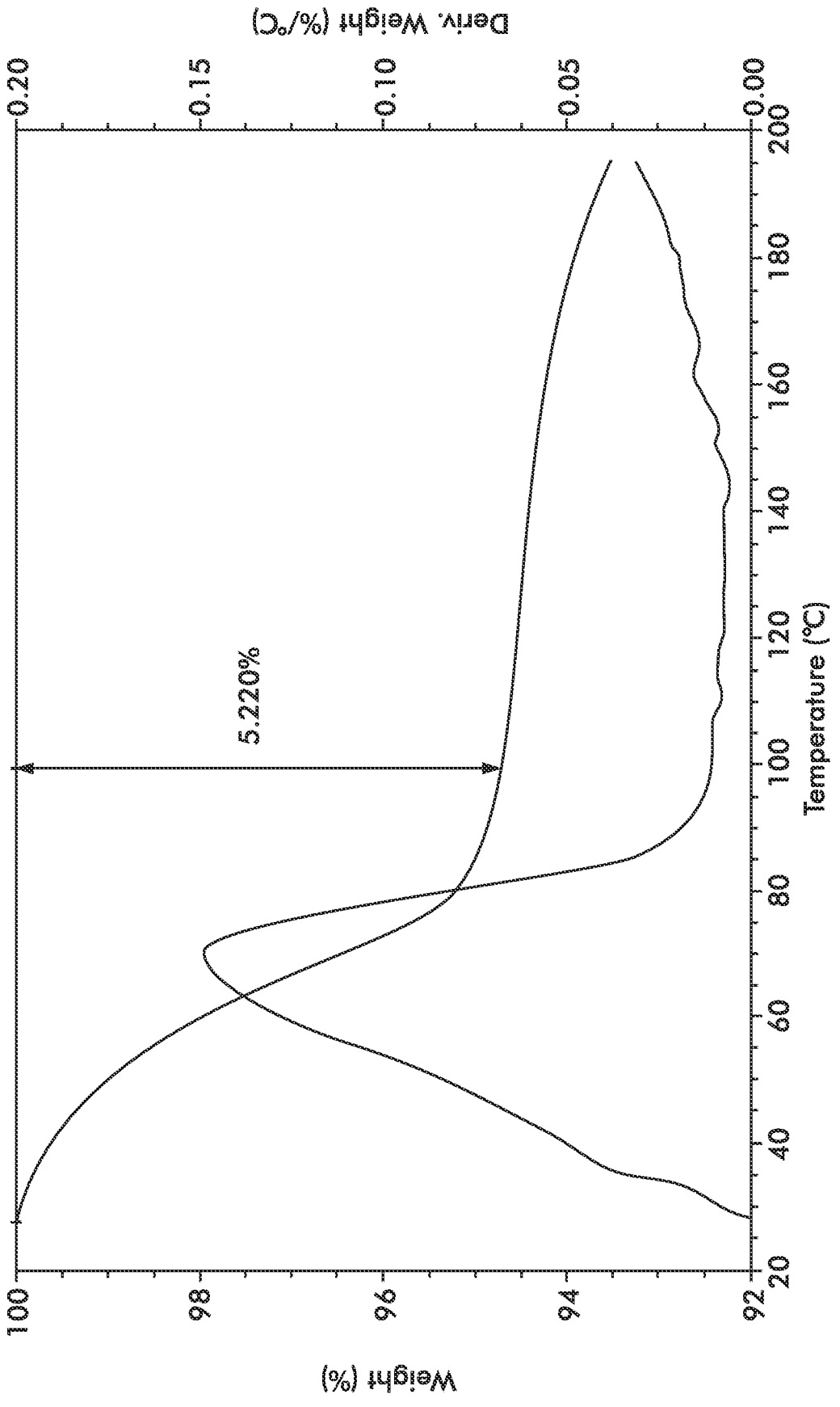

Deriv. Weight (%/°C)
0.20
0.15
0.10
0.05
0.00
200
180
160
140
120
100
80
60
40
20
Temperature (°C)
5.220%
100
98
96
94
92
Weight (%)

FIG. 8

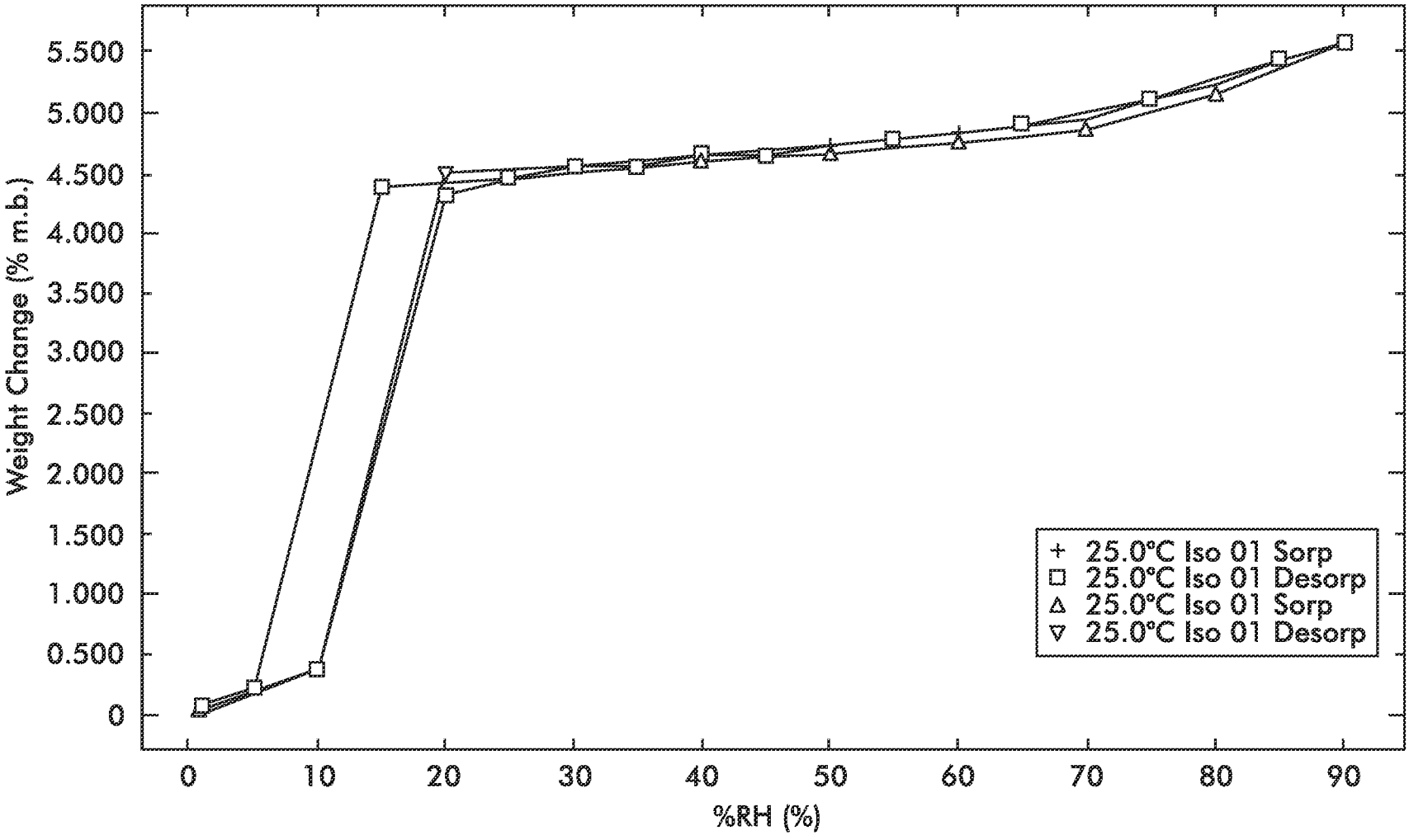

Weight Change (% m.b.)
5.500
5.000
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0
0
10
20
30
40
50
60
70
80
90
%RH (%)
25.0°C Iso 01 Sorp
25.0°C Iso 01 Desorp
25.0°C Iso 01 Sorp
25.0°C Iso 01 Desorp

FIG.9

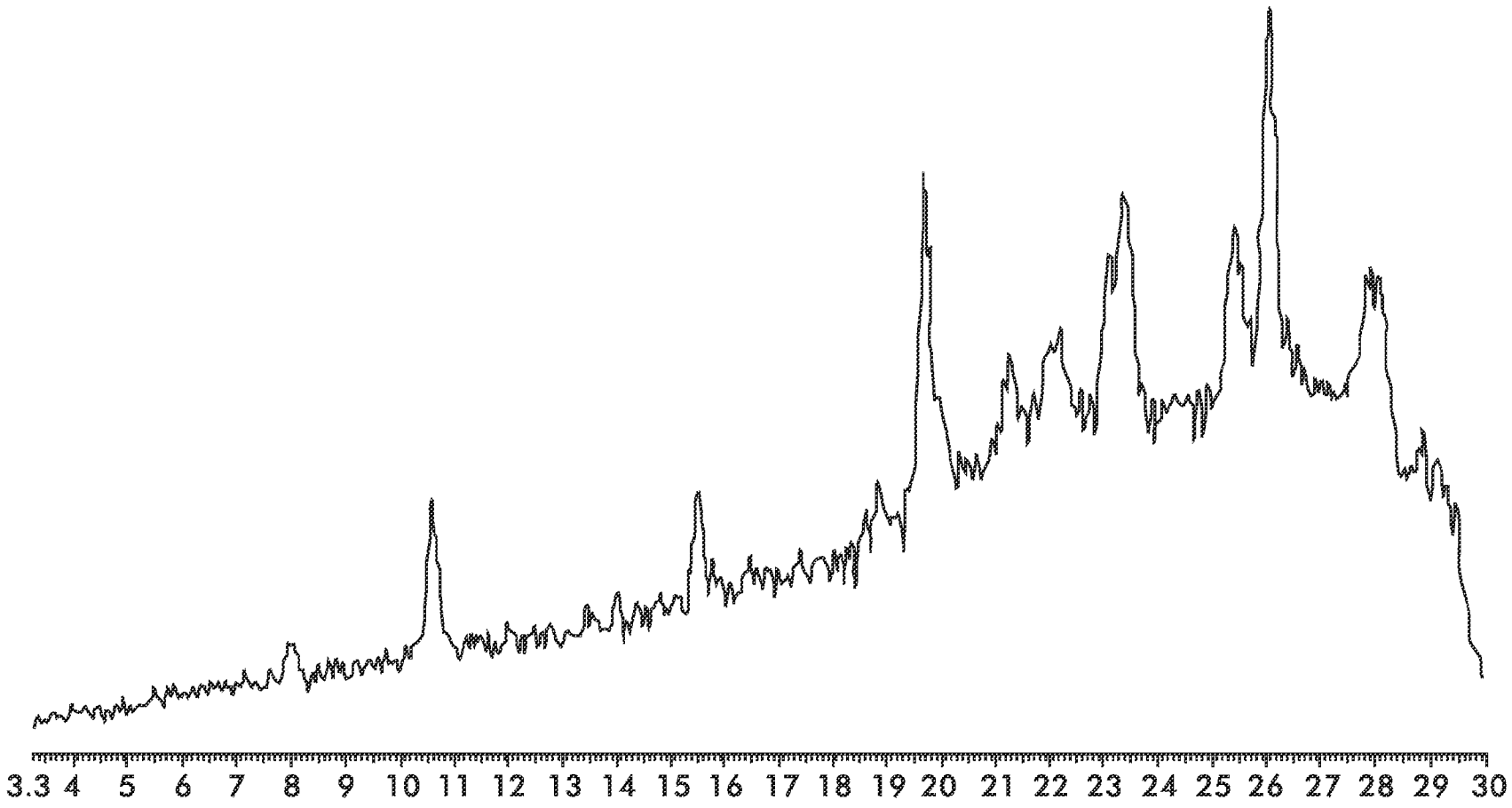

3.3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2 - Theta - Scale
Bendamustine HCl Form 3 - Addition of DCM as Anti-Solvent to a Solution in Methanol

FIG.10

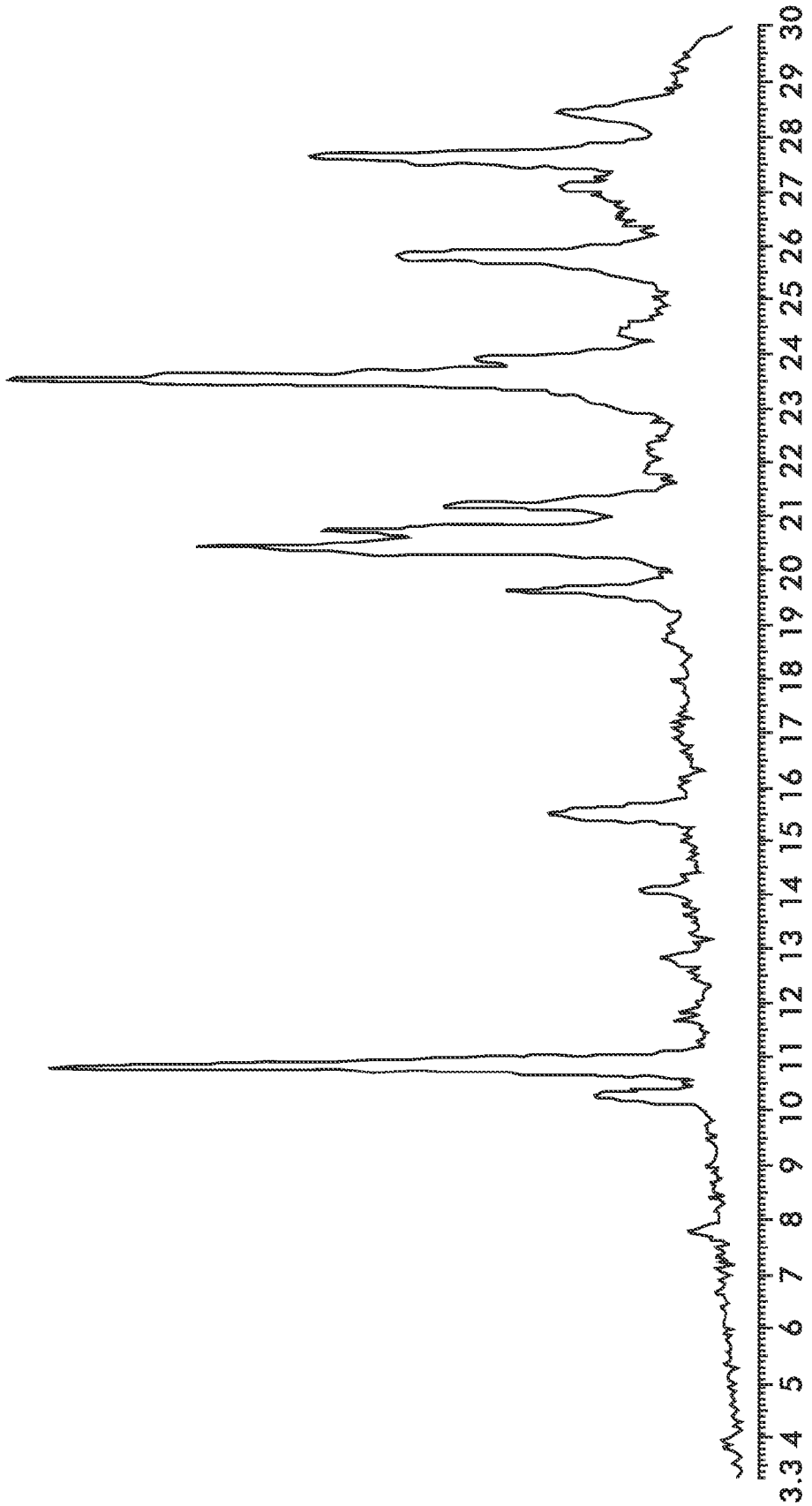

3.3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2 - Theta - Scale

FIG. 11

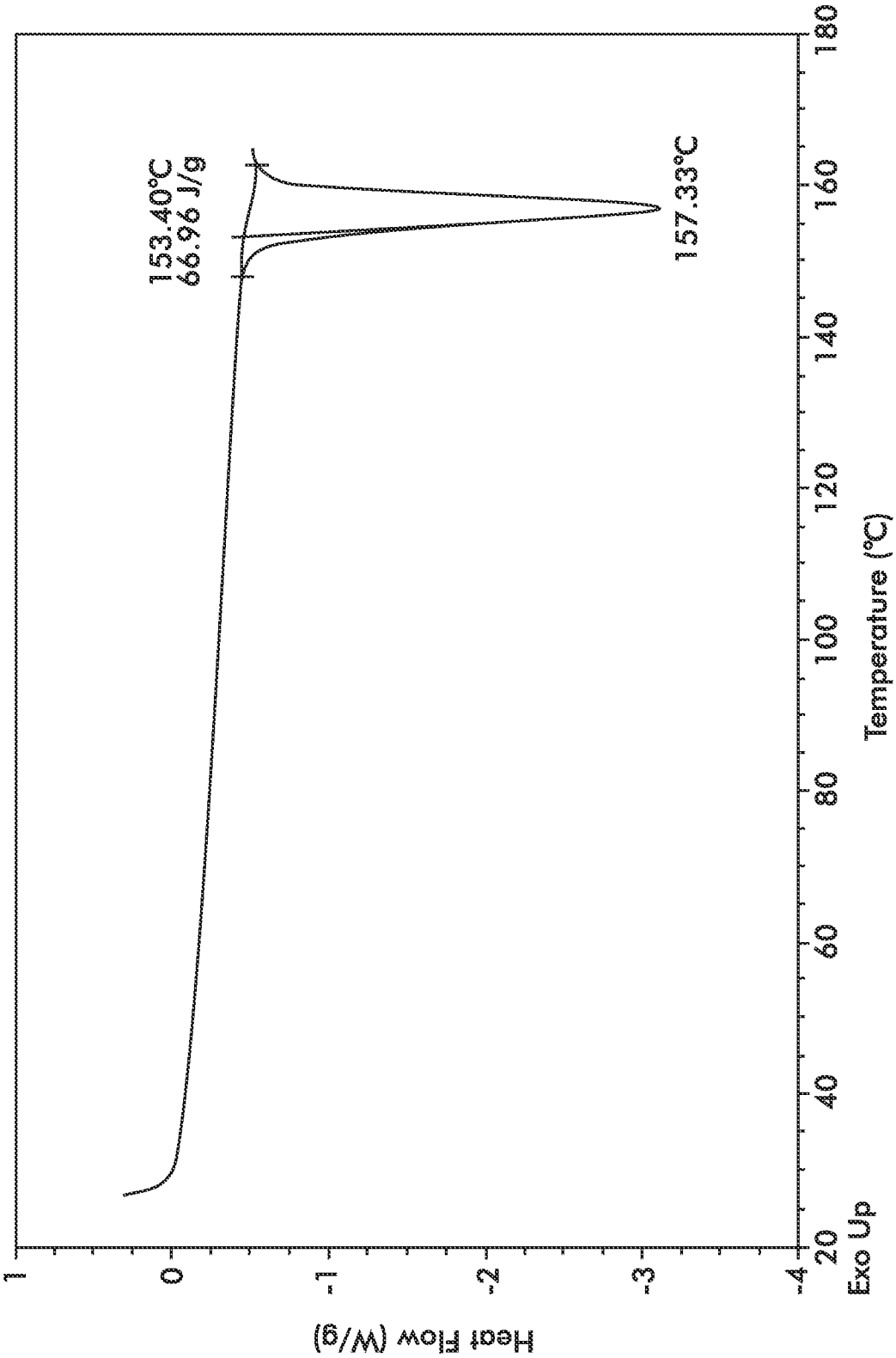

153.40°C
66.96 J/g
157.33°C
Heat Flow (W/g)
Exo Up
Temperature (°C)
1
0
-1
-2
-3
-4
20
40
60
80
100
120
140
160
180

FIG. 12

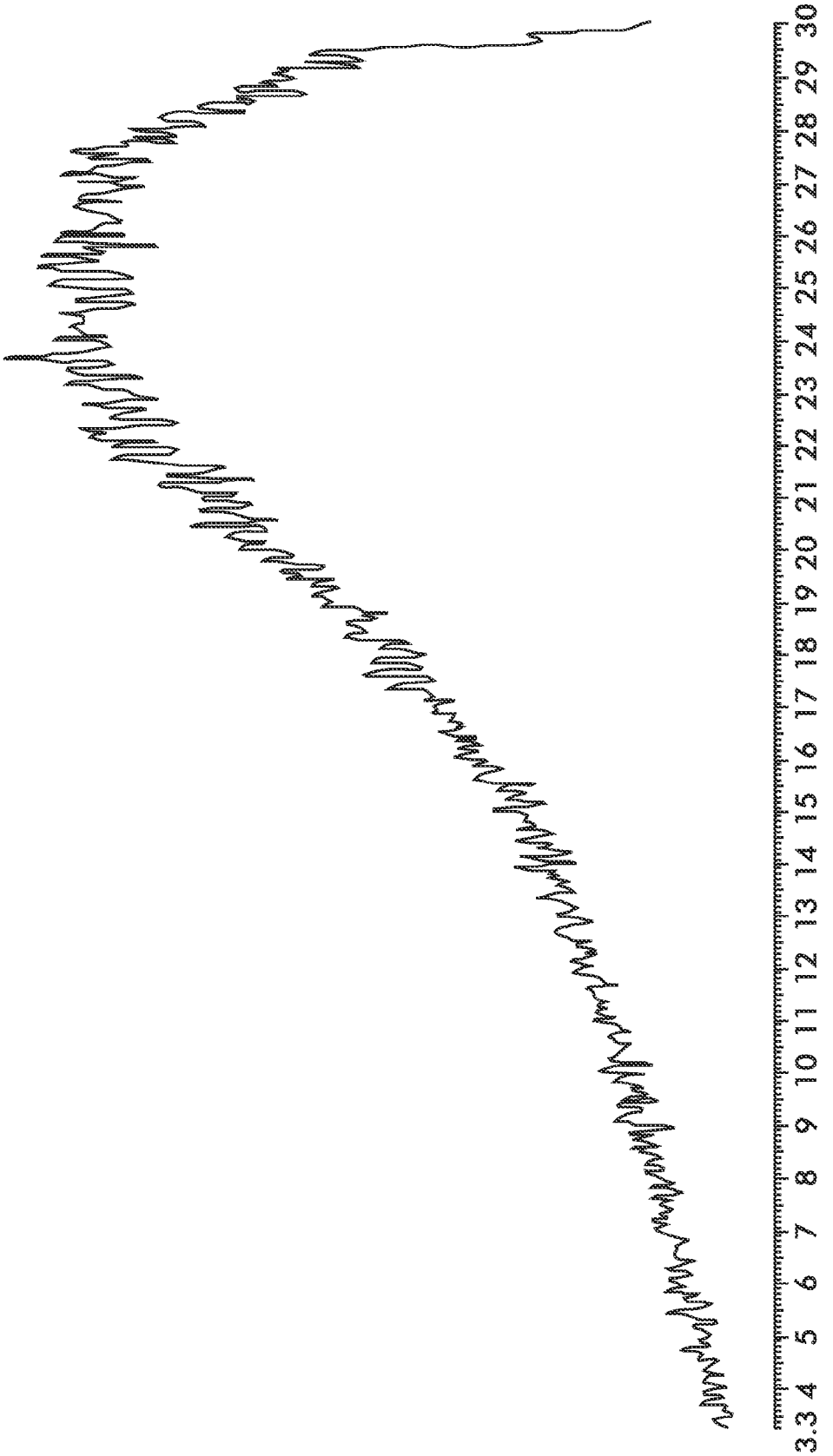

3.3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
2 - Theta - Scale

FIG. 13

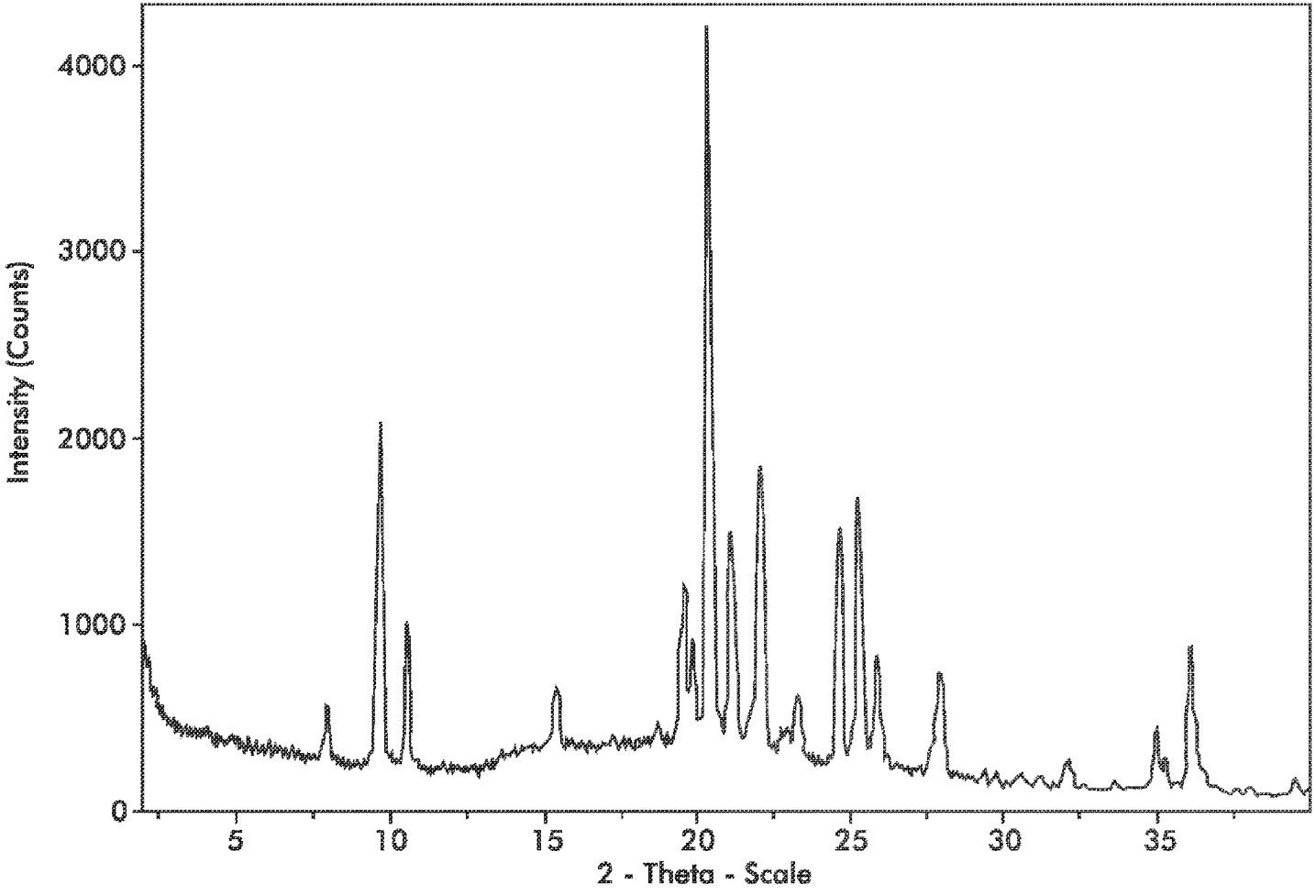

Intensity (Counts)
4000
3000
2000
1000
0
5
10
15
20
25
30
35
2 - Theta - Scale

FIG. 14

SOLID FORMS OF BENDAMUSTINE HYDROCHLORIDE

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. application Ser. No. 12/411,929, filed Mar. 26, 2009, which claims the benefit of U.S. Provisional Application No. 61/039,752, filed Mar. 26, 2008. The disclosures of these prior applications are incorporated herein by reference in their entireties for all purposes.

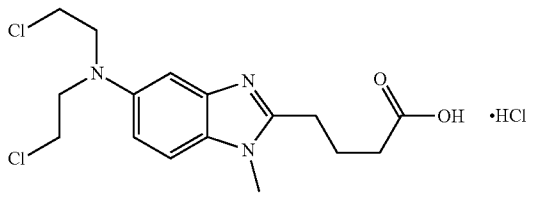
FIELD OF THE INVENTION

This invention pertains to bendamustine-containing compositions, pharmaceutical compositions comprising bendamustine, processes to reproducibly make them, and methods of treating patients using them.

BACKGROUND OF THE INVENTION

Active pharmaceutical ingredients (APIs) can be prepared in a variety of different forms, for example, chemical derivatives, solvates, hydrates, co-crystals, or salts. APIs may also be prepared in different solid forms, in that they may be amorphous, may exist as different crystalline polymorphs, and/or in different solvation or hydration states. By varying the form of an API, it is possible to vary the physical properties thereof. For instance, solid forms of an API typically have different solubilities such that a more thermodynamically stable solid form is less soluble than a less thermodynamically stable solid form. Solid forms can also differ in properties such as shelf-life, bioavailability, morphology, vapor pressure, density, color, and compressibility. Accordingly, variation of the solid state of an API is one of many ways in which to modulate the physical and pharmacological properties thereof.

Bendamustine, 4-{5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl}butyric acid:



Bendamustine Hydrochloride

was initially synthesized in 1963 in the German Democratic Republic (GDR) and was available from 1971 to 1992 there under the tradename Cytostasan®. See, e.g., W. Ozegowski and D. Krebs, IMET 3393 γ-[1-methyl-5-bis-(β-chloroethyl)-aminobenzimidazolo-(2)]-butyryl chloride, a new cytostatic agent of the group of benzimidazole nitrogen mustards. Zbl. Pharm. 110, (1971) Heft 10, 1013-1019, describing the synthesis of bendamustine hydrochloride monohydrate. Since that time, it has been marketed in Germany under the tradename Ribomustin®. Bendamustine is an alkylating agent that has been shown to have therapeutic utility in treating diseases such as chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer.

While bendamustine has been demonstrated as efficacious, it is known to be unstable, especially in aqueous solutions, leading to technical difficulties in its preparation and administration. Researchers, therefore, have investigated methods of improving the preparation and stability of bendamustine and its formulations. For example, German (GDR) Patent No. 159877 discloses a method for preparing bendamustine free base by reaction of the bis-hydroxyl precursor with thionyl chloride followed by recrystallization from water.

German (GDR) Patent No. 34727 discloses a method of preparing derivatives of bendamustine. The described derivatives differ from bendamustine in the substitution at the 1-position.

German (GDR) Patent No. 80967 discloses an injectable preparation of bendamustine hydrochloride monohydrate, ascorbic acid, and water. GDR 80967 describes that lyophilization of compounds such as bendamustine is only possible if the compound is of sufficient stability that it can withstand the processing conditions. The preparation described in GDR 80967 is not lyophilized.

German (GDR) Patent No. 159289 discloses a ready-to use, injectable solution of bendamustine hydrochloride that avoids lyophilization. GDR 159289 describes an anhydrous solution of bendamustine hydrochloride in 1,2-propylene glycol or ethanol.

U.S. application Ser. No. 11/330,868, filed Jan. 12, 2006, assigned to Cephalon, Inc., Frazer, Pa., discloses methods of preparing lyophilized pharmaceutical compositions comprising bendamustine hydrochloride.

In light of the potential benefits of different solid forms of APIs and in light of the efficacy of bendamustine, a need exists to identify and prepare novel solid forms of bendamustine hydrochloride.

SUMMARY OF THE INVENTION

Solid forms of bendamustine hydrochloride are described, as well as methods of their preparation. For example, in some embodiments, the invention is directed to a solid form of bendamustine hydrochloride that comprises at least one of bendamustine hydrochloride Form 1, bendamustine hydrochloride Form 3, bendamustine hydrochloride Form 4, amorphous bendamustine hydrochloride, or a mixture thereof. This solid form of bendamustine hydrochloride may be one that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 25.1, 24.9, 22.9, 22.0, and/or 14.1±0.2 degrees 2θ, or that produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.8, 17.5, 18.5, 24.9, and/or 28.3±0.2 degrees 2θ. Alternatively, the solid form of bendamustine hydrochloride may produce an X-ray powder diffraction pattern comprising one or more of the following reflections: 26.1, 27.9, and/or 28.1±0.2 degrees 2θ, or that further produces an X-ray powder diffraction pattern further comprising one or more of the following reflections: 10.6, 15.6, and/or 19.8±0.2 degrees 2θ. Other embodiments may produce an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.8, 15.5, 20.5, and/or 23.6±0.2 degrees 2θ, or that produce an X-ray powder diffraction pattern further comprising one or more of the following reflections: 10.3, 19.6, 20.7, 21.2, 25.8 and/or 27.6±0.2 degrees 2θ.

Another embodiment of the invention is directed to compositions comprising a solid form of bendamustine hydrochloride, such as described above. In certain embodiments, the composition is a pharmaceutical composition that further comprises at least one pharmaceutically acceptable excipient.

In other embodiments, the composition is a lyophilized composition. In certain embodiments the composition comprises a single solid form of bendamustine hydrochloride and is substantially free of other solid forms. Alternatively, the composition may contain a mixture of solid forms, such as a mixture of a crystalline form of bendamustine hydrochloride and amorphous bendamustine. Thus, the composition may, for example, be a lyophilized composition that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 7.98, 10.58, 15.43, 19.64, and/or 19.89±0.2 degrees 2θ.

Methods of preparing the compositions, and methods of using same for use in treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer are also described.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a ^{1}H NMR spectrum of bendamustine hydrochloride

FIG. **2** is an X-ray Powder Diffractogram (XRPD) of bendamustine hydrochloride Form 1

FIG. **3** is a Differential Scanning calorimetry (DSC) Thermogram of bendamustine hydrochloride Form 1

FIG. **4** is a Thermo-Gravimetric Analysis (TGA) Thermogram of bendamustine hydrochloride Form 1

FIG. **5** is a Gravimetric Vapor Sorption (GVS) trace of bendamustine hydrochloride Form 1

FIG. **6** is an X-ray Powder Diffractogram of bendamustine hydrochloride Form 2

FIG. **7**A is a DSC Thermogram of bendamustine hydrochloride Form 2

FIG. **7**B is a DSC Thermogram of bendamustine hydrochloride Form 2 using a 2° C. per minute heating rate.

FIG. **8** is a TGA Thermogram of bendamustine hydrochloride Form 2

FIG. **9** is a GVS trace of bendamustine hydrochloride Form 2

FIG. **10** is an X-ray Powder Diffractogram of bendamustine hydrochloride Form 3

FIG. **11** is an X-ray Powder Diffractogram of bendamustine hydrochloride Form 4

FIG. **12** is a DSC Thermogram of bendamustine hydrochloride Form 4

FIG. **13** is an X-ray Powder Diffractogram of amorphous bendamustine hydrochloride

FIG. **14** is an X-ray Powder Diffractogram of one embodiment of the present invention comprising amorphous bendamustine hydrochloride, bendamustine hydrochloride Form 3, and mannitol (Lot#426804).

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Four polymorphs of crystalline bendamustine hydrochloride are disclosed herein (referred to herein as Form 1, Form 2, Form 3, and Form 4). Also described is amorphous (i.e., non-crystalline) bendamustine hydrochloride. Spectral data relating to these solid forms of bendamustine hydrochloride is depicted in FIGS. **1-14**, and methods of preparing each of these forms is presented

In preferred embodiments are solid forms of bendamustine hydrochloride that comprise Form 1, Form 2, Form 3, Form 4, or mixtures thereof. More preferred embodiments are solid forms of bendamustine hydrochloride that are Form 1, Form 3, Form 4, amorphous bendamustine hydrochloride, or mixtures thereof. In other embodiments, solid forms of the invention may further comprise bendamustine hydrochloride Form 2. These polymorphic solid forms may be identified, for example, by X-ray powder diffraction and characterized by one, two, three, four, five, or more reflection peaks that are characteristic of each polymorphic form. The four crystalline polymorphs (Form 1, Form 2, Form 3, Form 4) and amorphous bendamustine ydrochloride can also be identified by reference to their DSC thermograms, TGA thermograms, and/or GVS traces, which are set forth in FIGS. **1-14**. Methods of making solid forms of bendamustine, including each of the described polymorphs, or a mixture of polymorphs, and amorphous bendamustine hydrochloride can be preformed using the techniques described herein.

Any of the solid forms of bendamustine hydrochloride described herein can be a component of a composition comprising bendmustine hydrochloride. In some embodiments, these compositions comprising at least one of the solid forms of bendamustine hydrochloride described herein are substantially free of other solid forms of bendamustine hydrochloride.

Certain of the preferred embodiments of the invention may be characterized, at least in part, by X-ray Powder Diffraction. As is known in the art, crystalline solids produce a distinctive diffraction pattern of peaks, represented in what is referred to as a diffractogram. The peak assignments for a given crystalline material, for example, degree 2θ values, may vary slightly, depending on the instrumentation used to obtain the diffractogram and certain other factors, for example, sample preparation. Nevertheless, these variations should not be more than +/−0.2 degrees 2θ and the relative spacing between the peaks in the diffractogram will always be the same, regardless of the instrumentation used or the method of sample preparation, and the like.

In preferred embodiments, compositions of the invention are pharmaceutical compositions that further comprise at least one pharmaceutically acceptable excipient. Preferred excipients include, for example, sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof. A more preferred pharmaceutical excipient is mannitol.

In another embodiment of the invention are pharmaceutical compositions comprising Form 1, Form 2, Form 3, Form 4, or mixtures thereof, of bendamustine hydrochloride. In more preferred embodiments are compositions, preferably pharmaceutical compositions, that comprise Form 1, Form 3, Form 4, amorphous, or mixtures thereof, of bendamustine hydrochloride. In other embodiments, the pharmaceutical compositions further comprise Form 2 or bendamustine hydrochloride. More preferred embodiments of the invention are pharmaceutical compositions comprising one or more of Form 1, Form 2, Form 3, and Form 4 with amorphous bendamustine hydrochloride.

In another embodiment of the invention are lyophilized compositions comprising at least one solid form of bendamustine hydrochloride as described herein. Preferred lyophilized compositions of the invention include those that comprise a mixture of amorphous bendamustine hydrochloride and at least one crystalline form of bendamustine hydrochloride. More preferred lyophilized compositions of the invention include those that comprise a mixture of amorphous bendamustine hydrochloride and bendamustine hydrochloride Form 4.

Lyophilized compositions of the invention can further include at least one pharmaceutically acceptable excipient. Preferred excipients include, for example, sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, gly-

cine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof. A more preferred pharmaceutical excipient is mannitol. A preferred lyophilized composition of the invention comprises a mixture of amorphous bendamustine hydrochloride, bendamustine hydrochloride Form 4, and at least one pharmaceutically acceptable excipient that is preferably mannitol. More preferred are lyophilized compositions consisting essentially of amorphous bendamustine hydrochloride, bendamustine hydrochloride Form 3, and mannitol. (See, e.g., FIG. **14**)

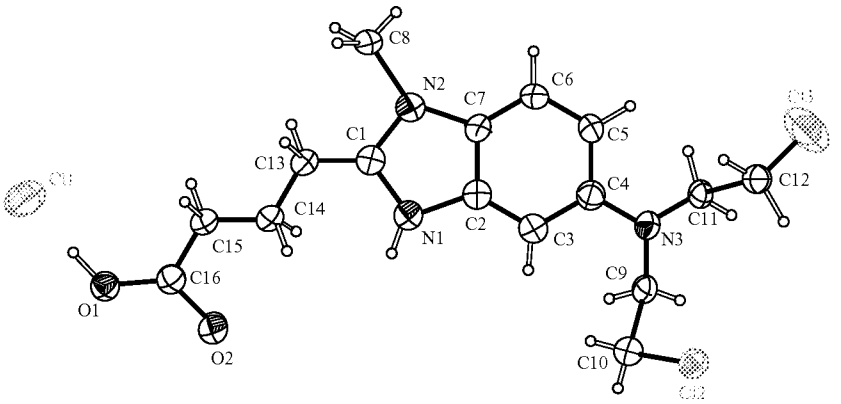
Form 1 was characterized as a white powder consisting of lath shaped particles. Form 1 was crystalline by X-ray Powder Diffraction (XRPD), the ^{1}H NMR spectrum was consistent with the structure of the molecule, and the purity was 97.2%. Thermal analysis showed an endotherm with onset 167° C. (ΔH 103 J/g) corresponding to a melting event. (Peak=170° C.). Degradation occurred above this temperature. The sample became amorphous by XRPD (FIG. **13**) on heating to 180° C. (melt) and remained amorphous on cooling to ambient temperature. Form 1 was found to have low hygroscopicity, showing a 0.7% weight increase between 0 and 90% relative humidity (RH). This did not lead to a significant change in XRPD pattern upon reanalysis under ambient conditions. There were no significant changes during 1 week of storage at 40° C./75% RH or 3 weeks of storage at 40° C./11% RH. The data from the XRPD is shown below.

XRPD Data for Bendamustine HCl Form 1

Angle (2-Theta)	d value (Angstrom)	Intensity (Counts)	Intensity (%)
8.349	10.59033	110	6.8
13.503	6.55757	129	8
14.049	6.30377	394	24.5
16.824	5.26978	190	11.8
17.51	5.06473	172	10.7
18.452	4.80825	167	10.4
20.239	4.38767	130	8.1
20.904	4.24957	257	16
21.544	4.12484	295	18.3
21.972	4.04537	980	60.9
22.354	3.97705	210	13.1
22.922	3.87977	1213	75.4
23.305	3.81696	215	13.4
23.672	3.7586	317	19.7
24.851	3.58278	833	51.8
25.122	3.54475	1608	100
25.858	3.44558	173	10.7
26.35	3.38229	254	15.8
27.082	3.29256	437	27.2
27.591	3.23295	343	21.3
28.327	3.15055	704	43.8
29.155	3.06303	144	8.9
29.356	3.04246	151	9.4

Form 1 converted to a hydrate of bendamustine hydrochloride (Form 2) during 2 months of storage at 25° C./94% RH. The aqueous solubility was 4.5 mg/ml with a solution pH of 2.16, but significant degradation occurred to the sample in this experiment. The pKa values found for this material by UV in aqueous conditions were 0.88 (Base), 4.17 (Acid) and 6.94 (Base). The Log P value found was 1.10 with a Log D at pH7.4 of 0.68. The single crystal structure of this form was obtained:

A View of the Single Crystal Structure of Form 1 of Bendamustine HCl



Unit Cell Data and Final Residuals for Bendamustine Hydrochloride Form 1

Crystal Data	Form 1	
Chemical Formula	$C_{16}H_{22}Cl_2N_3O_2$	
Molecular weight	394.7	
Crystal system	monoclinic	
Space group	C2/c	
	−193° C.	22° C.
a (Å)	23.0847(4)	23.080(5)
b (Å)	6.80560(10)	6.882(2)
c (Å)	25.5054(5)	25.504(6)
beta (°)	114.2480(10)	114.09(1)
volume (Å^3)	3653.52(11)	3693.8(4)
Z	8	
Density (calculated) (g/ml)	1.435	1.419
R(Fobs)	0.0382	
wR(all, Fsq)	0.1392	
S	1.006	

Form 1 was shown to be more stable to degradation in light, as compared to Form 2.

Form 2, a monohydrate, was characterized as a white powder consisting of rod shaped particles. Form 2 was crystalline by XRPD and the purity was 98.3%. The XRPD data is depicted below.

XRPD Data for Bendamustine HCl Form 2

Angle (2-Theta)	d value (Angstrom)	Intensity (Counts)	Intensity (%)
10.169	8.69836	167	8.5
10.638	8.31653	1274	64.6
11.443	7.73271	155	7.9
12.46	7.10378	162	8.2
13.662	6.48137	186	9.4
15.055	5.88491	234	11.9
18.828	4.71319	631	32
19.724	4.50101	206	10.5
20.115	4.41437	955	48.4
20.451	4.34275	1017	51.6
20.95	4.24033	654	33.2
21.45	4.14261	371	18.8
22.15	4.01325	301	15.3
23.105	3.84943	1972	100
23.449	3.79375	373	18.9
23.859	3.72952	236	12
24.101	3.6926	271	13.7
24.511	3.6317	317	16.1
24.849	3.58309	290	14.7
25.204	3.53342	434	22
25.498	3.49344	320	16.2
25.843	3.44749	257	13
26.538	3.35877	788	40
27.248	3.27289	382	19.4
27.695	3.22103	402	20.4
28.018	3.18459	243	12.3
28.256	3.15834	248	12.6
28.487	3.13331	297	15
29.046	3.07423	352	17.9
29.255	3.0527	244	12.4

Thermal analysis showed a broad endotherm with onset at 37° C. due to water loss. This corresponded with a 5.2% weight loss on heating between ambient and 100° C., equating to loss of 1.2 equivalents of water, and a conversion to Form 4. The sample showed a 4% uptake between 10 and 15% RH during GVS analysis, equating to 1 mole of water. On XRPD re-analysis after the GVS cycles a peak at 14° 2θ was observed. This peak is indicative of the presence of Form 1, suggesting that partial conversion occurred during the GVS experiment. A similar XRPD trace was obtained after storing pure Form 1 at 25° C./94% RH for one month as the sample was in the process of converting to Form 2. There were no significant changes to the sample by XRPD after one month of storage at 40° C./75% RH, but the sample became less crystalline during one month at 40° C./11% RH. A significant decrease in crystallinity and purity was observed during light stability experiments.

A review of the prior art indicates that a monohydrate of bendamustine hydrochloride has been prepared previously. See, W. Ozegowski and D. Krebs, supra. That monohydrate has a reported melting point of 152-56° C. This melting point is similar to that observed with bendamustine hydrochloride Form 2, which has an observed melting point of 153-157° C. While not conclusive, it is possible that Form 2 and the bendamustine hydrochloride monohydrate reported in the prior art are the same polymorph. But as no further characterization details, for example XRPD, have been reported or are available for the bendamustine hydrochloride monohydrate reported in the prior art, it is not known whether the monohydrate reported previously was Form 2 bendamustine hydrochloride.

Storage of Form 1, Form 2 and 1:1 mixtures for up to 6 weeks only showed a conversion of Form 1 to 2 after storage at high humidity (60° C./95% RH, 25° C. 94% RH and possibly 4° C./88% RH for 6, 6 and 2 weeks respectively). No conversion of Form 2 to Form 1 was noted in these studies after 6 weeks. Kinetic factors make it very difficult to determine the absolute thermodynamic stability in the 6 weeks studied and both forms were kinetically stable for 6 weeks at 4° C./34 to 76% RH, 25° C./43 to 75% RH and 60° C./11 to 75% RH.

Form 3 was characterized as a white powder which was partially crystalline by XRPD. No significant changes were observed on XRPD re-analysis after 1 month of storage under ambient conditions, but conversion to Form 2 occurred during 1 week at 40° C./75% RH. The purity was 95.9%. XRPD data for Form 3 is shown below.

XRPD Data for Bendamustine HCl Form 3

Angle (2-Theta)	d value (Angstrom)	Intensity (Counts)	Intensity (%)
3.85	22.95248	13.6	2.1
5.384	16.41406	16.3	2.5
5.75	15.37009	12.1	1.9
7.892	11.20261	40.4	6.2
10.575	8.36538	177	27.2
13.426	6.59478	30.1	4.6
13.636	6.49389	10.9	1.7
13.993	6.32893	36.3	5.6
14.7	6.0261	7.62	1.2
15.547	5.69958	121	18.6
15.734	5.63243	41.4	6.4
17.35	5.1112	25	3.8
17.608	5.0369	14.1	2.2
18.594	4.77186	55.1	8.5
18.85	4.70772	85.8	13.2
19.428	4.56899	80.2	12.3
19.749	4.49541	436	67
19.995	4.44068	173	26.6
21.3	4.17144	216	33.3
22.11	4.02037	233	35.8
23.328	3.81319	409	63
25.449	3.49996	393	60.5
25.571	3.48361	355	54.6
25.733	3.46204	294	45.3
26.083	3.41636	650	100
26.394	3.37675	305	46.9
26.61	3.34983	279	43
27.852	3.2032	393	60.5
27.977	3.1892	403	62
28.109	3.17455	392	60.3
29.039	3.07492	195	30

Form 4 was characterized as a white powder which was crystalline by XRPD. Thermal analysis showed an endotherm due to melting at 153° C. (Peak=157° C.). Form 4 converted

to Form 2 during 24 hours under ambient conditions. XRPD data for Form 4 is depicted below.

XRPD Data for Bendamustine HCl Form 4

Angle (2-Theta)	d value (Angstrom)	Intensity (Counts)	Intensity (%)
3.86	22.88824	63.2	4.6
7.794	11.34336	120	8.8
10.267	8.61623	293	21.4
10.831	8.16867	1297	95
11.624	7.61314	149	10.9
11.804	7.4972	134	9.8
12.806	6.91286	169	12.4
14.077	6.29121	209	15.3
15.521	5.70899	376	27.5
16.038	5.5262	135	9.9
18.748	4.73313	168	12.3
19.636	4.52097	455	33.3
20.447	4.34345	1021	74.7
20.734	4.28411	793	58.1
21.227	4.18563	557	40.8
21.865	4.06498	202	14.8
22.263	3.99311	198	14.5
23.1	3.85031	306	22.4
23.579	3.77323	1366	100
23.95	3.71555	513	37.5
24.39	3.64947	250	18.3
24.548	3.62633	237	17.3
25.477	3.49624	266	19.5
25.81	3.45184	659	48.3
26.559	3.35619	258	18.9
27.101	3.29025	363	26.6
27.627	3.22885	818	59.9
28.415	3.14102	364	26.6

Amorphous bendamustine hydrochloride had a glass transition temperature of about 50° C. and became gummy during 24 hours under ambient conditions, showing it is hygroscopic. Also, partial crystallization occurred during 1 week at 40° C./75% RH, possibly to a mixture of Forms 2 and 3. After subjection to GVS humidity cycle, amorphous bendamustine hydrochloride converted to Form 2.

Preferred pharmaceutical compositions of the invention comprise amorphous bendamustine hydrochloride. The bendamustine hydrochloride may be provided as compositions consisting primarily of an amorphous form of bendamustine hydrochloride or as compositions comprising amorphous bendamustine hydrochloride as well as a crystalline form, such as crystalline bendamustine hydrochloride Form 1, Form 2, Form 3, Form 4, or mixtures thereof. Preferred pharmaceutical compositions of the invention comprise bendamustine hydrochloride substantially free from crystalline bendamustine hydrochloride.

In preferred embodiments, pharmaceutical compositions comprising at least one of Form 1, Form 2, Form 3, Form 4, and amorphous bendamustine hydrochloride, as well as at least one pharmaceutically acceptable excipient, are provided. Preferably, the pharmaceutical compositions comprise at least one of Form 1, Form 3, Form 4, and amorphous bendamustine hydrochloride, as well as at least one pharmaceutically acceptable excipient. More preferred are pharmaceutical compositions that comprise amorphous bendamustine hydrochloride, Form 4, and at least one pharmaceutically acceptable excipient.

Pharmaceutically acceptable excipients are known in the art and include those described in, for example, U.S. application Ser. No. 11/267,010, the content of which is incorporate herein in its entirety. These pharmaceutical compositions may be prepared as injectables, either as liquid solutions or suspensions, as well as solid forms, for example, capsules, tablets, lozenges, pastilles, powders, suspensions, and the like.

In preferred embodiments, the pharmaceutical compositions are sublimed, preferably freeze-dried or lyophilized, compositions. Methods of preparing such sublimed, preferably freeze-dried or lyophilized, preparations of bendamustine hydrochloride that contain Form 1, Form 2, Form 3, Form 4, or a mixture thereof, are also within the scope of the invention. Methods of preparing such sublimed, preferably freeze-dried or lyophilized, preparations of bendamustine hydrochloride that contain Form 1, Form 3, Form 4, amorphous bendamustine hydrochloride, or a mixture thereof, are also within the scope of the invention. Methods of preparing such sublimed, preferably freeze-dried or lyophilized, preparations of bendamustine hydrochloride that further contain Form 2, are also within the scope of the invention.

Lyophilization involves the addition of water to a compound, followed by freezing of the resultant suspension or solution, and sublimation of the water from the compound. In preferred embodiments, at least one organic solvent is added to the suspension/solution. In other preferred embodiments, the suspension/solution further comprises a lyophilization excipient. The lyophilized preparations of bendamustine hydrochloride of the present invention may further comprise amorphous bendamustine hydrochloride.

In a typical lyophilization procedure, water, a pharmaceutically acceptable lyophilizing excipient, an organic solvent, and a compound are combined to form a solution, which is then sterilized, preferably using sterile filtration methodology. This solution is then lyophilized using standard lyophilization equipment and techniques.

While preferred embodiments of the present invention include lyophilization of bendamustine hydrochloride, it is envisioned that other sublimation techniques may also be used. For example, one of more of the described forms of bendamustine hydrochloride may be dissolved, dispersed or suspended in a solvent, the resulting mixture (be it a solution, dispersion or suspension) frozen, and the solvent removed by sublimation.

A lyophilization excipient can be any pharmaceutically acceptable excipient that, when used during the lyophilization process, results in a lyophilzed product that has improved properties, for example, improved handling properties, solubility properties, and the like. A lyophilization excipient can be, for example, a bulking agent; suitable bulking agents are known in the art. Examples of suitable lyophilization excipients include, for example, sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or mixtures thereof. A lyophilization excipient may also comprise a pharmaceutically acceptable antioxidant, such as, for example, ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxylanisole, butyl-hydroxytoluene, or alpha-tocopherol acetate. A preferred lyophilization excipient is mannitol.

Solvents for use in the present invention include water and organic solvents that form stable solutions with bendamustine hydrochloride without appreciably degrading the bendamustine, and which are capable of being evaporated/sublimed through lyophilization. Examples of suitable organic solvents include, for example, methanol, ethanol, n-propanol, iso-propanol, n-butanol, tert-butanol, or mixtures thereof. A preferred organic solvent is tert-butanol.

In one embodiment of the invention are methods of preparing lyophilized compositions that comprise at least one crys-

talline form of bendamustine hydrochloride. Preferably, the crystalline form of bendamustine hydrochloride is bendamustine hydrochloride Form 1, bendamustine hydrochloride Form 2, bendamustine hydrochloride Form 3, bendamustine hydrochloride Form 4, or a mixture thereof. In other embodiments of the invention, the lyophilized compositions further comprise amorphous bendamustine hydrochloride. More preferred methods of the invention produce lyophilized compositions comprising a mixture of bendamustine Form 4 and amorphous bendamustine hydrochloride.

Preferred methods of preparing lyophilized compositions comprising at least one crystalline form of bendamustine hydrochloride comprise combining bendamustine hydrochloride with at least one solvent to form a solution and then lyophilizing the solution. In some embodiments, the solution further comprises at least one lyophilization excipient. Preferred lyophilization excipients include, for example, sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof. More preferably, the pharmaceutically acceptable excipient is mannitol. In some embodiments, the solvent is water, an organic solvent, or a mixture thereof. Preferably, the organic solvent is methanol, ethanol, n-propanol, iso-propanol, n-butanol, tert-butanol, or a mixture thereof. More preferably, the organic solvent is tert-butanol. In certain embodiments, the solvent is a mixture of water and an organic solvent, for example, a mixture having a ratio of water to organic solvent of from about 1:1 to about 3:1 (v/v), preferably about 7:3 (v/v).

Lyophilized compositions produced according to any of the methods described herein are also within the scope of the invention. An X-ray Powder Diffractogram of one such composition, prepared in accordance with the lyophilization procedures described herein and comprising amorphous bendamustine hydrochloride, bendamustine hydrochloride Form 3, and mannitol is shown in FIG. **14**. The XRPD data corresponding to this Diffractogram is shown below.

Angle (2-Theta)	d value (Angstrom)	Intensity (Counts)	Intensity (%)
7.98	11.07642	231	6.3
9.75	9.06671	1710	47.0
10.58	8.35697	751	20.7
13.68	6.46585	30	0.8
15.43	5.73932	286	7.9
18.69	4.74293	91	2.5
19.48	4.55224	474	13.1
19.64	4.51705	799	22.0
19.89	4.45920	416	11.5
20.45	4.33901	3635	100.0
21.12	4.20296	1052	29.0
21.30	4.16740	545	15.0
22.15	4.01060	1349	37.1
22.76	3.90380	95	2.6
23.34	3.80874	293	8.1
24.72	3.59834	1153	31.7
25.30	3.51781	1396	38.4
25.43	3.50023	899	24.7
25.91	3.43569	454	12.5
27.95	3.19006	534	14.7
29.39	3.03627	35	1.0
29.73	3.00276	40	1.1
30.64	2.91594	38	1.1
31.20	2.86471	39	1.1
32.22	2.77642	109	3.0
33.65	2.66154	37	1.0
35.00	2.56159	287	7.9
35.34	2.53782	117	3.2
36.11	2.48539	682	18.8
36.23	2.47719	538	14.8
36.58	2.45430	105	2.9
38.04	2.36363	27	0.8
39.53	2.27806	36	1.0

Also within the scope of the invention are methods of treating diseases, such as, for example, chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, or breast cancer, with a pharmaceutical composition of the present invention. Preferably, the solid forms of the invention are used to treat chronic lymphocytic leukemia. Also preferred are methods of using the solid forms of the invention to treat indolent B-cell non-Hodgkin's lymphoma, in particular, indolent B-cell non-Hodgkin's lymphoma that has progressed during or within six months of treatment with, for example, rituximab or a rituximab-containing regimen. In certain embodiments, the method comprises administering a therapeutically effective amount of a pharmaceutical composition of the present invention directly to the patient (for example, when the pharmaceutical composition is a tablet or capsule). In other embodiments, the method comprises modifying a pharmaceutical composition of the present invention before administration, such as by dissolving the composition in water or another solvent prior to administration. In these embodiments, the method comprises administering to the patient a therapeutically effective amount of a preparation prepared from a pharmaceutical composition of the present invention. Preferably, the preparation is an injectable preparation. The injectable preparation may be administered subcutaneously, intracutaneously, intravenously, intramuscularly, intra-articularly, intrasynovially, intrasternally, intrathecally, intralesionally, intracranially or via infusion. Other conditions amenable to treatment utilizing the compositions and injectable preparations of the present invention include small cell lung cancer, hyperproliferative disorders, and autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, and lupus.

Preferably, the dose administered is about 100 mg/m^2 or about 120 mg/m^2, administered intravenously. Dosages of about 25 mg/m^2, 60 mg/m^2, 50 mg/m^2 and 90 mg/m^2, administered intravenously, are also within the scope of the invention. Preferably, the dosage is administered intravenously over about 30 minutes or over about 60 minutes. Also preferred are methods of administration wherein the dosage is administered on days 1 and 2 of a 28-day cycle. In some embodiments, the dosage is administered in from 1 to 6 or from 1 to 8 cycles.

The injectable preparations described herein are in the form of a sterile injectable preparation, for example, as a sterile, injectable aqueous or oleaginous suspension or solution formulated according to techniques known in the art. Typically, the pharmaceutical compositions of the present invention, containing at least one of Form 1, Form 2, Form 3, Form 4, or amorphous bendamustine hydrochloride, preferably at least one of Form 1, Form 3, Form 4, or amorphous bendamustine hydrochloride, are formulated as lyophilized powders which may be provided, for example, in vials containing 100 mg of drug per 50 mL or 20 mL vial. The injectable preparation may be prepared by reconstitution of a freeze-dried or lyophilized composition with Sterile Water for Injection and then further dilution with a pharmaceutically acceptable intraveneous solution, such as, for example,

0.9% sodium Chloride, 5% dextrose in water (D5W), Lactated Ringers solution, or 0.45% Sodium Chloride/2.5% dextrose.

Preferably, the pharmaceutical compositions of bendamustine hydrochloride described herein are reconstituted into an injectable preparation, for example, with sterile water, in less than about 20 minutes. More preferably, reconstitution occurs in less than about 10 minutes, most preferably about 5 minutes.

A typical reconstitution process would include reconstituting, preferably aseptically, 100 mg bendamustine hydrochloride with 20 mL Sterile Water for Injection. This yields a clear, colorless to pale yellow solution having a bendamustine HCl concentration of 5 mg/mL. If lyophilized bendamustine hydrochloride is being reconstituted, the bendamustine hydrochloride should completely dissolve in about 5 minutes. The volume needed for the required dose (based on 5 mg/mL concentration) can be aseptically withdrawn and transferred to a 500 mL infusion bag of 0.9% Sodium Chloride (or other pharmaceutically acceptable intravenous solution) for injection. Preferably, the reconstituted solution is transferred to the infusion bag within 30 minutes of reconstitution. After transfer, the contents of the infusion bag are thoroughly mixed. Administration by intravenous infusion is typically provided over a time period of from about 30 to about 60 minutes.

It is envisioned that the pharmaceutical compositions of the present invention can be administered in combination with one or more anti-neoplastic agents where the anti-neoplastic agent is given prior to, concurrently with, or subsequent to the administration of the composition of the present invention. Pharmaceutically acceptable anti-neoplastic agents are known in the art. Preferred anti-neoplastic agents are those disclosed in co-pending U.S. application Ser. No. 11/330,868, filed Jan. 12, 2006, the entirety of which is incorporated herein by reference.

Therapeutically effective amounts of bendamustine can be readily determined by an attending diagnostician by use of conventional techniques. The effective dose can vary depending upon a number of factors, including type and extent of progression of the disease or disorder, overall health of a particular patient, biological efficacy of bendamustine, formulation of bendamustine, and route of administration of the forms of bendamustine. Bendamustine can also be administered at lower dosage levels with gradual increases until the desired effect is achieved.

Terminology

The term "anti-solvent," as used herein, means a solvent in which a compound is substantially insoluble.

The term "crystalline," as used herein, means having a regularly repeating arrangement of molecules or external face planes.

The term "crystalline composition," as used in herein, refers to a solid chemical compound or mixture of compounds that provides a characteristic pattern of peaks when analyzed by x-ray powder diffraction; this includes, but is not limited to, polymorphs, solvates, hydrates, co-crystals, and desolvated solvates.

The term "isolating" as used herein, means separating a compound from a solvent, anti-solvent, or a mixture of solvent and anti-solvent to provide a solid, semisolid or syrup. This is typically accomplished by means such as centrifugation, filtration with or without vacuum, filtration under positive pressure, distillation, evaporation or a combination thereof. Isolating may or may not be accompanied by purifying during which the chemical, chiral or chemical and chiral purity of the isolate is increased. Purifying is typically conducted by means such as crystallization, distillation, extraction, filtration through acidic, basic or neutral alumina, filtration through acidic, basic or neutral charcoal, column chromatography on a column packed with a chiral stationary phase, filtration through a porous paper, plastic or glass barrier, column chromatography on silica gel, ion exchange chromatography, recrystallization, normal-phase high performance liquid chromatography, reverse-phase high performance liquid chromatography, trituration and the like.

The term "pharmaceutically acceptable excipient," as used herein, includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art, such as in *Remington: The Science and Practice of Pharmacy,* 20th ed.; Gennaro, A. R., Ed.; Lippincott Williams & Wilkins: Philadelphia, Pa., 2000. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "solution," as used herein, refers to a mixture containing at least one solvent and at least one compound that is at least partially dissolved in the solvent.

The term "solvate," as used herein, means a crystalline composition of variable stoichiometry formed by a solute and an organic solvent as defined herein.

The term "solvent," as used herein, means a substance, typically a liquid, that is capable of completely or partially dissolving another substance, typically a solid. Solvents for the practice of this invention include, but are not limited to, water, acetic acid, acetone, acetonitrile, benzene, chloroform, carbon tetrachloride, dichloromethane, dimethylsulfoxide, 1,4-dioxane, ethanol, ethyl acetate, butanol, tert-butanol, N,N-dimethylacetamide, N,N-dimethylformamide, formamide, formic acid, heptane, hexane, isopropanol, methanol, methyl ethyl ketone (butanone), 1-methyl-2-pyrrolidinone, mesitylene, nitromethane, polyethylene glycol, propanol, 2-propanone, propionitrile, pyridine, tetrahydrofuran, toluene, xylene, mixtures thereof and the like.

The term "sublimation," as used herein, refers to the transition from the solid phase to the gas phase with no intermediate liquid stage.

The term "substantially free," as used herein with regard to compositions that contain a particular form of bendamustine hydrochloride while being "substantially free" of other forms of the compound, means that the recited form is associated with less than 10%, preferably less than 5%, in particular less than 2% and most preferably less than 1% of the other recited forms of bendamustine hydrochloride.

The term "therapeutically effective amount," as used herein, refers to the amount determined to be required to produce the physiological effect intended and associated with a given drug, as measured according to established pharmacokinetic methods and techniques, for the given administration route. Appropriate and specific therapeutically effective amounts can be readily determined by the attending diagnostician, as one skilled in the art, by the use of conventional techniques. The effective dose will vary depending upon a number of factors, including the type and extent of progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the active agent with appropriate excipients, and the route of administration.

Instrumentation

X-Ray Powder Diffraction (XRPD)

The novel crystalline forms of bendamustine hydrochloride have been characterized by XRPD which produces a fingerprint of the particular crystallite form. Measurements of 2θ values typically are accurate to within ±0.2 degrees.

Bruker AXS/Diemens D5000

X-Ray Powder Diffraction patterns were collected on a Siemens D5000 diffractometer using CuKα radiation (40 kV, 40 mA), θ-θ goniometer, automatic divergence and receiving slits, a graphite secondary monochromator and a scintillation counter. The instrument is performance checked using a certified corundum standard (NIST 1976).

Ambient Conditions

Samples run under ambient conditions were prepared as flat plate specimens. Approximately 35 mg of the sample was gently packed into a cavity cut into polished, zero-background (510) silicon wafer and a Mylar cover was placed over the sample. The sample was rotated in its own plane during analysis.

Bruker AXS C2 GADDS

X-Ray Powder Diffraction patterns were collected on a Bruker AXS C2 GADDS diffractometer using CuKα radiation (40 kV, 40 mA), automated XYZ stage, laser video microscope for autosample positioning and a HiStar 2-dimensional area detector. X-ray optics consists of a single Göbel multilayer mirror coupled with a pinhole collimator of 0.3 mm.

The beam divergence, i.e. the effective size of the X-ray beam on the sample, was approximately 5 mm. A θ-θ continuous scan mode was employed with a sample-detector distance of 20 cm which gives an effective 2θ range of 3.2°-29.7°. Typically, the sample would be exposed to the X-ray beam for 120 seconds.

Ambient Conditions

Samples run under ambient conditions were prepared as flat plate specimens using powder without grinding. Approximately 1-2 mg of the sample was lightly pressed on a glass slide to obtain a flat surface.

Non-Ambient Conditions

Samples run under non-ambient conditions were mounted on a silicon wafer with heatconducting compound. The sample was then heated to the appropriate temperature at ca. 20° C. min^{-1} and subsequently held isothermally for ca 1 minute before data collection was initiated.

Single Crystal X-Ray Diffraction (SCXRD)

The crystals chosen were coated with paratone oil and flash frozen on a (Bruker SMART CCD diffractometer. Data were collected on a Bruker AXS 1K SMART CCD diffractometer equipped with an Oxford Cryosystems Cryostream cooling device. Structures were solved using either the SHELXS or SHELXD programs and refined with the SHELXL program as part of the Bruker AXS SHELXTL suite. Unless otherwise stated, hydrogen atoms attached to carbon were placed geometrically and allowed to refine with a riding isotropic displacement parameter. Hydrogen atoms attached to a heteroatom were located in a difference Fourier synthesis and were allowed to refine freely with an isotropic displacement parameter.

^{1}H NMR

^{1}H NMR spectra were collected on a Bruker 400 MHz instrument equipped with an auto-sampler and controlled by a DRX400 console. Automated experiments were acquired using ICON-NMR v4.0.4 (build 1) running with Topspin v 1.3 (patch level 6) using the standard Bruker loaded experiments. For non-routine spectroscopy, data were acquired through the use of Topspin alone. Samples were prepared in d6-DMSO, unless otherwise stated. Off-line analysis was carried out using ACD SpecManager v 9.09 (build 7703).

Differential Scanning Calorimetry (DSC)

DSC data were collected on a TA Instruments Q1000 equipped with a 50 position auto-sampler. The instrument was calibrated for energy and temperature calibration using certified indium. Typically 0.5-2 mg of each sample, in a pin-holed hermetically sealed aluminium pan, was heated at 10° C.min-1 from 25° C. to 200° C. A nitrogen purge at 50 ml.min-1 was maintained over the sample. The instrument control software was Thermal Advantage v4.6.6 and the data were analyzed using Universal Analysis v4.3A.

Thermo-Gravimetric Analysis (TGA)

TGA data were collected on a TA Instruments Q500 TGA, equipped with a 16 position autosampler. The instrument was temperature calibrated using certified Alumel. Typically 1-2 mg of each sample was loaded into a pin-holed hermetically sealed aluminum DSC pan on a pre-tared platinum crucible, and was heated at 10° C. min^{-1} from ambient temperature to 200° C. A nitrogen purge at 60 ml min^{-1} was maintained over the sample. The instrument control software was Thermal Advantage v4.6.6 and the data were analyzed using Universal Analysis v4.3A.

Purity Analysis

Purity analysis was performed on an Agilent HP1100 series system equipped with a diode array detector and using ChemStation software vB.02.01-SR1.

Type of method	Normal Phase	Reverse Phase	✓
	Isocratic	Gradient	✓
Column:	Zorbax Bonus-RP C14, 150 × 4.6 mm, 5 µm		
Column Temperature (° C.):	30		
Test Sample Make-Up:	NMP/mobile phase A 1:1		
Injection (µl):	2		
Detection: Wavelength, Bandwidth(nm):	254, 8		
Flow Rate (ml · min−1):	1.0		
Phase A:	0.1% TFA in water		
Phase B:	0.1% TFA in acetonitrile		

Timetable:	Time (min)	% Phase A	% Phase B
	0	93	7
	5	93	7
	13	73	27
	16	73	27
	25	43	57
	26	10	90
	31	10	90

Thermodynamic Aqueous Solubility by HPLC

Aqueous solubility was determined by suspending sufficient compound in 0.25 ml of water to give a maximum final concentration of ≧10 mg.ml^{-1} of the parent free-form of the compound. The suspension was equilibrated at 25° C. for 24 hours (unless otherwise stated) after which the pH was measured. The suspension was then filtered through a glass fibre C filter into a 96 well plate. The filtrate was then diluted by a factor of 100 times. Quantitation was by HPLC with reference to a standard solution of approximately 0.1 mg.ml-1 in DMSO. Different volumes of the standard, diluted and undiluted sample solutions were injected. The solubility was calculated using the peak areas determined by integration of the peak found at the same retention time as the principal peak in the standard injection.

Type of method:	Reverse phase with gradient elution
Column:	Phenomenex Luna, C18 (2) 5 μm 50 × 4.6 mm
Column Temperature (° C.):	25
Injection (μl):	5, 8 and 50
Detection: Wavelength, Bandwidth (nm):	260, 80
Flow Rate (ml · min−1):	2
Phase A:	0.1% TFA in water
Phase B:	0.085% TFA in acetonitrile

Timetable:	Time (min)	% Phase A	% Phase B
	0.0	95	5
	1.0	80	20
	2.3	5	95
	3.3	5	95
	3.5	95	5
	4.4	95	5

Gravimetric Vapor Sorption (GVS)

Sorption isotherms were obtained using a Hiden IGASorp moisture sorption analyser, controlled by CFRSorp software. The sample temperature was maintained at 25° C. by a Huber recirculating water bath. The humidity was controlled by mixing streams of dry and wet nitrogen, with a total flow rate of 250 ml.min-1. The relative humidity was measured by a calibrated Vaisala RH probe (dynamic range of 0-95% RH), located near the sample. The weight change, (mass relaxation) of the sample as a function of % RH was constantly monitored by the microbalance (accuracy±0.001 mg). Typically 1-3 mg of sample was placed in a tared mesh stainless steel basket under ambient conditions. The sample was loaded and unloaded at 40% RH and 25° C. (typical room conditions). A moisture sorption isotherm was performed as outlined below (2 scans giving 1 complete cycle). The standard isotherm was performed at 25° C. at 10% RH intervals over a 0-90% RH range.

Parameter	Values
Adsorption -Scan 1	40-90
Desorption/Adsorption -Scan 2	85-Dry, Dry-40
Intervals (% RH)	10
Number of Scans	2
Flow rate (ml · min^{-1})	250
Temperature (° C.)	25
Stability (° C. · min^{-1})	0.05
Minimum Sorption Time (hours)	1
Maximum Sorption Time (hours)	4
Mode	AF2
Accuracy (%)	98

The software uses a least squares minimization procedure together with a model of the mass relaxation, to predict an asymptotic value. The measured mass relaxation value must be within 5% of that predicted by the software before the next % RH value is selected. The minimum equilibration time was set to 1 hour and the maximum to 4 hours.

pKa Determination and Prediction

Data were collected on a Sirius GlpKa instrument with a D-PAS attachment. Measurements were made at 25° C. in aqueous solution by UV. The compound was initially dissolved in DMSO at 5 mg/ml of which 50 μl (0.25 mg) was used for the titration from pH 1.3 to 9.0. The titration media was ionic-strength adjusted (ISA) with 0.15 M KCl (aq). The data were refined using Refinement Pro software v1.0. Prediction of pKa values was made using ACD pKa prediction software v9.

Log P Determination

Data were collected by potentiometric titration on a Sirius GlpKa instrument using three ratios of octanol: ionic-strength adjusted (ISA) water to generate Log P, Log Pion, and Log D values. The data were refined using Refinement Pro software v1.0. Prediction of Log P values was made using ACD v9 and Syracuse KOWWIN v1.67 software.

Preparation of Bendamustine Hydrochloride (Crude)

Step 1:

4-{5-[Bis-(2-hydroxy-ethyl)-amino]-1-methyl-1H-benzoimidazol-2-yl}-butyric acid ethyl ester (27.0 kg) was dissolved in 270 kg chloroform. After cooling to 0 to 5° C., 19.2 kg thionyl chloride was added over about 1 hour. The mixture was warmed to 25° C.±5° C. and stirred for 20 to 24 hours. 75.6 kg hydrochloric acid (32% aqueous solution) was then added. After phase separation, the organic (lower) phase was removed. The product remained in the aqueous phase.

Step 2:

A suspension of activated charcoal in hydrochloric acid was added to the aqueous phase obtained in step 1. The mixture was heated over 1 hour to 85 to 90° C. and stirred for 4 to 5 hours at reflux. The suspension was then filtered and rinsed with aqueous hydrochloric acid. The solvent was distilled off under reduced pressure at a temperature not exceeding 65° C. 108 kg to 324 kg (108 kg preferred) of warm (35 to 45° C.) deionized water was added to induce crystallization.

After crystallization, the mixture was cooled to 20 C±5° C. and stirred for an additional 1 to 2 hours or overnight. The product was collected by filtration on a filter dryer, washed with three portions each of 108 to 324 kg (108 kg preferred) deionized water and 108 to 216 kg (108 kg preferred) of cold acetone. The crude product was treated four times each with 54 to 108 kg (54 kg preferred) acetone at reflux for at least 1 hour, in the filter dryer. The suspension was filtered and the product dried at a temperature not higher than 40° C. under reduced pressure, to give 21.4 kg±2.1 kg bendamustine hydrochloride crude (70%±10%, calculated as dried substance).

Step 3 (Optional):

The product obtained from step 2 was dissolved in hydrochloric acid (32% aqueous solution) and heated to reflux (85 to 90° C.) for at least 4 hours. To improve color, activated charcoal can be added to the hydrochloric acid and the mixture heated to reflux (85 to 90° C.) for at least 4 hours. With activated charcoal, the suspension was filtered and rinsed with aqueous hydrochloric acid. Solvent was distilled off under reduced pressure at a temperature not exceeding 65° C. The mixture was then diluted with deionized water. If no crystallization occurred within 15 min, the mixture was seeded. After crystallization, the suspension was stirred at 40° C.±5° C. for one hour, then cooled to 20° C.±5° C. After stirring an additional 1 to 2 hours at 20° C.±5° C. collected by filtration, washed three times with cold deionized water, and at least three times with cold acetone. The crude product was treated four times with acetone at reflux for at least 1 hour. The suspension was filtered and the product dried at a temperature not higher than 40° C., under reduced pressure. Yield was of crude bendamustine hydrochloride was 80%±10%.

Preparation of Purified Bendamustine Hydrochloride

Bendamustine HCl crude (15.0 kg) was suspended with 0.45 kg activated charcoal in ethanol/water (vol/vol=97/3) at room temperature. The mixture was quickly warmed to 75 to 80° C. and stirred for not more than 10 min under reflux conditions. The mixture was filtered to remove the activated charcoal. After filtration, 33.0 kg of filtered acetone was added quickly at 40-50° C. to induce crystallization.

After crystallization, the mixture was stirred for 30 to 60 min at 40-50° C., then cooled to 0 to 5° C., and stirred for at least an additional 30 min or overnight. The product was collected by filtration and washed with three 45 kg of cold acetone. After that, the crude product was treated 4 times each with 30 kg acetone at reflux for at least 1 hour. The suspension was filtered and the product dried at a temperature not higher than 40° C. under reduced pressure providing 11.3±1.5 kg bendamustine hydrochloride (75%±10%).

Preparation of Bulk Solution (1 L) of Bendamustine Hydrochloride

Under sterile conditions, Water for Injection ("WFI," ~65% of total batch size) was transferred to a stainless steel compounding vessel equipped with a mixer. The temperature of the WFI in the compounding tank was adjusted to 15 to 25° C. Mannitol (25.5 g) was added to the compounding vessel and mixed at for a minimum of 5 minutes while maintaining the solution temperature at 15 to 25° C. Tertiary butyl alcohol ("TBA," 234.2 g) was added to the compounding vessel. The solution was mixed for a minimum of 5 minutes at 15 to 25° C. Purified bendamustine HCl (15.0 g) was added to the compounding vessel and mixed for a minimum of 10 minutes while maintaining the solution temperature between 15 to 25° C. Water for Injection, USP, sufficient to bring the batch to 1 L was added and mixed for a minimum of 10 minutes. The bulk solution was sterilized by filtration through a 0.22 μm filter using nitrogen at 1-2 bar.

Lyophilization of Filtered Bulk Solution of Bendamustine Hydrochloride

Step 1:

The formulated, sterile filtered bendamustine HCl bulk solution was filled by a fully automated filling/stoppering machine. The vials continued to the stoppering station, where they were partially stoppered with pre-sterilized stoppers. Bendamustine HCl drug product was filled to approximately 6.47 g (6.67 mL) in a 20-cc Type I borosilicate tubing glass amber vial. Filled and partially stoppered vials were transferred to the lyophilizer located in the lyophilization area.

Step 2:

The filled and partially stoppered vials from step 1 are transferred to the lyophilizer equipped with eight shelves that can be loaded with product-filled trays. The filled and partially stoppered drug product vials were lyophilized. A summary of the freeze drying cycle used during lyophilization of bendamustine HCl drug product is provided in the Table 1 below.

TABLE 1

Lyophilization Cycle for Bendamustine HCl

Process parameters	Target Setpoint
Loading temperature	5° C.
Freezing temperature	Hold at −50° C. for 4 hours
Primary drying vacuum	150 microns
Primary drying temperature	Hold at −15° C. for 27 hours
Intermediate drying temperature	Hold at −12° C. for 7 hours
Secondary drying vacuum	50 microns
Secondary drying temperature	Hold at 40° C. for 15 hours

At the end of the lyophilization cycle, the chamber pressure was raised to ~0.6 bar with sterile filtered nitrogen. The vials were hydraulically stoppered by adjusting the shelves to the stoppering position under sterile filtered nitrogen atmosphere. After the vials were stoppered, the shelves were raised, and the chamber was backfilled with sterile filtered air to atmospheric pressure for unloading. This procedure results in about 100 mg of bendamustine HCl/vial.

Preparation of Solutions of Bendamustine Hydrochloride

50 mg of bendamustine hydrochloride Form 1 was weighed into a screw-top vial. Solvent was added in aliquots (with heating to 50° C.) until a clear solution was obtained. Observations are recorded in Table 2.

TABLE 2

Solubility of Bendamustine Hydrochloride

Solvent	Volume Added	Solution Obtained?
Ethanol	1 ml	Yes (50° C.)
Acetic acid	1 ml	Yes (50° C.)
Methanol	100 μl	Yes (50° C.)
Formamide	1 ml	Yes (50° C.)
DMF	500 μl	Yes (50° C.)
DMSO	100 μl	Yes (50° C.)
DMA	500 μl	Yes (50° C.)

Maturation Experiment

Approximately 10 mg of Form 1 bendamustine hydrochloride was slurried in the solvents list in Table 3. The slurries were shaken for 48 hours with alternating 4 hour periods at 50 C and ambient temperature. Any solid material was then isolated by filtration and analyzed by XRPD. Solutions were allowed to evaporate. Results are shown in Table 3 below.

TABLE 3

Assignment of XRPD Results from Maturation of Bendamustine Hydrochloride

Solvent	XRPD Analysis	Solvent	XRPD Analysis
Ethanol	Form 1	DCM	Form 1
Ethyl acetate	Form 1		
TBME	Form 1	Methyl acetate	Form 1
IPA	Form 1	DMF	Hydrate (Form 2)
Isopropyl acetate	Form 1		
Acetone	Form 1	Dioxane	Form 1
THF	Form 1	Diethyl ether	Form 1
Acetonitrile	Form 1	Anisole	Form 1
Heptane	Form 1	MIBK	Form 1
Water	degradant	Nitromethane	Form 1
Toluene	Form 1	DIPE	Form 1
Methanol	Mix of Form 1 and hydrate (Form 2)	DMA	Hydrate (Form 2)

Crystallization of Bendamustine by Fast Evaporation

Solutions of Bendamustine Hydrochloride in ethanol, acetic acid, methanol, formamide, DMF, DMSO, and DMA were allowed to evaporate under ambient conditions by allowing the uncapped vials of solution to evaporate to dryness (referred to herein as "rapid evaporation"). Resulting solids were analyzed by XRPD. Results are shown in Table 4.

TABLE 4

Assignment of XRPD Results from Crystallization of Bendamustine Hydrochloride by Fast Evaporation

Solvent	XRPD Analysis
Ethanol	Form 1
Acetic acid	Hydrate (Form 2)
Methanol	Mix of Form 1 and hydrate (Form 2)
DMF	Form 1
DMSO	Form 1
DMA	Form 1

Crystallization of Bendamustine by Slow Evaporation

Solutions of Bendamustine Hydrochloride in ethanol, acetic acid, methanol, formamide, DMF, DMSO, and DMA were allowed to evaporate under ambient conditions by allowing the capped vials of solution, the vial caps having pinholes, to evaporate to dryness under ambient conditions. The rate of evaporation was constrained by use of air tight film covers containing small holes. Resulting solids were analyzed by XRPD. Results are shown in Table 5.

TABLE 5

Assignment of XRPD Results from Crystallization of Bendamustine Hydrochloride

Solvent	XRPD Analysis
Ethanol	Form 1
Acetic acid	Form 1
Methanol	Mix of Form 1 and hydrate (Form 2)
Formamide	No solid obtained
DMF	Insufficient material
DMSO	Form 1*
DMA	No solid obtained

*Single crystal data presented herein for Form 1 was obtained from a sample recrystallized from DMSO

Crystallization by Anti-Solvent

Toluene was added as anti-solvent to solutions of Bendamustine Hydrochloride in ethanol, acetic acid, methanol, formamide, DMF, DMSO, and DMA to encourage crystallization The volume of toluene added and observations on anti-solvent addition are recorded in Table 6. Solids were isolated by filtration. The Resulting solids were analyzed by XRPD. Results are shown in Table 6.

TABLE 6

Assignment of XRPD Results from Crystallization of Bendamustine Hydrochloride by Anti-Solvent Addition

Solvent	Anti-Solvent Used	Volume of Anti-solvent	Observations	XRPD Analysis
Ethanol	Toluene	10 ml	No precipitate - evaporated	Form 1
Acetic acid	Toluene	0.5 ml	Precipitate	Form 1
DMF	Toluene	0.5 ml	Precipitate	Form 1
DMSO	Toluene	1 ml	Precipitate	Form 1
DMA	Toluene	0.5 ml	Precipitate	Form 1

Preparation of Form 2 from Form 1 of Bendamustine Hydrochloride

One mL of water was added 30 mg of bendamustine hydrochloride Form 1 and the mixture warmed to 25° C. to provide a clear solution. After about 4 minutes, Form 2 precipitated from solution as a white solid. The solid was collected by filtration.

Stability of Forms 1 and 2 of Bendamustine Hydrochloride

10 mg of bendamustine hydrochloride Form 1 (A), bendamustine hydrochloride Form 2 (B), and a 1:1 mixture of Forms 1 and 2 (C) were stored under the conditions listed in Table 7. Samples were analyzed by XRPD at 1 day, 2 week, and 6 week time points. The results are shown in Table 7A. Under high humity conditions (~90% RH), conversion of Form 1 of bendamustine hydrochloride to Form 2 was observed. The rate of this conversion appears to increase with temperature. The purity of Forms 1 and 2 after storage at 4° C./87% RH (5) and 60° C./75% RH (13) for 6 weeks was measured. No large purity decreases were observed.

TABLE 7

Bendamustine Hydrochloride Stability Study Conditions

Condition	Temperature (° C.)	Relative Humidity (% RH)
1	4	33.6 (Magnesium Chloride)
2	4	43.1 (Potassium Carbonate)
3	4	58.9 (Magnesium Nitrate)
4	4	75.7 (Sodium Chloride)
5	4	87.7 (Potassium Chloride)
6	25	43.2 (Potassium Carbonate)
7	25	57.6 (Sodium Bromide)
8	25	75.3 (Sodium Chloride)
9	25	93.6 (Potassium Nitrate)
10	60	11.0 (Lithium Chloride)
11	60	29.3 (Magnesium Chloride)
12	60	~43 (Potassium Carbonate)
13	60	74.5 (Sodium Chloride)
14	60	~95 (Potassium Sulphate)

TABLE 7A

XRPD Analysis of Stability Study Samples of Bendamustine Hydrochloride

Condition	XRPD Analysis after 1 Day	XRPD Analysis after 2 Weeks	XRPD Analysis after 6 Weeks
1	No changes	No changes	No changes
2	No changes	No changes	No changes
3	No changes	No changes	No changes
4	No changes	No changes	No changes
5	No changes	C) Fully converted to Form 2	C) Some Form 1 now present
6	No changes	No changes	No changes
7	No changes	No changes	No changes
8	No changes	No changes	No changes
9	No changes	A) Partially converted to Form 2 C) Fully converted to Form 2	A) Partially converted to Form 2 C) Fully converted to Form 2
10	No changes	No changes	No changes
11	No changes	No changes	No changes
12	No changes	No changes	No changes
13	Not performed	No changes	No changes
14	Not performed	A) Partially converted to Form 2 B) Sample deliquesced C) Fully converted to Form 2	A) Fully converted to Form 2 B) Sample deliquesced C) Fully converted to Form 2

Light Stability of Bendamustine Hydrochloride

Samples of Form 1 and Form 2 of Bendamustine Hydrochloride were stressed in a Suntest Light Box with a light intensity of 250 watts/m2 for 1 week with the black body temperature set to 25° C. A blank of each sample, wrapped in foil for protection, was also included in the experiment. After the experiment, samples were analyzed by XRPD and the purity was determined by HPLC. A significant decrease in both crystallinity and purity was observed for Form 2 during the light stress test. In contrast, Form 1 showed only a slight decrease in purity. See Table 8.

TABLE 8

XRPD and Purity Analysis of Stability Study Samples of Bendamustine Hydrochloride

Sample	XRPD	Purity (%)
Form 1 blank	No change	97.3
Form 1	No change (sample brown in colour)	95.9

TABLE 8-continued

XRPD and Purity Analysis of Stability Study Samples of Bendamustine Hydrochloride

Sample	XRPD	Purity (%)
Form 2 blank	No change	95.6
Form 2	Less crystalline (sample brown in colour)	68.7

In certain embodiments, the invention is directed to a pharmaceutical composition comprising bendamustine hydrochloride Form 1, bendamustine hydrochloride Form 2, bendamustine hydrochloride Form 3, bendamustine hydrochloride Form 4, or a mixture thereof. The invention is also directed to those pharmaceutical compositions wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 1. The invention is also directed to those pharmaceutical compositions wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 2. The invention is also directed to those pharmaceutical compositions wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 3. The invention is also directed to those pharmaceutical compositions wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 4. The invention is also directed to those pharmaceutical compositions, further comprising amorphous bendamustine hydrochloride.

Other embodiments of the invention are directed to a crystalline form of bendamustine hydrochloride that is bendamustine hydrochloride Form 1, bendamustine hydrochloride Form 2, bendamustine hydrochloride Form 3, bendamustine hydrochloride Form 4, or a mixture thereof. The invention is also directed to crystalline forms, wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 1 The invention is also directed to crystalline forms, wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 2. The invention is also directed to crystalline forms, wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 3. The invention is also directed to crystalline forms, wherein the bendamustine hydrochloride is bendamustine hydrochloride Form 4.

Other embodiments of the invention are directed to a crystalline form of bendamustine hydrochloride that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 25.12, 24.85, 22.92, 21.97, and/or 14.05±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride that produce an X-ray powder diffraction pattern further comprising one or more of the following reflections: 16.82, 17.51, 18.45, 24.85, and/or 28.33±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride having an X-ray powder diffraction pattern substantially as depicted in FIG. **2**. The invention is also directed to pharmaceutical compositions comprising the crystalline form of bendamustine hydrochloride as set forth herein.

Other embodiments of the invention are directed to a crystalline form of bendamustine hydrochloride that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.64, 20.12, 20.45, and/or 23.11±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride that produce an X-ray powder diffraction pattern further comprising one or more of the following reflections: 10.17, 15.06, 18.82, 20.95, 25.20, 26.54, and/or 29.05±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride having an X-ray powder diffraction pattern substantially as depicted in FIG. **6**. The invention is also directed to pharmaceutical compositions comprising the crystalline form of bendamustine hydrochloride as set forth herein.

Other embodiments of the invention are directed to a crystalline form of bendamustine hydrochloride that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 26.08, 27.85, and/or 28.11±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride that produce an X-ray powder diffraction pattern further comprising one or more of the following reflections: 10.58, 15.55, and/or 19.75±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride having an X-ray powder diffraction pattern substantially as depicted in FIG. **10**. The invention is also directed to pharmaceutical compositions comprising the crystalline form of bendamustine hydrochloride as set forth herein.

Other embodiments of the invention are directed to a crystalline form of bendamustine hydrochloride that produces an X-ray powder diffraction pattern comprising one or more of the following reflections: 10.83, 15.52, 20.45, and/or 23.58±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride that produce an X-ray powder diffraction pattern further comprising one or more of the following reflections: 10.27, 19.64, 20.73, 21.23, 25.81 and/or 27.63±0.2 degrees 2θ. The invention is also directed to crystalline forms of bendamustine hydrochloride having an X-ray powder diffraction pattern substantially as depicted in FIG. **11**. The invention is also directed to pharmaceutical compositions comprising the crystalline form of bendamustine hydrochloride as set forth herein.

Other embodiments of the invention are directed to a lyophilized composition comprising bendamustine hydrochloride Form 1, bendamustine hydrochloride Form 2, bendamustine hydrochloride Form 3, bendamustine hydrochloride Form 4, or a mixture thereof. In certain embodiments, the bendamustine hydrochloride is bendamustine Form 1. In other embodiments, the bendamustine hydrochloride is bendamustine Form 2. In other embodiments, the bendamustine hydrochloride is bendamustine Form 3. In other embodiments, the bendamustine hydrochloride is bendamustine Form 4. The invention is also directed to lyophilized compositions described herein further comprising amorphous bendamustine hydrochloride.

A preferred embodiment of the invention includes a lyophilized composition as described herein, comprising amorphous bendamustine hydrochloride, bendamustine hydrochloride Form 2, and a pharmaceutically acceptable excipient.

Also within the scope of the invention is a method for preparing a lyophilized composition comprising a crystalline form of bendamustine hydrochloride comprising the steps of combining bendamustine hydrochloride with at least one solvent to form a mixture; and

lyophilizing the mixture. Preferably, methods of the invention include those wherein the solution further comprises a lyophilization excipient. Preferably, the lyophilization excipient is sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof More preferably, the lyophilization excipient is mannitol. Preferably, methods of the invention include those wherein the solvent is water, an organic solvent, or a mixture thereof. Preferably, the organic solvent is methanol, ethanol, n-propanol, iso-propanol, n-butanol, tert-butanol, or a mixture thereof More preferably, the organic solvent is tert-butanol. In other methods of the invention, the solvent is a mixture of water and an organic solvent. In preferred meth-

ods of the invention, the ratio of the water to the organic solvent is about 1:1 (v/v). In preferred methods of the invention, the ratio of the water to the organic solvent is about 2:1 (v/v) In preferred methods of the invention, the ratio of the water to the organic solvent is about 3:1 (v/v) In preferred methods of the invention, the ratio of the water to the organic solvent is about 7:3 (v/v).

In preferred methods of the invention, the crystalline form of bendamustine hydrochloride is Form 1. In other preferred methods of the invention, the crystalline form of bendamustine hydrochloride is Form 2. In still other preferred methods of the invention, the crystalline form of bendamustine hydrochloride is Form 3. In yet other preferred methods of the invention, the crystalline form of bendamustine hydrochloride is Form 4. Other preferred methods of the invention include those wherein the lyophilized composition further comprises amorphous bendamustine hydrochloride.

Also within the scope of the invention are method of treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer comprising administering to a patient in need thereof a therapeutically effective amount of a preparation prepared from a composition as described herein.

Also within the scope of the invention are methods of preparing Form 1 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in ethanol, ethyl acetate, tert-butyl methyl ether, iso-propyl alcohol, isopropyl acetate, dichloromethane, methyl acetate, acetone, tetrahydrofuran, acetonitrile, heptane, toluene, methanol, dioxane, diethyl ether, anisole, nitromethane, or di-isopropyl ether, and evaporating the solution under ambient conditions.

Also within the scope of the invention are methods of preparing Form 1 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in ethanol, methanol, dimethylformamide, dimethylsulfoxide, or dimethylamine, and rapidly evaporating the solution to dryness under ambient conditions.

Also within the scope of the invention are methods of preparing Form 1 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in ethanol, acetic acid, methanol, or dimethylsulfoxide, and slowly evaporating the solution to dryness under ambient conditions.

Also within the scope of the invention are methods of preparing Form 1 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in acetic acid, formamide, dimethylformamide, dimethylsulfoxide, or dimethylamine, and adding a sufficient quantity of toluene to induce crystallization.

Also within the scope of the invention are methods of preparing Form 2 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in dimethylformamide, methanol, or dimethylamine and evaporating the solution under ambient conditions.

Also within the scope of the invention are methods of preparing Form 2 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in acetic acid or methanol, and rapidly evaporating the solution to dryness under ambient conditions.

Also within the scope of the invention are methods of preparing Form 2 bendamustine hydrochloride comprising providing a solution of bendamustine hydrochloride in methanol and slowly evaporating the solution to dryness under ambient conditions.

Also within the scope of the invention are methods of preparing Form 2 bendamustine hydrochloride comprising providing an amount of Form 1 bendamustine hydrochloride and storing the amount at a relative humidity of at least about 88% for a period of time sufficient to convert Form 1 to Form 2.

Also within the scope of the invention are methods of preparing Form 2 bendamustine hydrochloride comprising combining bendamustine hydrochloride Form 1 with water to form a solution and allowing Form 2 to precipitate from the solution.

Also within the scope of the invention are methods of preparing Form 3 bendamustine hydrochloride comprising providing an amount of amorphous bendamustine hydrochloride and storing the amount at about 40° C. and about 75% relative humidity for a period of time sufficient to convert amorphous bendamustine hydrochloride to Form 3.

Also within the scope of the invention are methods of preparing Form 4 bendamustine hydrochloride comprising providing an amount of Form 2 bendamustine hydrochloride and heating Form 2 to about 100° C. for a period of time sufficient to convert Form 2 to Form 4.

Also within the scope of the invention are methods of preparing a pharmaceutical composition of bendamustine hydrochloride comprising the steps of: preparing bendamustine hydrochloride Form 1; and combining the Form 1 with a pharmaceutically acceptable excipient.

Also within the scope of the invention are methods of preparing a pharmaceutical composition of bendamustine hydrochloride comprising the steps of: preparing bendamustine hydrochloride Form 2; and combining the Form 2 with a pharmaceutically acceptable excipient.

Also within the scope of the invention are methods of preparing a pharmaceutical composition of bendamustine hydrochloride comprising the steps of: preparing bendamustine hydrochloride Form 3; and combining the Form 3 with a pharmaceutically acceptable excipient

Also within the scope of the invention are methods of preparing a pharmaceutical composition of bendamustine hydrochloride comprising the steps of: preparing bendamustine hydrochloride Form 4; and combining the Form 4 with a pharmaceutically acceptable excipient

Also within the scope of the invention are methods of preparing a lyophilized composition of bendamustine hydrochloride comprising the steps of combining Form 1 bendamustine hydrochloride with a solvent to form a mixture; and lyophilizing the mixture. According to the invention, the Form 1 bendamustine hydrochloride is prepared according to any of the methods described herein.

Also within the scope of the invention are methods of preparing a lyophilized composition of bendamustine hydrochloride comprising the steps of combining Form 2 bendamustine hydrochloride a solvent to form a mixture; and lyophilizing the mixture. According to the invention, the Form 1 bendamustine hydrochloride is prepared according to any of the methods described herein.

Also within the scope of the invention are methods of preparing a lyophilized composition of bendamustine hydrochloride comprising the steps of combining Form 3 bendamustine hydrochloride with a solvent to form a mixture; and lyophilizing the mixture. In certain methods of the invention, the Form 3 bendamustine hydrochloride is prepared by providing an amount of amorphous bendamustine hydrochloride and storing the amount at about 40° C. and about 75% relative humidity for a period of time sufficient to convert amorphous bendamustine hydrochloride to Form 3.

Also within the scope of the invention are methods of preparing a lyophilized composition of bendamustine hydrochloride comprising the steps of: combining Form 4 bendamustine hydrochloride with a solvent to form a mixture; and

lyophilizing the mixture. In certain methods of the invention, the Form 4 bendamustine hydrochloride is prepared by providing an amount of Form 2 bendamustine hydrochloride and heating Form 2 to about 100° C. for a period of time sufficient to convert Form 2 to Form 4.

Also within the scope of the invention are lyophilized compositions comprising amorphous bendamustine hydrochloride, wherein said composition is substantially free of any crystalline bendamustine hydrochloride.

In preferred methods of preparing a lyophilized composition of bendmustine hydrochloride, the described mixtures further comprise a lyophilization excipient. Preferably, the lyophilization excipient is sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof In more preferred methods, the lyophilization excipient is mannitol.

In preferred methods of preparing a lyophilized composition of bendmustine hydrochloride, the solvent is water, an organic solvent, or a mixture thereof Preferably, the organic solvent is methanol, ethanol, n-propanol, iso-propanol, n-butanol, tert-butanol, or a mixture thereof In more preferred methods, the organic solvent is tert-butanol.

In preferred methods of preparing a lyophilized composition of bendmustine hydrochloride, the solvent is a mixture of water and an organic solvent. Preferably, the ratio of the water to the organic solvent is about 1:1 (v/v). Also preferred are those methods wherein the ratio of the water to the organic solvent is about 2:1 (v/v). In other preferred methods, the ratio of the water to the organic solvent is about 3:1 (v/v). In other preferred methods, the ratio of the water to the organic solvent is about 7:3 (v/v).

As those skilled in the art will appreciate, numerous modifications and variations of the present invention are possible in view of the above teachings. It is therefore understood that within the scope of the appended claims, the invention can be practiced otherwise than as specifically described herein, and the scope of the invention is intended to encompass all such variations.

What is claimed is:

1. A solid form of bendamustine hydrochloride, designated as bendamustine hydrochloride Form 1, that produces an X-ray powder diffraction pattern comprising the following reflections: 8.3, 16.8, and 18.5±0.2 degrees 2θ.

2. The solid form of bendamustine hydrochloride according to claim **1** that produces an X-ray powder diffraction pattern further comprising the following reflections: 14.0, 22.0, 22.9, 25.1, and 28.3±0.2 degrees 2θ.

3. The solid form of bendamustine hydrochloride according to claim **1** that produces an X-ray powder diffraction pattern further comprising a reflection at 14.0±0.2 degrees 2θ.

4. A composition comprising the solid form of bendamustine hydrochloride according to claim **1**.

5. A composition comprising the solid form of bendamustine hydrochloride according to claim **1**, wherein the composition is substantially free of other solid forms of bendamustine hydrochloride.

6. The composition according to claim **4** wherein the composition is a pharmaceutical composition and further comprises at least one pharmaceutically acceptable excipient.

7. The composition of claim **6** wherein the pharmaceutically acceptable excipient is sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof.

8. The composition of claim **7** wherein the excipient is mannitol.

9. The composition according to claim **5** wherein the composition is a pharmaceutical composition and further comprises at least one pharmaceutically acceptable excipient.

10. The composition of claim **9** wherein the pharmaceutically acceptable excipient is sodium phosphate, potassium phosphate, citric acid, tartaric acid, gelatin, glycine, mannitol, lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose, hetastarch, or a mixture thereof.

11. The composition of claim **10** wherein the excipient is mannitol.

12. A method of treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer in a patient comprising administering to the patient a lyophilized composition comprising the solid form of bendamustine hydrochloride according to claim **1**.

13. The method according to claim **12** wherein the non-Hodgkin's lymphoma is indolent B-cell non-Hodgkin's lymphoma.

14. A method for preparing a lyophilized composition comprising bendamustine hydrochloride comprising:

combining the bendamustine hydrochloride Form 1, according to claim **1**, mannitol, water, and an organic solvent to form a solution; and

lyophilizing the solution to form the lyophilized composition comprising bendamustine hydrochloride.

15. The method of claim **14**, wherein the solvent is methanol, n-propanol, isopropanol, n-butanol, tert-butanol, or a mixture thereof.

16. The method of claim **14**, wherein the solvent is tert-butanol.

17. The method of claim **14**, wherein the lyophilization step comprises the following lyophilization cycle:

Process parameters	Target Setpoint
Loading temperature	5° C.
Freezing temperature	Hold at −50° C. for 4 hours
Primary drying vacuum	150 microns
Primary drying temperature	Hold at −15° C. for 27 hours
Intermediate drying temperature	Hold at −12° C. for 7 hours
Secondary drying vacuum	50 microns
Secondary drying temperature	Hold at 40° C. for 15 hours.

18. A lyophilized composition comprising bendamustine hydrochloride prepared according to the method of any one of claims **14** to **17**.

19. A method of treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma or breast cancer in a patient comprising administering to the patient an injectable preparation prepared from the lyophilized composition of claim **18**.

* * * * *

Exhibit B

US008436190B2

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

(10) **Patent No.: US 8,436,190 B2**
(45) **Date of Patent: May 7, 2013**

(54) **BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS**

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(52) **U.S. Cl.**
USPC **548/304.7**; 34/284

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See application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

The present invention provides pharmaceutical formulations of lyophilized bendamustine suitable for pharmaceutical use. The present invention further provides methods of producing lyophilized bendamustine. The pharmaceutical formulations can be used for any disease that is sensitive to treatment with bendamustine, such as neoplastic diseases.

9 Claims, 6 Drawing Sheets

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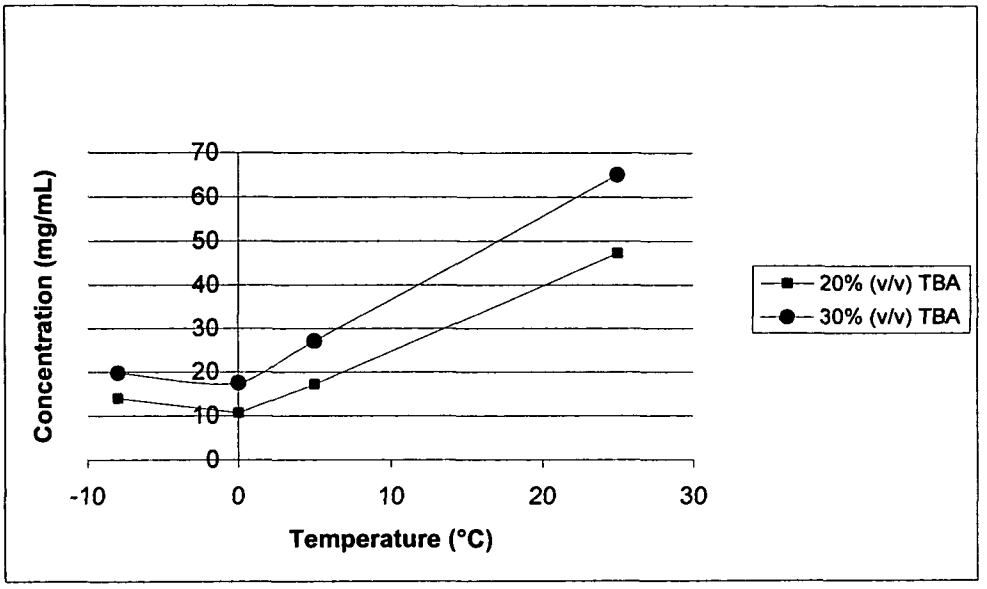

Concentration (mg/mL)
70
60
50
40
30
20
10
0
-10
0
10
20
30
Temperature (°C)
20% (v/v) TBA
30% (v/v) TBA

Fig. 1

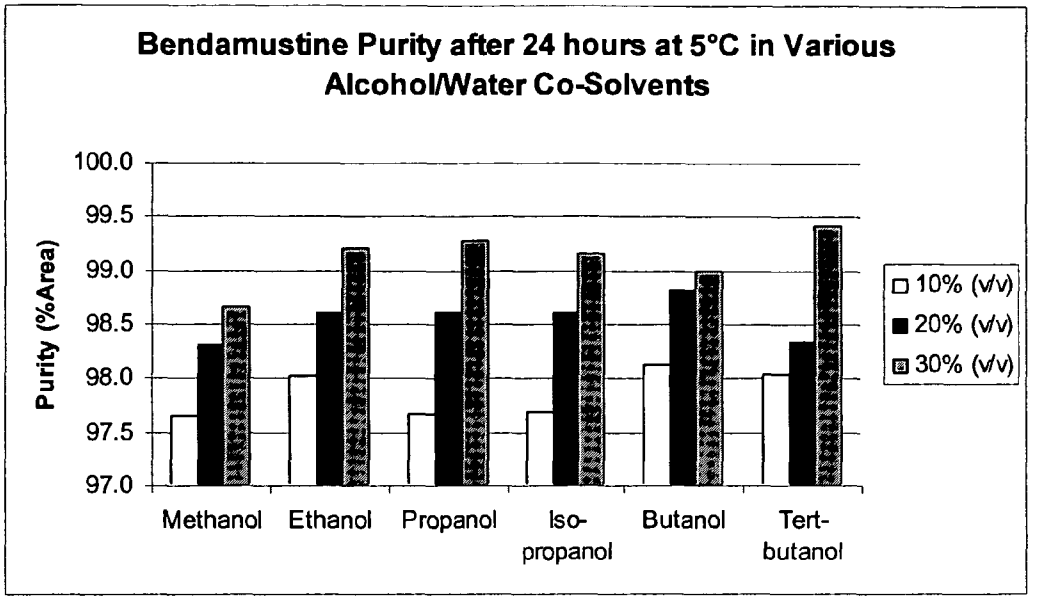

Bendamustine Purity after 24 hours at 5°C in Various Alcohol/Water Co-Solvents
Purity (%Area)
100.0
99.5
99.0
98.5
98.0
97.5
97.0
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol
10% (v/v)
20% (v/v)
30% (v/v)

Fig 2

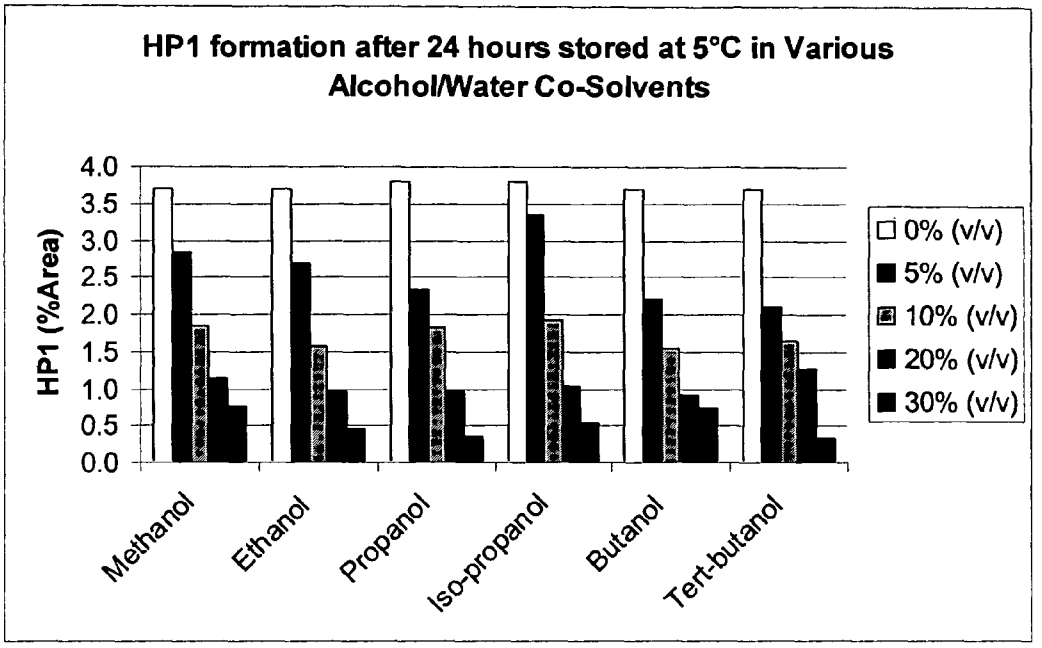
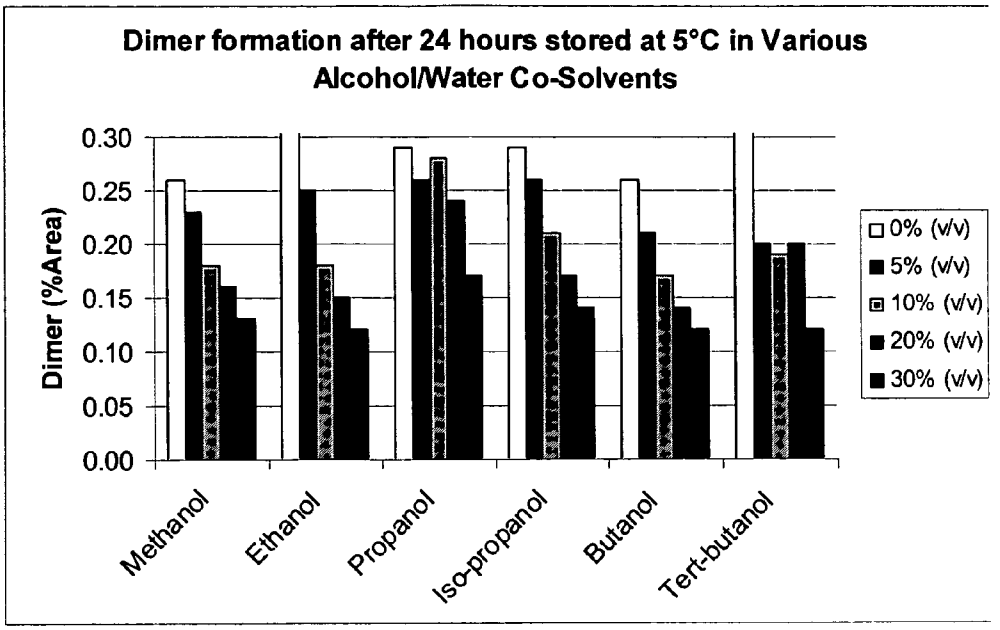

HP1 formation after 24 hours stored at 5°C in Various Alcohol/Water Co-Solvents
HP1 (%Area)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol
0% (v/v)
5% (v/v)
10% (v/v)
20% (v/v)
30% (v/v)

Fig 3

Figure 4.



The numerical values for Figure 4 are provided in Tables 3-9 in Appendix 1.

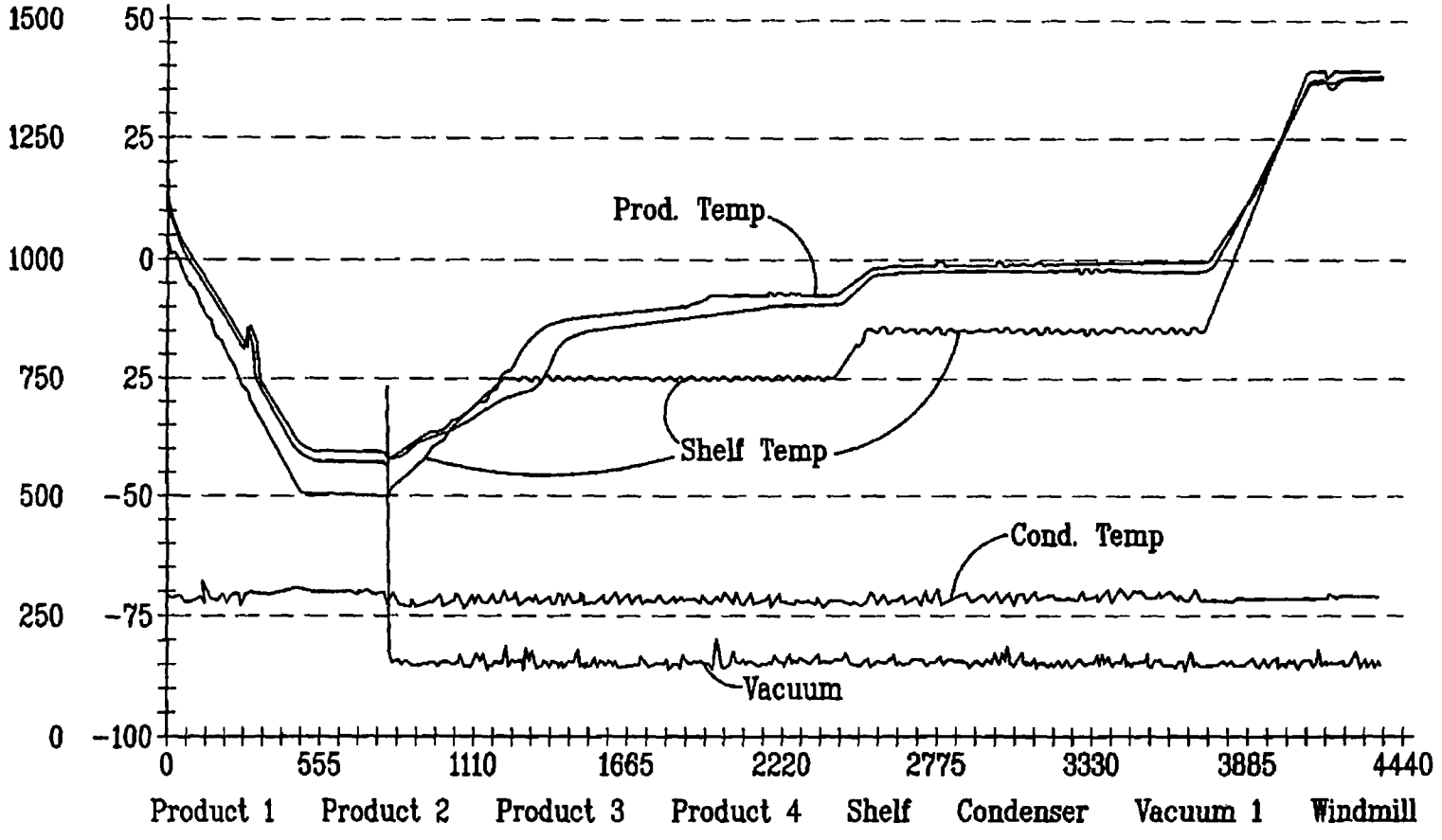

1500
1250
1000
750
500
250
0
50
25
0
25
-50
-75
-100
Prod. Temp
Shelf Temp
Cond. Temp
Vacuum
0
555
1110
1665
2220
2775
3330
3885
4440
Product 1
Product 2
Product 3
Product 4
Shelf
Condenser
Vacuum 1
Windmill

FIG. 5

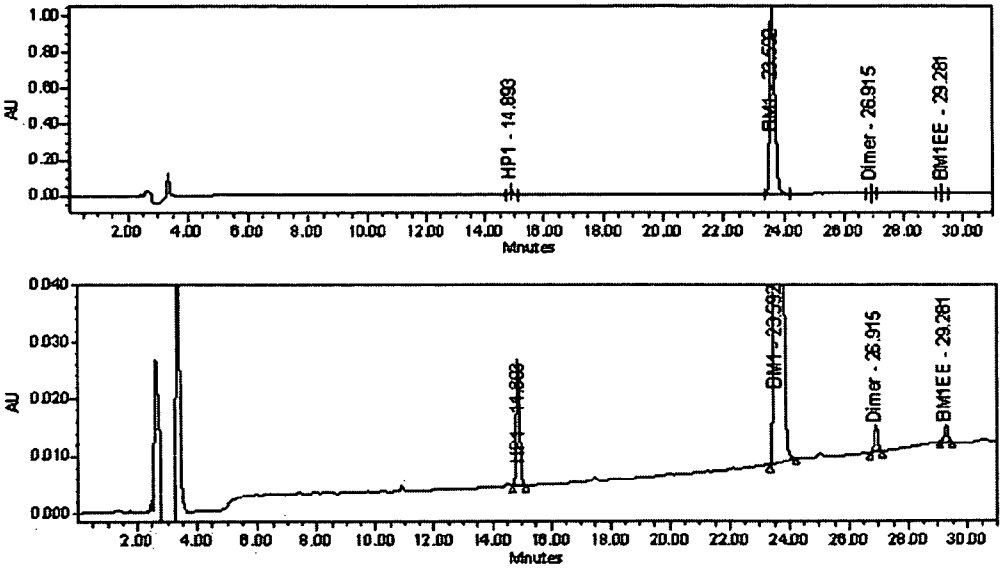

AU
HP1 - 14.893
Dimer - 26.915
BM1EE - 29.281
Minutes
AU
Dimer - 26.915
BM1EE - 29.281
Minutes

Fig. 6

BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

The present invention pertains to the field of pharmaceutical compositions for the treatment of various disease states, especially neoplastic diseases and autoimmune diseases. Particularly, it relates to pharmaceutical formulations comprising nitrogen mustards, particularly the nitrogen mustard bendamustine, e.g., bendamustine HCl.

BACKGROUND OF THE INVENTION

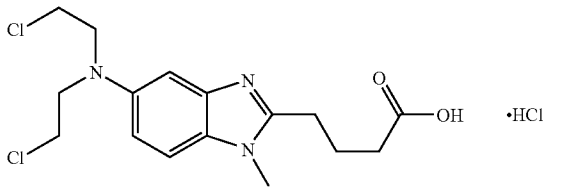
The present invention claims the benefit of and priority to U.S. Ser. No. 60/644,354, filed Jan. 14, 2005, entitled, "Bendamustine Pharmaceutical Compositions," which is incorporated herein by reference in its entirety, including figures and claims.

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

Because of their high reactivity in aqueous solutions, nitrogen mustards are difficult to formulate as pharmaceuticals and are often supplied for administration in a lyophilized form that requires reconstitution, usually in water, by skilled hospital personal prior to administration. Once in aqueous solution, nitrogen mustards are subject to degradation by hydrolysis, thus, the reconstituted product should be administered to a patient as soon as possible after its reconstitution.

Bendamustine, (4-{5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl}butyric acid, is an atypical structure with a benzimidazole ring, whose structure includes an active nitrogen mustard (see Formula I, which shows bendamustine hydrochloride).

Formula I



Bendamustine was initially synthesized in 1963 in the German Democratic Republic (GDR) and was available from 1971 to 1992 in that location under the name Cytostasan®. Since that time, it has been marketed in Germany under the tradename Ribomustin®. It has been widely used in Germany to treat chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer.

Due to its degradation in aqueous solutions (like other nitrogen mustards), bendamustine is supplied as a lyophilized product. The current lyophilized formulation of bendamustine (Ribomustin®) contains bendamustine hydrochloride and mannitol in a sterile lyophilized form as a white powder for intravenous use following reconstitution. The finished lyophilisate is unstable when exposed to light. Therefore, the product is stored in brown or amber-colored glass bottles. The current lyophilized formulation of bendamustine contains degradation products that may occur during manufacturing of the drug substance and/or during the lyophilization process to make the finished drug product.

Currently bendamustine is formulated as a lyophilized powder for injection with 100 mg of drug per 50 mL vial or 25 mg of drug per 20 mL vial. The vials are opened and reconstituted as close to the time of patient administration as possible. The product is reconstituted with 40 mL (for the 100 mg presentation) or 10 mL (for the 25 mg presentation) of Sterile Water for Injection. The reconstituted product is further diluted into 500 mL, q.s., 0.9% Sodium Chloride for Injection. The route of administration is by intravenous infusion over 30 to 60 minutes.

Following reconstitution with 40 mL Sterile Water for Injection, vials of bendamustine are stable for a period of 7 hours under room temperature storage or for 6 days upon storage at 2-8° C. The 500 mL admixture solution must be administered to the patient within 7 hours of vial reconstitution (assuming room temperature storage of the admixture).

The reconstitution of the present bendamustine lyophilized powder is difficult. Reports from the clinic indicate that reconstitution can require at least fifteen minutes and may require as long as thirty minutes. Besides being burdensome and time-consuming for the healthcare professional responsible for reconstituting the product, the lengthy exposure of bendamustine to water during the reconstitution process increases the potential for loss of potency and impurity formation due to the hydrolysis of the product by water.

Thus, a need exists for lyophilized formulations of bendamustine that are easier to reconstitute and which have a better impurity profile than the current lyophilate (lyophilized powder) formulations of bendamustine.

German (GDR) Patent No. 34727 discloses a method of preparing ω-[5-bis-(β-chloroethyl)-amino-benzimidazolyl-(2)]-alkane carboxylic acids substituted in the 1-position.

German (GDR) Patent No. 80967 discloses an injectable preparation of γ-[1-methyl-5-bis-(β-chloroethyl)-amino-benzimaidazolyl-(2)]-butric acid hydrochloride.

German (GDR) Patent No. 159877 discloses a method for preparing 4-[1-methyl-5-bis(2-chloroethyl)amino-benzimidazolyl-2)-butyric acid.

German (GDR) Patent No. 159289 discloses an injectable solution of bendamustine.

Ribomustin® bendamustine Product monograph (updated January 2002) http://www.ribosepharm.de/pdf/ribomustin_bendamustin/productmonograph.pdf provides information about Ribomustin® including product description.

Ni et al. report that the nitrosourea SarCNU was more stable in pure tertiary butanol than in pure acetic acid, dimethyl sulfoxide, methylhydroxy, water or in TBA/water mixtures (Ni et al. (2001) *Intl. J. Phamaceutics* 226:39-46).

Lyophilized cyclophoshamide is known in the art see e.g., U.S. Pat. Nos. 5,418,223; 5,413,995; 5,268,368; 5,227,374; 5,130,305; 4,659,699; 4,537,883; and 5,066,647.

The lyophilized nitrogen mustard Ifosfamide is disclosed in International Publication No. WO 2003/066027; U.S. Pat. Nos. 6,613,927; 5,750,131; 5,972,912; 5,227,373; and 5,204,335.

Teagarden et al. disclose lyophilized formulations of prostaglandin E-1 made by dissolving PGE-1 in a solution of lactose and tertiary butyl alcohol (U.S. Pat. No. 5,770,230).

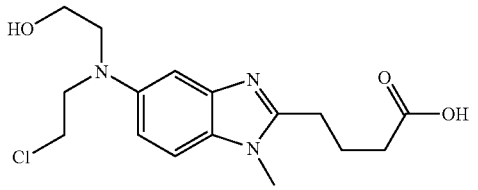
SUMMARY OF THE INVENTION

The present invention is directed to stable pharmaceutical compositions of nitrogen mustards, in particular lyophilized

bendamustine and its use in treatment of various disease states, especially neoplastic diseases and autoimmune diseases.

An embodiment of the invention is a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1, as shown in Formula II,

Formula II



at the time of release or where the HP1 is the amount of HP1 present at time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as described herein. In a preferred embodiment is a pharmaceutical composition of bendamustine containing not more than about 0.5% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%.

Another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.1% to about 0.3% bendamustine dimer as shown in Formula III at release or at time zero after reconstitution

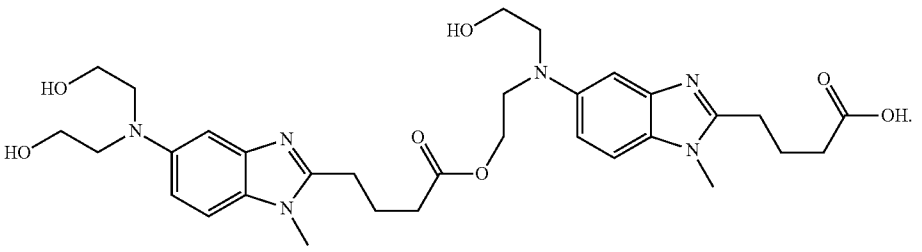
bendamustine ethylester (Formula IV) is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the lyophilized preparation.

In another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1 at the time of drug product release. In a preferred embodiment is a lyophilized preparation of bendamustine containing not more than about 0.50% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%. An aspect of this embodiment is lyophilized preparations of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 at the time of release of drug product where the lyophilized preparation is packaged in a vial or other pharmaceutically acceptable container.

In yet another aspect of the invention, the lyophilized preparations of bendamustine are stable with respect to the amount of HP1 for at least about 6 months, preferably 12 months, preferably 24 months, to about 36 months or greater when stored at about 2° to about 30°. Preferred temperatures for storage are about 5° C. and about room temperature.

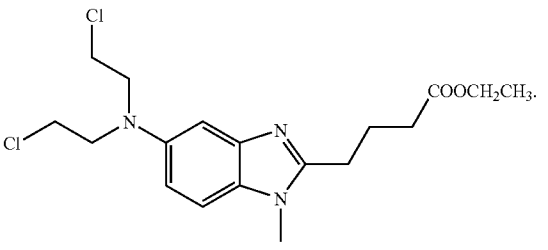
Another embodiment of the invention is a pharmaceutical dosage form that includes a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% HP1, preferably not more than about 0.50%, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%,

Formula III



Yet another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5%, preferably 0.15% to about 0.5%, bendamustine ethylester, as shown in Formula IV at release or at time zero after reconstitution

Formula IV



Yet another embodiment of the invention is a lyophilized preparation of bendamustine wherein the concentration of

even more preferably not more than 0.30%, where the HP1 is the amount of HP1 present at release or at time zero after reconstitution of a lyophilized preparation of bendamustine of the present invention. In preferred aspects of the invention, the dosage form can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

Yet another embodiment of the invention is a pharmaceutical dosage form that includes a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1. Preferred dosage forms can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

In still another embodiment, the invention includes a pharmaceutical composition of bendamustine including bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine), preferably not more than about 0.50%, preferably not more than about 0.45%, more

preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%, and a trace amount of one or more organic solvents, wherein said HP1 is the amount of HP1 present at release or time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as disclosed herein. In different aspects of this embodiment, the organic solvent is selected from one or more of tertiary butanol, n-propanol, n-butanol, isopropanol, ethanol, methanol, acetone, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, 1-pentanol, methyl acetate, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, and cyclohexane. Preferred organic solvents include one or more of ethanol, methanol, propanol, butanol, isopropanol, and tertiary butanol. A more preferred organic solvent is tertiary butanol, also known as TBA, t-butanol, tert-butyl alcohol or tertiary butyl alcohol.

The present invention involves a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a release specification for bendamustine degradants at less than about 4.0%, preferably about 2.0% to about 4.0%, (area percent bendamustine) or otherwise to achieve the pharmaceutical compositions described herein. An aspect of this embodiment is a method for obtaining agency approval for a bendamustine product which includes setting a release specification for HP1 to be less than or equal to 1.5% (area percent Bendamustine). The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment is a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a shelf-life specification for bendamustine degradants at less than about 7.0%, preferably about 5.0% to about 7.0%, (area percent bendamustine) where the product is stored at about 2° C. to about 30° C. Preferred temperatures for storage are about 5° C. and about room temperature. The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment of the invention is a process for manufacturing a lyophilized preparation of bendamustine which includes controlling for the concentration of bendamustine degradants in the final product, such that the concentration of bendamustine degradants is less than about 4.0%, preferably no more than about 2.0% to about 4.0%, (area percent of bendamustine) at release or otherwise to achieve the pharmaceutical compositions described herein. The bendamustine product herein contains not more than about 0.5% to about 0.9%, preferably about 0.5%, (area percent of bendamustine) HP1 at release.

The present invention discloses a process for manufacturing a lyophilized preparation of bendamustine which comprises controlling for the concentration of bendamustine degradants in the final product, such that, at release, the concentration of HP1 is less than 0.9%, preferably 0.5%, (area percent of bendamustine) and, at the time of product expiration, the concentration of bendamustine degradants is less than about 7.0%, preferably no more than about 5.0% to about 7.0%; wherein said product is stored at about 2° C. to about 30° C.

Another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of HP1 produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% to about 0.9% (area percent of bendamustine) preferably 0.50%, preferably 0.45%, more preferably 0.40%, more preferably 0.35%, even more preferably 0.30%. An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% (area percent bendamustine). An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester (as shown in Formula IV) produced during lyophilization from about 0 to 24 hours is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the pre-lyophilization solution. A preferred organic solvent is tertiary butanol.

The invention also discloses methods for preparing a bendamustine lyophilized preparation that includes dissolving bendamustine in a stabilizing concentration of an alcohol solvent of between about 5% to about 100% (v/v alcohol to form a pre-lyophilization solution; and lyophilizing the pre-lyophilization solution; wherein the bendamustine lyophilized preparation made from such methods contains not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 as shown in Formula II, wherein said HP1 is the amount of HP1 present at release or at time zero after reconstitution of the lyophilized pharmaceutical composition of bendamustine. Other alcohol concentrations include about 5% to about 99.9%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 40%, about 20% to about 35%. Preferred concentrations of alcohol are from about 20% to about 30%. Preferred alcohols include one or more of methanol, ethanol, propanol, iso-propanol, butanol, and tertiary-butanol. A more preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

In a preferred method for preparing a bendamustine lyophilized preparation, lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to a temperature below about −40° C., preferably −50° C., to form a frozen solution; ii) holding the frozen solution at or below −40° C., preferably −50° C., for at least 2 hours; iii) ramping the frozen solution to a primary drying temperature between about −40° C. and about −10° C. to form a dried solution; iv) holding for about 10 to about 70 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vii) holding for about 5 to about 40 hours to form a bendamustine lyophilized preparation. In a more preferred method lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to about −50° C. to form a frozen solution; ii) holding the frozen solution at about −50° C. for at least 2 hours to about 4 hours; iii) ramping to a primary drying temperature between

about −20° C. and about −12° C. to form a dried solution; iv) holding at a primary drying temperature for about 10 to about 48 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vi) holding at a secondary drying temperature for at least 5 hours up to about 20 hours. A preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

Another embodiment of the invention is the lyophilized powder or preparation obtained from the methods of preparing a bendamustine lyophilized preparation disclosed herein.

The invention also involves bendamustine formulations for lyophilization that include an excipient and a stabilizing concentration of an organic solvent. A preferred formulation includes bendamustine at a concentration of about 15 mg/mL, mannitol at a concentration of about 25.5 mg/mL, tertiary-butyl alcohol at a concentration of about 30% (v/v) and water. Included in this embodiment of the invention are the lyophilized preparations made from such bendamustine formulations.

Included in the inventions are methods of treating a medical condition in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition. Some conditions amenable to treatment with the compositions of the invention include chronic lymphocytic leukemia (CLL), Hodgkin's disease, non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), breast cancer, small cell lung cancer, hyperproliferative disorders, and an autoimmune disease. Preferred conditions include NHL, CLL, breast cancer, and MM. Preferred autoimmune diseases include rheumatoid arthritis, multiple sclerosis or lupus.

Included in the inventions are the use of the pharmaceutical compositions or pharmaceutical preparations of the invention in the manufacture of a medicament for the treatment of a medical condition, as defined herein, in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition.

Also included in the invention are methods of treating in which the pharmaceutical compositions of the invention are in combination with one or more anti-neoplastic agents where the antineoplastic agent is given prior, concurrently, or subsequent to the administration of the pharmaceutical composition of the invention. Preferred antineoplastic agents are antibodies specific for CD20.

Another embodiment of the invention is a lyophilization cycle for producing lyophilized bendamustine preparations of the invention. A preferred lyophilization cycle includes a) freezing to about −50° C. over about 8 hours; b) holding at −50° C. for about 4 hours; c) ramping to −25° C. over about 3 hours; d) holding at −10° C. for 30 hours; e) ramping to between about 25° C. and about 40° C. or higher for about 3 hours; f) holding between about 25° C. and about 40° C. for about 25 hours; g) ramping to about 20° C. in 1 hour; h) unloading at about 20° C., at a pressure of 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. An aspect of this cycle involves step (e) which is ramped to about 30-35° C. for 3 hours and then ramped to 40° C. for 5 hours. Another aspect of this embodiment is the lyophilized powered prepared from such lyophilization cycles. A more preferred lyophilization cycle includes i) starting with a shelf temperature of about 5° C. for loading; ii) freezing to about −50° C. over about 8 hours; iii) holding at −50° C. for about 4 hours; iv) ramping to about −20° C. over about 3 hours; v) holding at about −20° C. for 6 hours; ramping to about −15° C. over about 1 hour; vi) holding at −15° C. for about 20 hours; vii) ramping to about −15° C. over about 1 hour; viii) holding at about −15° C. for about 20 hours; ix) ramping to about −12° C. over about 0.5 hours; x) holding at about −12° C. for about 15.5 hours; xi) ramping to between about 25° C. and about 40° C. or higher for about 15 hours; xii) holding between about 25° C. and about 40° C. for about 10 hours; xiii) ramping to about 40° C. over about 1 hour; and xiv) holding at about 40° C. for about 5 hours; unloading at about 5° C., at a pressure of about 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. In a preferred embodiment step (xi) is ramped to about 30-35° C. for about 15 hours.

The invention also encompasses a pharmaceutical dosage form of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1 (area percent of bendamustine) wherein said dosage form comprises a vial or other pharmaceutically acceptable container, wherein said HP1 is the amount of HP1 present pre-reconstitution or at time zero after reconstitution of said dosage form. Preferred concentrations of bendamustine include about 10 to about 500 mg/container, about 100 mg/container, about 5 mg to about 2 g/container and about 170 mg/container.

The present invention also includes pre-lyophilized pharmaceutical compositions of bendamustine. A preferred pre-lyophilized composition includes bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and water.

These and other embodiments of the invention are described hereinbelow or are evident to persons of ordinary skill in the art based on the following disclosures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the solubility of bendamustine at various temperatures for two different solutions of bendamustine in tertiary butanol.

FIG. **2** shows the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the bendamustine peak.

FIG. **3** shows HP1 (Formula II) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **4** shows dimer (Formula III) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **5**-*shows* a lyophilization cycle for bendamustine using a TBA/water co-solvent.

FIG. **6** shows a chromatogram for Ribomustin® using HPLC method No. 1.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the terms "formulate" refers to the preparation of a drug, e.g., bendamustine, in a form suitable for administration to a mammalian patient, preferably a human. Thus, "formulation" can include the addition of pharmaceutically acceptable excipients, diluents, or carriers.

As used herein, the term "lyophilized powder" or "lyophilized preparation" refers to any solid material obtained by lyophilization, i.e., freeze-drying of an aqueous solution. The aqueous solution may contain a non-aqueous solvent, i.e. a solution composed of aqueous and one or more non-aqueous solvent(s). Preferably, a lyophilized preparation is one in which the solid material is obtained by freeze-drying a solution composed of aqueous and one or more non-aqueous solvents, more preferably the non-aqueous solvent is an alcohol.

By "stable pharmaceutical composition" is meant any pharmaceutical composition having sufficient stability to have utility as a pharmaceutical product. Preferably, a stable pharmaceutical composition has sufficient stability to allow storage at a convenient temperature, preferably between −20° C. and 40° C., more preferably about 2° C. to about 30° C., for a reasonable period of time, e.g., the shelf-life of the product which can be as short as one month but is typically six months or longer, more preferably one year or longer even more preferably twenty-four months or longer, and even more preferably thirty-six months or longer. The shelf-life or expiration can be that amount of time where the active ingredient degrades to a point below 90% purity. For purposes of the present invention stable pharmaceutical composition includes reference to pharmaceutical compositions with specific ranges of impurities as described herein. Preferably, a stable pharmaceutical composition is one which has minimal degradation of the active ingredient, e.g., it retains at least about 85% of un-degraded active, preferably at least about 90%, and more preferably at least about 95%, after storage at 2-30° C. for a 2-3 year period of time.

By "stable lyophilized preparation" is meant any lyophilized preparation having sufficient stability, such characteristics as similarly defined herein for a stable pharmaceutical composition, to have utility as a pharmaceutical product

By "degraded" is meant that the active has undergone a change in chemical structure.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered that will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of neoplasms, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer. Therapeutically effective amount can also mean preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment). Further, therapeutically effective amount can be that amount that increases the life expectancy of a patient afflicted with a terminal disorder. Typical therapeutically effective doses for bendamustine for the treatment of non-Hodgkin's lymphoma can be from about 60-120 mg/m^2 given as a single dose on two consecutive days. The cycle can be repeated about every three to four weeks. For the treatment of chronic lymphocytic leukemia (CLL) bendamustine can be given at about 80-100 mg/m^2 on days 1 and 2. The cycle can be repeated after about 4 weeks. For the treatment of Hodgkin's disease (stages II-IV), bendamustine can be given in the "DBVBe regimen" with daunorubicin 25 mg/m^2 on days 1 and 15, bleomycin 10 mg/m^2 on days 1 and 15, vincristine 1.4 mg/m^2 on days 1 and 15, and bendamustine 50 mg/m^2 on days 1-5 with repetition of the cycle about every 4 weeks. For breast cancer, bendamustine (120 mg/m^2) on days 1 and 8 can be given in combination with methotrexate 40 mg/m^2 on days 1 and 8, and 5-fluorouracil 600 mg/m^2 on days 1 and 8 with repetition of the cycle about every 4 weeks. As a second-line of therapy for breast cancer, bendamustine can be given at about 100-150 mg/m^2 on days 1 and 2 with repetition of the cycle about every 4 weeks.

As used herein "neoplastic" refers to a neoplasm, which is an abnormal growth, such growth occurring because of a proliferation of cells not subject to the usual limitations of growth. As used herein, "anti-neoplastic agent" is any compound, composition, admixture, co-mixture, or blend which inhibits, eliminates, retards, or reverses the neoplastic phenotype of a cell.

As used herein "hyperproliferation" is the overproduction of cells in response to a particular growth factor. "Hyperproliferative disorders" are diseases in which the cells overproduce in response to a particular growth factor. Examples of such "hyperproliferative disorders" include diabetic retinopathy, psoriasis, endometriosis, cancer, macular degenerative disorders and benign growth disorders such as prostate enlargement.

As used herein, the term "vial" refers to any walled container, whether rigid or flexible.

"Controlling" as used herein means putting process controls in place to facilitate achievement of the thing being controlled. For example, in a given case, "controlling" can mean testing samples of each lot or a number of lots regularly or randomly; setting the concentration of degradants as a release specification; selecting process conditions, e.g., use of alcohols and/or other organic solvents in the pre-lyophilization solution or dispersion, so as to assure that the concentration of degradants of the active ingredient is not unacceptably high; etc. Controlling for degradants by setting release specifications for the amount of degradants can be used to facilitate regulatory approval of a pharmaceutical product by a regulatory agency, such as the U.S. Food and Drug Administration and similar agencies in other countries or regions ("agency").

The term "pharmaceutically acceptable" as used herein means that the thing that is pharmaceutically acceptable, e.g., components, including containers, of a pharmaceutical composition, does not cause unacceptable loss of pharmacological activity or unacceptable adverse side effects. Examples of pharmaceutically acceptable components are provided in The United States Pharmacopeia (USP), The National Formulary (NF), adopted at the United States Pharmacopeial Convention, held in Rockville, Md. in 1990 and FDA Inactive Ingredient Guide 1990, 1996 issued by the U.S. Food and Drug Administration (both are hereby incorporated by reference herein, including any drawings). Other grades of solutions or components that meet necessary limits and/or specifications that are outside of the USP/NF may also be used.

The term "pharmaceutical composition" as used herein shall mean a composition that is made under conditions such that it is suitable for administration to humans, e.g., it is made under GMP conditions and contains pharmaceutically acceptable excipients, e.g., without limitation, stabilizers, bulking agents, buffers, carriers, diluents, vehicles, solubilizers, and binders. As used herein pharmaceutical composition includes but is not limited to a pre-lyophilization solution or dispersion as well as a liquid form ready for injection or infusion after reconstitution of a lyophilized preparation.

A "pharmaceutical dosage form" as used herein means the pharmaceutical compositions disclosed herein being in a container and in an amount suitable for reconstitution and administration of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. Preferably, a "pharma-

ceutical dosage form" as used herein means a lyophilized pharmaceutical composition disclosed herein in a container and in an amount suitable for reconstitution and delivery of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. The pharmaceutical dosage form can comprise a vial or syringe or other suitable pharmaceutically acceptable container. The pharmaceutical dosage form suitable for injection or infusion use can include sterile aqueous solutions or dispersions or sterile powders comprising an active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol such as glycerol, propylene glycol, or liquid polyethylene glycols and the like, vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The prevention of the growth of microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

As used herein, the term "excipient" means the substances used to formulate active pharmaceutical ingredients (API) into pharmaceutical formulations; in a preferred embodiment, an excipient does not lower or interfere with the primary therapeutic effect of the API. Preferably, an excipient is therapeutically inert. The term "excipient" encompasses carriers, diluents, vehicles, solubilizers, stabilizers, bulking agents, and binders. Excipients can also be those substances present in a pharmaceutical formulation as an indirect or unintended result of the manufacturing process. Preferably, excipients are approved for or considered to be safe for human and animal administration, i.e., GRAS substances (generally regarded as safe). GRAS substances are listed by the Food and Drug administration in the Code of Federal Regulations (CFR) at 21 CFR §182 and 21 CFR §184, incorporated herein by reference. Preferred excipients include, but are not limited to, hexitols, including mannitol and the like.

As used herein "a stabilizing concentration of an organic solvent" or "a stabilizing concentration of an alcohol" means that amount of an organic solvent or alcohol that reduces the level of degradation of bendamustine to achieve a specified level of degradants in the final drug product. For example, with respect to the degradant HP1, a stabilizing concentration of an organic solvent is that amount which results in an HP1 concentration (area percent of bendamustine) of less than about 0.5%, preferably less than 0.45%, preferably less than 0.40%, more preferably less than 0.35%, more preferably less than 0.30%, and even more preferably less than 0.25%. With respect to the overall or total degradant concentration of the final drug product, a stabilizing concentration of an organic solvent is that amount that results in a total degradant concentration (at the time of drug product release) of less than about 7% (area percent bendamustine), preferably less than about 6%, more preferably less than about 5%, and even more preferably less than about 4.0%. By "area percent of bendamustine" is meant the amount of a specified degradant, e.g., HP1, relative to the amount of bendamustine as determined, e.g., by HPLC.

The term "organic solvent" means an organic material, usually a liquid, capable of dissolving other substances.

As used herein, "trace amount of an organic solvent" means an amount of solvent that is equal to or below recommended levels for pharmaceutical products, for example, as recommended by ICH guidelines (International Conferences on Harmonization, Impurities—Guidelines for Residual Solvents. Q3C. Federal Register. 1997; 62(247):67377). The lower limit is the lowest amount that can be detected.

The term "release" or "at release" means the drug product has met the release specifications and can be used for its intended pharmaceutical purpose.

A. General

The invention provides stable, pharmaceutically acceptable compositions prepared from bendamustine. In particular, the invention provides formulations for the lyophilization of bendamustine HCl. The lyophilized powder obtained from such formulations is more easily reconstituted than the presently available lyophilized powder of bendamustine. Further, the lyophilized products of the present invention have a better impurity profile than Ribomustin® with respect to certain impurities, in particular HP1, bendamustine dimer, and bendamustine ethylester, prior to reconstitution, upon storage of the lyophilate, or following reconstitution and admixture.

The present invention further provides formulations of bendamustine useful for treating neoplastic diseases. The formulations described herein can be administered alone or in combination with at least one additional anti-neoplastic agent and/or radioactive therapy.

An aspect of the invention is conditions and means for enhancing the stability of bendamustine prior to and during the lyophilization process, upon shelf storage or upon reconstitution.

Anti-neoplastic agents which may be utilized in combination with the formulations of the invention include those provided in the Merck Index 11, pp 16-17, Merck & Co., Inc. (1989) and The Chemotherapy Source Book (1997). Both books are widely recognized and readily available to the skilled artisan.

There are large numbers of antineoplastic agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for treatment of neoplasia by combination drug chemotherapy. Such antineoplastic agents fall into several major categories, namely, antibiotic-type agents, covalent DNA-binding drugs, antimetabolite agents, hormonal agents, including glucocorticoids such as prednisone and dexamethasone, immunological agents, interferon-type agents, differentiating agents such as the retinoids, pro-apoptotic agents, and a category of miscellaneous agents, including compounds such as antisense, small interfering RNA, and the like. Alternatively, other antineoplastic agents, such as metallomatrix proteases (MMP) inhibitors, SOD mimics or $alpha_v$ $beta_3$ inhibitors may be used.

One family of antineoplastic agents which may be used in combination with the compounds of the inventions consists of antimetabolite-type antineoplastic agents. Suitable antimetabolite antineoplastic agents may be selected from the group consisting of alanosine, AG2037 (Pfizer), 5-FU-fibrinogen, acanthifolic acid, aminothiadiazole, brequinar sodium, carmofur, Ciba-Geigy CGP-30694, cyclopentyl cytosine, cytarabine phosphate stearate, cytarabine conjugates, Lilly DATHF, Merrel Dow DDFC, dezaguanine, dideoxycytidine, dideoxyguanosine, didox, Yoshitomi DMDC, doxifluridine, Wellcome EHNA, Merck & Co. EX-015, fazarabine, floxuridine, fludarabine phosphate, 5-fluorouracil, N-(2'-furanidyl)-5-fluorouracil, Daiichi Seiyaku FO-152, isopropyl pyrrolizine, Lilly LY-188011, Lilly LY-264618, methobenzaprim, methotrexate, Wellcome MZPES, norspermidine, NCI NSC-127716, NCI NSC-264880, NCI NSC-39661, NCI NSC-612567, Warner-Lambert PALA, pentostatin, piritrexim, plicamycin, Asahi Chemical PL-AC, Takeda TAC-788,

thioguanine, tiazofurin, Erbamont TIF, trimetrexate, tyrosine kinase inhibitors, tyrosine protein kinase inhibitors, Taiho UFT and uricytin.

A second family of antineoplastic agents which may be used in combination with the compounds of the invention consists of covalent DNA-binding agents. Suitable alkylating-type antineoplastic agents may be selected from the group consisting of Shionogi 254-S, aldo-phosphamide analogues, altretamine, anaxirone, Boehringer Mannheim BBR-2207, bestrabucil, budotitane, Wakunaga CA-102, carboplatin, cannustine, Chinoin-139, Chinoin-153, chlorambucil, cisplatin, cyclophosphamide, American Cyanamid CL-286558, Sanofi CY-233, cyplatate, Degussa D-19-384, Sumimoto DACHP(Myr)2, diphenylspiromustine, diplatinum cytostatic, Erba distamycin derivatives, Chugai DWA-2114R, ITI E09, elmustine, Erbamont FCE-24517, estramustine phosphate sodium, fotemustine, Unimed G-6-M, Chinoin GYKI-17230, hepsul-fam, ifosfamide, iproplatin, lomustine, mafosfamide, melphalan, mitolactol, Nippon Kayaku NK-121, NCI NSC-264395, NCI NSC-342215, oxaliplatin, Upjohn PCNU, prednimustine, Proter PTT-119, ranimustine, semustine, SmithKline SK&F-101772, Yakult Honsha SN-22, spiromustine, Tanabe Seiyaku TA-077, tauromustine, temozolomide, teroxirone, tetraplatin and trimelamol.

Another family of antineoplastic agents which may be used in combination with the compounds disclosed herein consists of antibiotic-type antineoplastic agents. Suitable antibiotic-type antineoplastic agents may be selected from the group consisting of Taiho 4181-A, aclarubicin, actinomycin D, actinoplanone, alanosine, Erbamont ADR-456, aeroplysinin derivative, Ajinomoto AN-201-II, Ajinomoto AN-3, Nippon Soda anisomycins, anthracycline, azino-mycin-A, bisucaberin, Bristol-Myers BL-6859, Bristol-Myers BMY-25067, Bristol-Myers BMY-25551, Bristol-Myers BMY-26605, Bristol-Myers BMY-27557, Bristol-Myers BMY-28438, bleomycin sulfate, bryostatin-1, Taiho C-1027, calichemycin, chromoximycin, dactinomycin, daunorubicin, Kyowa Hakko DC-102, Kyowa Hakko DC-79, Kyowa Hakko DC-88A, Kyowa Hakko DC89-Al, Kyowa Hakko DC92-B, ditrisarubicin B, Shionogi DOB-41, doxorubicin, doxorubicin-fibrinogen, elsamicin-A, epirubicin, erbstatin, esorubicin, esperamicin-Al, esperamicin-Alb, Erbamont FCE-21954, Fujisawa FK-973, fostriecin, Fujisawa FR-900482, glidobactin, gregatin-A, grincamycin, herbimycin, idarubicin, illudins, kazusamycin, kesarirhodins, Kyowa Hakko KM-5539, Kirin Brewery KRN-8602, Kyowa Hakko KT-5432, Kyowa Hakko KT-5594, Kyowa Hakko KT-6149, American Cyanamid LL-D49194, Meiji Seika ME 2303, menogaril, mitomycin, mitoxantrone, SmithKline M-TAG, neoenactin, Nippon Kayaku NK-313, Nippon Kayaku NKT-01, SRI International NSC-357704, oxalysine, oxaunomycin, peplomycin, pilatin, pirarubicin, porothramycin, pyrindamycin A, Tobishi RA-I, rapamycin, rhizoxin, rodorubicin, sibanomicin, siwenmycin, Sumitomo SM-5887, Snow Brand SN-706, Snow Brand SN-07, sorangicin-A, sparsomycin, SS Pharmaceutical SS-21020, SS Pharmaceutical SS-7313B, SS Pharmaceutical SS-9816B, steffimycin B, Taiho 4181-2, talisomycin, Takeda TAN-868A, terpentecin, thrazine, tricrozarin A, Upjohn U-73975, Kyowa Hakko UCN-10028A, Fujisawa WF-3405, Yoshitomi Y-25024 and zorubicin.

A fourth family of antineoplastic agents which may be used in combination with the compounds of the invention include a miscellaneous family of antineoplastic agents selected from the group consisting of alpha-carotene, alpha-difluoromethyl-arginine, acitretin, arsenic trioxide, Avastin® (bevacizumab), Biotec AD-5, Kyorin AHC-52, alstonine, amonafide, amphethinile, amsacrine, Angiostat, ankinomycin, anti-neoplaston A10, antineoplaston A2, antineoplaston A3, antineoplaston A5, antineoplaston AS2-1, Henkel APD, aphidicolin glycinate, asparaginase, Avarol, baccharin, batracylin, benfluron, benzotript, Ipsen-Beaufour BIM-23015, bisantrene, Bristo-Myers BMY-40481, Vestar boron-10, bromofosfamide, Wellcome BW-502, Wellcome BW-773, caracemide, carmethizole hydrochloride, Ajinomoto CDAF, chlorsulfaquinoxalone, Chemes CHX-2053, Chemex CHX-100, Warner-Lambert CI-921, Warner-Lambert CI-937, Warner-Lambert CI-941, Warner-Lambert CI-958, clanfenur, claviridenone, ICN compound 1259, ICN compound 4711, Contracan, Yakult Honsha CPT-ll, crisnatol, curaderm, cytochalasin B, cytarabine, cytocytin, Merz D-609, DABIS maleate, dacarbazine, datelliptinium, didemnin-B, dihaematoporphyrin ether, dihydrolenperone, dinaline, distamycin, Toyo Pharmar DM-341, Toyo Pharmar DM-75, Daiichi Seiyaku DN-9693, elliprabin, elliptinium acetate, epothionesTsumura EPMTC, erbitux, ergotamine, erlotnib, etoposide, etretinate, fenretinide, Fujisawa FR-57704, gallium nitrate, genkwadaphnin, Gleevec® (imatnib), Chugai GLA-43, Glaxo GR-63178, gefitinib, grifolan NMF-5N, hexadecylphosphocholine, Green Cross HO-221, homoharringtonine, hydroxyurea, BTG ICRF-187, indanocine, ilmofosine, isoglutamine, isotretinoin, Otsuka JI-36, Ramot K-477, Otsuak K-76COONa, Kureha Chemical K-AM, MECT Corp KI-8110, American Cyanamid L-623, leukoregulin, lonidamine, Lundbeck LU-23-112, Lilly LY-186641, NCI (US) MAP, marycin, mefloquine, Merrel Dow MDL-27048, Medco MEDR-340, merbarone, merocyanine derivatives, methylanilinoacridine, Molecular Genetics MGI-136, minactivin, mitonafide, mitoquidone, mopidamol, motretinide, Zenyaku Kogyo MST-16, N-(retinoyl)amino acids, Nisshin Flour Milling N-021, N-acylated-dehydroalanines, nafazatrom, Taisho NCU-190, nocodazole derivative, Normosang, NCI NSC-145813, NCI NSC-361456, NCI NSC-604782, NCI NSC-95580, octreotide, Ono ONO-112, oquizanocine, Akzo Org-10172, paclitaxel, pancratistatin, pazelliptine, Warner-Lambert PD-111707, Warner-Lambert PD-115934, Warner-Lambert PD-131141, Pierre Fabre PE-1001, ICRT peptide D, piroxantrone, polyhaematoporphyrin, polypreic acid, Efamol porphyrin, probimane, procarbazine, proglumide, Invitron protease nexin I, Tobishi RA-700, razoxane, Sapporo Breweries RBS, restrictin-P, retelliptine, retinoic acid, Rhone-Poulenc RP-49532, Rhone-Poulenc RP-56976, Rituxan® (and other anti CD20 antibodies, e.g. Bexxar®, Zevalin®), SmithKline SK&F-104864, statins (Lipitor® etc.), Sumitomo SM-108, Kuraray SMANCS, SeaPharm SP-10094, spatol, spirocyclopropane derivatives, spirogermanium, Unimed, SS Pharmaceutical SS-554, strypoldinone, Stypoldione, Suntory SUN 0237, Suntory SUN 2071, superoxide dismutase, Thalidomide, Thalidomide analogs, Toyama T-506, Toyama T-680, taxol, Teijin TEI-0303, teniposide, thaliblastine, Eastman Kodak TJB-29, tocotrienol, Topostin, Teijin TT-82, Kyowa Hakko UCN-01, Kyowa Hakko UCN-1028, ukrain, Eastman Kodak USB-006, vinblastine sulfate, vincristine, vindesine, vinestramide, vinorelbine, vintriptol, vinzolidine, withanolides and Yamanouchi YM-534, Zometa®.

Examples of radioprotective agents which may be used in the combination chemotherapy of this invention are AD-5, adchnon, amifostine analogues, detox, dimesna, 1-102, MM-159, N-acylated-dehydroalanines, TGF-Genentech, tiprotimod, arnifostine, WR-151327, FUT-187, ketoprofen transdermal, nabumetone, superoxide dismutase (Chiron and Enzon).

Methods for preparation of the antineoplastic agents described above may be found in the literature. Methods for preparation of doxorubicin, for example, are described in U.S. Pat. Nos. 3,590,028 and 4,012,448. Methods for preparing metallomatrix protease inhibitors are described in EP 780386. Methods for preparing .alpha$_v$ .beta$_3$ inhibitors are described in WO 97/08174.

Preferred anti-neoplastic agents include, without limitation, one or more of daunorubicin, bleomycin, vincristine, doxorubicin, dacarbazine, prednisolone, mitoxantrone, prednisone, methotrexate, 5-flurouracil, dexamethasone, thalidomide, thalidomide derivatives, 2ME2, Neovastat, R 11 5777, arsenic trioxide, bortezomib, tamoxifen, G3139 (antisense), and SU5416, mitomycin, anti-CD20 antibodies, such as Rituxan® and R-etodolac.

Preferred drug regimens for which the present formulation may be used in conjunction with or as a replacement for one or more of the components includes, without limitation, ABVD (doxorubicin, bleomycin, vincristine, dacarbazine), DBV (daunorubicin, belomycin, vincristine), CVPP (cyclophosphamide, vinblastine, procarbazine, prednisolone), COP (cyclophosphamide, vincristine, prednisolone), CHOP (cyclophosphamide, doxorubicin,

vincristine and prednisone) and CMF (cyclophosphamide, methotrexate, 5-fluorouracil). Additional regimens are given in Table A below.

TABLE A

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
AC	Doxorubicin & Cyclophosphamide	Breast cancer
CFM (CF, FNC)	Cyclophosphamide, Fluorouracil, Mitaxantrone	Breast cancer
CMF	Cyclophosphamide, Methotrexate, Fluorouracil	Breast cancer
NFL	Mitoxantrone, Fluorouracil, Leucovorin	Breast cancer
Sequential Dox-CMF	Doxorubicin	Breast cancer
VATH	Vinblastine, Doxorubicin, Thiotepa, Fluoxymesterone	Breast cancer
EMA-86	Etoposide, Mitoxantrone, Cytarabine	AML (induction)
7 + 3	Cytarabine WITH Daunorubicin OR Idarobicin OR Mitoxantrone	AML (induction)
5 + 2	Cytarabine WITH Daunorubicin OR Mitoxantrone	AML (induction)
HiDAC	Cytarabine	AML (post-remission)
ABVD	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine	Hodgkin's
ChlVPP	Chlorambucil, Vinblastine, Procarbazine, Prednisone	Hodgkin's
EVA	Etoposide, Vinblastine, Doxorubicin	Hodgkin's
MOPP	Mechlorethamine, Vincristine, Procarbazine, Prednisone	Hodgkin's
MOPP/ABV Hybrid	Mechlorethamine, Vincristine, Procarbazine, Prednisone, Doxorubicin, Bleomycin, Vinblastine	Hodgkin's
MOPP/ABVD	Mechlorethamine, Doxorubicin, Vinblastine, Bleomycin, Etoposide, Prednisone	Hodgkin's
CNOP	Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone	Non-Hodgkin's

TABLE A-continued

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
COMLA	Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, Cytarabine	Non-Hodgkin's
DHAP	Dexamethasone, Cisplatin, Cytarabine	Non-Hodgkin's
ESHAP	Etoposide, Methylprednisilone, Cisplatin, Cytarabine	Non-Hodgkin's
MACOP-B	Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Vincristine, Prednisone, Bleomycin, Septra, Ketoconazole	Non-Hodgkin's
m-BACOD	Methotrexate, Leucovorin, Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, Dexamethasone	Non-Hodgkin's
MINE-ESHAP	Mesna, Ifosfamide, Mitoxantrone, Etoposide	Non-Hodgkin's
NOVP	Mitoxantrone, Vinblastine, Prednisone, Vincristine	Non-Hodgkin's
ProMACE/cytaBOM	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Cytarabine, Bleomycin, Vincristine, Methotrexate, Leucovorin, Septra	Non-Hodgkin's
M2	Vincristine, Carmustine, Cyclophosphamide, Melphalan, Prednisone	Multiple Myeloma
MP	Melphalan, Prednisone	Multiple Myeloma
VAD	Vincristine, Doxorubicin, Dexamethasone	Multiple Myeloma
VBMCP	Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone	Multiple Myeloma

As described herein, a lyophilized formulation of bendamustine is achieved following removal of an organic solvent in water. The most typical example of the solvent used to prepare this formulation is tertiary butanol (TBA). Other organic solvents can be used including ethanol, n-propanol, n-butanol, isoproponal, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, acetone, 1-pentanol, methyl acetate, methanol, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, cyclohexane. These preceding solvents may be used individually or in combination. Useful solvents must form stable solutions with bendamustine and must not appreciably degrade or deactivate the API. The solubility of bendamustine in the selected solvent must be high enough to form commercially useful concentrations of the drug in solvent. Additionally, the solvent should be capable of being removed easily from an aqueous dispersion or solution of the drug product, e.g., through lyophilization or vacuum drying. Preferably, a solution having a concentration of about 2-80 mg/mL, preferably about 5 to 40 mg/mL, more preferably 5-20 mg/mL and even more preferably 12 to 17 mg/mL bendamustine is used.

A pharmaceutically acceptable lyophilization excipient can be dissolved in the aqueous phase. Examples of excipients useful for the present invention include, without limitation, sodium or potassium phosphate, citric acid, tartaric acid, gelatin, glycine, and carbohydrates such as lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose and hetastarch. Mannitol is a preferred excipient. Other excipients that may

be used if desired include antioxidants, such as, without limitation, ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxyanisole, butyl-hydroxytoluene or alpha-tocopherol acetate, or chelators.

A typical formulation and lyophilization cycle useful in accordance with the present invention is provided below. Lyophilization can be carried out using standard equipment as used for lyophilization or vacuum drying. The cycle may be varied depending upon the equipment and facilities used for the fill/finish.

In accordance with a typical embodiment of the present invention, an aqueous pre-lyophilization solution or dispersion is first formulated in a pharmaceutically acceptable compounding vessel. The solution is aseptically filtered into a sterile container, filled into an appropriate sized vial, partially stoppered and loaded into the lyophilizer. Using lyophilization techniques described herein the solution is lyophilized until a moisture content in the range of about 0.1 to about 8.0 percent is achieved. The resulting lyophilization powder is stable as a lyophilized powder for about six months to greater than about 2 years, preferably greater than about 3 years at about 5° C. to about 25° C. and can be readily reconstituted with Sterile Water for Injection, or other suitable carrier, to provide liquid formulations of bendamustine, suitable for internal administration e.g., by parenteral injection. For intravenous administration, the reconstituted liquid formulation, i.e., the pharmaceutical composition, is preferably a solution.

The pre-lyophilization solution or dispersion normally is first formulated in a pharmaceutically acceptable container by: 1) adding an excipient, such as mannitol (about 0 to about 50 mg/mL) with mixing to water (about 65% of the total volume) at ambient temperature, 2) adding an organic solvent (0.5-99.9% v/v), such as TBA to the aqueous solution with mixing at about 20°-35° C., 4) adding bendamustine HCl to the desired concentration with mixing, 5) adding water to achieve the final volume, and 6) cooling the solution to about 1° C. to about 30° C., preferably about 5° C. Although the preceding steps are shown in a certain order, it is understood that one skilled in the art can change the order of the steps and quantities as needed. Quantities can be prepared on a weight basis also.

The pre-lyophilization solution or dispersion can be sterilized prior to lyophilization, sterilization is generally performed by aseptic filtration, e.g., through a 0.22 micron or less filter. Multiple sterilization filters can be used. Sterilization of the solution or dispersion can be achieved by other methods known in the art, e.g., radiation.

In this case, after sterilization, the solution or dispersion is ready for lyophilization. Generally, the filtered solution will be introduced into a sterile receiving vessel, and then transferred to any suitable container or containers in which the formulation may be effectively lyophilized. Usually the formulation is effectively and efficiently lyophilized in the containers in which the product is to be marketed, such as, without limitation, a vial, as described herein and as known in the art.

A typical procedure for use in lyophilizing the pre-lyophilization solutions or dispersions is set forth below. However, a person skilled in the art would understand that modifications to the procedure or process may be made depending on such things as, but not limited to, the pre-lyophilization solution or dispersion and lyophilization equipment.

Initially, the product is placed in a lyophilization chamber under a range of temperatures and then subjected to temperatures well below the product's freezing point, generally for several hours. Preferably, the temperature will be at or below about −40° C. for at least 2 hours. After freezing is complete, the chamber and the condenser are evacuated through vacuum pumps, the condenser surface having been previously chilled by circulating refrigerant. Preferably, the condenser will have been chilled below the freezing point of the solution preferably to about −40°, more preferably to about −50° C. or lower, even more preferably to about −60° C. or lower. Additionally, evacuation of the chamber should continue until a pressure of about 10 to about 600 microns, preferably about 50 to about 150 microns is obtained.

The product composition is then warmed under vacuum in the chamber and condenser. This usually will be carried out by warming the shelves within the lyophilizer on which the product rests during the lyophilization process at a pressure ranging from about 10 to about 600 microns. The warming process will optimally take place very gradually, over the course of several hours. For example, the product temperature should initially be increased from about −30° C. to about −101° C. and maintained for about 10-70 hours. Additionally, the product temperature can be increased from the freezing temperature to about 25° C.-40° C. over a period of 30-192 hours. To prevent powder ejection of the lyophilate from vials, complete removal of the organic solvent and water should be done during the initial drying phase. Complete drying can be confirmed by stabilization of vacuum, condenser temperature and product shelf temperature. After the initial drying, the product temperature should be increased to about 25° C.-40° C. and maintained for about 5-40 hours.

Once the drying cycle is completed, the pressure in the chamber can be slowly released to atmospheric pressure (or slightly below) with sterile, dry-nitrogen gas (or equivalent gas). If the product composition has been lyophilized in containers such as vials, the vials can be stoppered, removed and sealed. Several representative samples can be removed for purposes of performing various physical, chemical, and microbiological tests to analyze the quality of the product.

The lyophilized bendamustine formulation is typically marketed in pharmaceutical dosage form. The pharmaceutical dosage form of the present invention, although typically in the form of a vial, may be any suitable container, such as ampoules, syringes, co-vials, which are capable of maintaining a sterile environment. Such containers can be glass or plastic, provided that the material does not interact with the bendamustine formulation. The closure is typically a stopper, most typically a sterile rubber stopper, preferably a bromobutyl rubber stopper, which affords a hermetic seal.

After lyophilization, the bendamustine lyophilization powder may be filled into containers, such as vials, or alternatively the pre-lyophilization solution can be filled into such vials and lyophilized therein, resulting in vials which directly contain the lyophilized bendamustine formulation. Such vials are, after filling or lyophilization of the solution therein, sealed, as with a stopper, to provide a sealed, sterile, pharmaceutical dosage form. Typically, a vial will contain a lyophilized powder including about 10-500 mg/vial, preferably about 100 mg/vial, bendamustine and about 5 mg-2 g/vial, preferably about 170 mg/vial, mannitol.

The lyophilized formulations of the present invention may be reconstituted with water, preferably Sterile Water for Injection, or other sterile fluid such as co-solvents, to provide an appropriate solution of bendamustine for administration, as through parenteral injection following further dilution into an appropriate intravenous admixture container, for example, normal saline.

B. Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of alcohols commonly used in lyophilization, e.g., methanol, ethanol, pro-

panol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, combined with mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions at room temperature (see Table 1). Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

The results shown in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For the alcohols tested, the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time. Bendamustine did not precipitate immediately with any alcohol, but crystallized after storage at 5° C. Alcohols varied in their effect on solubility. Without wishing to be bound to any particular theory, smaller alcohols such as methanol and ethanol have less of an effect on solubility as compared with larger alcohols (tertiary-butanol and n-butanol). However, the shape of the alcohol is also important. For example n-propanol was found to be better than iso-propanol in preventing precipitation in this system. The two alcohols with the greatest effect on solubility were n-propanol and tertiary-butanol.

TABLE 1

Bendamustine solubility over a 24 hour period in various alcohols when stored at 5° C.

	Zero Time	3 Hours	6 Hours	24 Hours
Methanol (v/v)				
0% (Water Only)	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	Precipitate
30%	CCS	CCS	CCS	CCS
Ethanol (v/v)				
1.9%	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
n-Propanol (v/v/)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
Iso-propanol (v/v)				
5%	CCS	Precipitate	Precipitate	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
n-Butanol (v/v)				
5%	CCS	CCS	CCS	CCS
10%	CCS	CCS	CCS	CCS
20%	2 layers	2 layers	2 layers	2 layers
30%	2 layers	2 layers	2 layers	2 layers
Tert-Butanol (v/v)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS

CCS stands for clear colorless solution

Experiments to quantitatively determine the solubility of bendamustine at various temperatures for three different solutions are summarized in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment was based on stability studies (results described below). For both solutions tested, the solubility of bendamustine decreased linearly with temperatures from 25° C. to 0° C. This experiment confirmed the data shown in Table 1 and highlights the difference in bendamustine solubility for 20% and 30% TBA solutions.

TABLE 2

Solubility of bendamustine in TBA

	−8° C.	0° C.	5° C.	25° C.
20% (v/v) TBA				
25.5 mg/mL mannitol Water, q.s. to desired volume	14 mg/mL	11 mg/mL	17 mg/mL	47 mg/mL
30% (v/v) TBA				
25.5 mg/mL mannitol Water, q.s. to desired volume	20 mg/mL	18 mg/mL	27 mg/mL	65 mg/mL

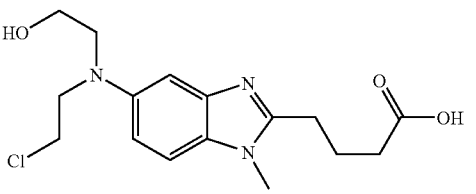
C. Stability

Because of its instability in aqueous solutions due to hydrolysis with water, bendamustine requires lyophilization in order to make a product suitable for pharmaceutical use. However, during the manufacturing of lyophilized drug products, aqueous solutions are commonly needed for filling, prior to lyophilization. Thus, the use of aqueous solutions during the compounding and fill processes for bendamustine and other nitrogen mustards can result in degradation of the drug product. Consequently, the effect of various alcohols on the degradation of bendamustine was evaluated to determine if formulations could be found that would allow longer fill-finish times, provide lyophilate powders that could be reconstituted more quickly than the current Ribomustin® formulation, and/or provide lyophilized preparations of bendamustine with a better impurity profile with respect to certain impurities, e.g., HP1, and BM1 dimer than Ribomustin®.

Preferably, a lyophilized preparation of the invention is stable with respect to HP1, i.e., the amount of HP1 does not increase appreciably (does not exceed the shelf-life specifications), for 6 months, more preferably 12 months, and even more preferably greater than 24 months, e.g., 36 months, when stored at about 2° C. to about 30° C., preferably 5° C.

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades rapidly in water alone and forms predominantly the hydrolysis product, HP1 (monohydroxy bendamustine).

Formula II



Monohydroxy bendamustine (HP1)

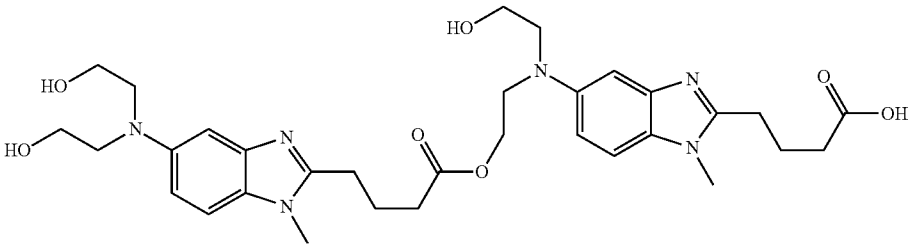
TABLE 3

Stability of bendamustine in water

	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
0% Alcohol, i.e., Water Alone	0 hours	99.11	0.60	0.11
	3 hours	98.83	0.86	0.13
	6 hours	98.44	1.22	0.17
	24 hours	95.67	3.81	0.29

The other major degradant observed during this study and other long term stability studies was the dimer of bendamustine.

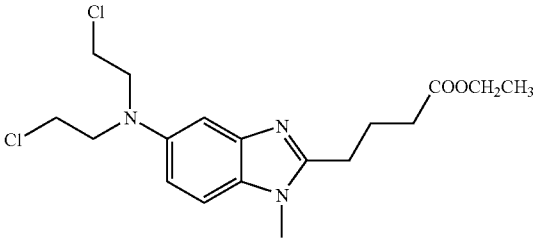
Formula III



Bendamustine Dimer (BM1 Dimer)

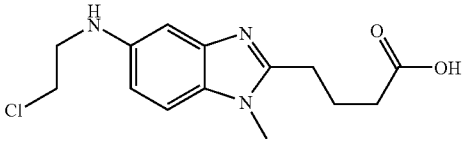
Other degradants contained in the Ribomustin lyophilized product are bendamustine ethylester (BM1EE) (Formula IV) and BM1DCE (Formula V). BM1EE is formed when bendamustine reacts with ethyl alcohol.

Formula IV



Bendamustine ethylester (BM1EE)

Formula V



BM1DCE

FIG. **2** summarizes the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the total peak area. The numerical values for FIG. **2** are provided in Tables 3-9. The purity was highest in solutions containing higher concentration of alcohols, regardless of the alcohol. Of the alcohols evaluated, bendamustine degraded the least in a solution containing about 30% (v/v) TBA. In about 10% and about 20% alcohol solutions, n-butanol was superior in preventing degradation of bendamustine. At 20% and 30% (v/v), n-butanol in water resulted in a biphasic system due to the insolubility of n-butanol in water at these concentrations.

FIGS. **3** and **4** show the amount of degradation of bendamustine as measured by HP1 and dimer formation quantified by HPLC (as described herein). HP1 and dimer formation increased as the amount of alcohol concentration decreased regardless of the alcohol. This increase in impurities occurred with an anticipated time dependence (see Tables 3-9). Tert-butanol and n-butanol appeared superior to other alcohols in preventing degradation of the product. As seen in Table 10, mannitol had no effect on the stabilization of bendamustine with TBA.

TABLE 4

HPLC stability results for the stability of bendamustine in various ethyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

V/V alcohol	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
1.9% Ethanol	0 hours	99.11	0.64	0.12
	3 hours	98.83	0.90	0.14
	6 hours	98.60	1.12	0.15
	24 hours	96.16	3.41	0.27
5% Ethanol	0 hours	99.31	0.44	0.12
	3 hours	99.10	0.64	0.13
	6 hours	98.87	0.86	0.14
	24 hours	96.89	2.68	0.25
10% Ethanol	0 hours	99.44	0.33	0.11
	3 hours	99.28	0.48	0.12
	6 hours	99.10	0.65	0.12
	24 hours	98.03	1.57	0.18
20% Ethanol	0 hours	99.54	0.22	0.10
	3 hours	99.45	0.30	0.11
	6 hours	99.36	0.39	0.11
	24 hours	98.61	0.96	0.15
30% Ethanol	0 hours	99.62	0.15	0.10
	3 hours	99.56	0.21	0.11
	6 hours	99.52	0.24	0.12
	24 hours	99.21	0.45	0.12

TABLE 5

HPLC stability results for bendamustine in various Tert-butanol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Tert-butanol	0 hours	99.34	0.41	0.12
	3 hours	99.10	0.64	0.14
	6 hours	98.85	0.88	0.13
	24 hours	97.58	2.09	0.20
10% Tert-butanol	0 hours	99.46	0.30	0.11
	3 hours	99.26	0.48	0.12
	6 hours	99.05	0.69	0.13
	24 hours	98.04	1.64	0.19

TABLE 5-continued

HPLC stability results for bendamustine in various Tert-butanol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
20% Tert-butanol	0 hours	99.59	0.17	0.11
	3 hours	99.48	0.29	0.11
	6 hours	99.35	0.40	0.12
	24 hours	98.35	1.27	0.20
30% Tert-butanol	0 hours	99.63	0.13	0.10
	3 hours	99.60	0.16	0.10
	6 hours	99.58	0.18	0.11
	24 hours	99.42	0.34	0.12

TABLE 6

HPLC stability results for various n-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% n-Propanol	0 hours	99.25	0.43	0.13
	3 hours	99.00	0.66	0.15
	6 hours	98.72	0.94	0.16
	24 hours	97.24	2.33	0.26
10% n-Propanol	0 hours	99.34	0.33	0.15
	3 hours	99.17	0.48	0.14
	6 hours	98.92	0.70	0.16
	24 hours	97.67	1.83	0.28
20% n-Propanol	0 hours	99.45	0.33	0.13
	3 hours	99.42	0.26	0.13
	6 hours	99.29	0.39	0.14
	24 hours	98.60	0.97	0.24
30% n-Propanol	0 hours	99.53	0.15	0.13
	3 hours	99.51	0.15	0.15
	6 hours	99.44	0.20	0.11
	24 hours	99.27	0.36	0.17

TABLE 7

HPLC stability results for bendamustine in various iso-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Iso-propanol	0 hours	99.21	0.48	0.13
	3 hours	98.65	0.72	0.14
	6 hours	98.56	1.02	0.14
	24 hours	96.14	3.35	0.26
10% Iso-propanol	0 hours	99.32	0.37	0.12
	3 hours	99.11	0.55	0.14
	6 hours	98.85	0.75	0.16
	24 hours	97.68	1.92	0.21
20% Iso-propanol	0 hours	99.49	0.21	0.11
	3 hours	99.39	0.31	0.12
	6 hours	99.22	0.42	0.13
	24 hours	98.61	1.04	0.17
30% Iso-propanol	0 hours	99.56	0.15	0.10
	3 hours	99.47	0.20	0.12
	6 hours	99.40	0.24	0.11
	24 hours	99.15	0.52	0.14

TABLE 8

HPLC stability results for bendamustine in various methyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Methanol	0 hours	99.35	0.40	0.12
	3 hours	98.97	0.70	0.14
	6 hours	98.66	0.95	0.14
	24 hours	96.65	2.83	0.23
10% Methanol	0 hours	99.42	0.34	0.11
	3 hours	99.01	0.59	0.12
	6 hours	98.86	0.80	0.12
	24 hours	97.65	1.85	0.18
20% Methanol	0 hours	99.56	0.22	0.11
	3 hours	99.31	0.38	0.11
	6 hours	98.99	0.50	0.12
	24 hours	98.31	1.15	0.16
30% Methanol	0 hours	99.59	0.18	0.10
	3 hours	99.43	0.27	0.11
	6 hours	99.25	0.34	0.11
	24 hours	98.65	0.76	0.13

TABLE 9

HPLC stability results for bendamustine in various n-butyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Butanol	0 hours	99.25	0.49	0.13
	3 hours	98.94	0.73	0.14
	6 hours	98.76	0.91	0.14
	24 hours	97.46	2.20	0.21
10% Butanol	0 hours	99.44	0.30	0.11
	3 hours	99.18	0.49	0.12
	6 hours	99.03	0.64	0.12
	24 hours	98.13	1.55	0.17
20% Butanol[a]	0 hours	99.54	0.23	0.10
	3 hours	99.45	0.31	0.11
	6 hours	99.30	0.40	0.11
	24 hours	98.81	0.91	0.14
30% Butanol[a]	0 hours	99.55	0.24	0.10
	3 hours	99.40	0.29	0.10
	6 hours	99.40	0.37	0.11
	24 hours	99.00	0.74	0.12

[a]Both solutions had 2 layers/phases of liquids in the vial. Solutions were vortexed prior to sample preparation.

The results in Tables 1-9 indicate that the stability of bendamustine HCl with respect to HP1 and dimer improves with increasing alcohol concentration.

TABLE 10

HPLC stability results for bendamustine in TBA with and without mannitol over a 24 hour period.

Sample	Purity (% Area)	HP1 (%)
TBA 20% (v/v) with Mannitol		
0 hours	99.59	0.17
24 hours @ 5° C.	99.35	1.27
TBA 20% (v/v) without Mannitol		
0 hours	100.0	0.00
24 hours @ 5° C.	98.80	1.21

NOTE:
The samples analyzed without mannitol were analyzed by HPLC using a normal phase method while the samples analyzed with mannitol used a reverse phase HPLC method. Slight variability may be seen in other samples analyzed between the two methods.

D. Lyophilization Cycle Development

Different pre-lyophilization formulations were prepared at various concentrations of bendamustine, mannitol, and alcohols in water. The cycle development was changed and optimized at each step for freezing (fast vs. slow), primary drying (both temperature and pressure), and secondary drying as described herein.

Based upon all of the information detailed above on solubility, stability, and ease of lyophilization, preferred formulations include the following:

Ingredients	Concentration
Bendamustine	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Alcohol	about 0.5%-40% (v/v)
Water, q.s. to	desired volume
wherein the alcohol is selected from methanol, n-propanol, or isopropanol	
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	1-20% (v/v)
Water, q.s. to	desired volume
wherein the alcohol is selected from methanol, n-propanol, or isopropanol	
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	5-40% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Alcohol	about 5-15% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Alcohol	about 10% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Butanol	about 0.5-20% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Butanol	about 10% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-100% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99.9% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 90-99% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Tertiary butanol	about 5-80% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Tertiary butanol	about 10-50% (v/v)
Water, q.s. to	desired volume

-continued

Ingredients	Concentration
Bendamustine HCl	about 12.5-15 mg/mL
Mannitol	about 0-30 mg/mL
Ethanol	about 20-30% (v/v)
Water, q.s. to	desired volume
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Tertiary butanol	about 30% (v/v)
Water, q.s. to	desired volume

EXAMPLES

The following Examples are provided to illustrate certain aspects of the present invention and to aid those of skill in the art in practicing the invention. These Examples are in no way to be considered to limit the scope of the invention in any manner.

Materials:

Bendamustine HCl, (Degussa, Lot #s 0206005 and 0206007)
Mannitol, NF or equivalent (Mallinckrodt)
Ethyl Alcohol Dehydrated (200 proof), USP or equivalent (Spectrum)
Tertiary-butyl alcohol, ACS (EM Science)
Methanol (Spectrum and EMD)
Propanol (Spectrum)
Iso-propanol (Spectrum)
Butanol (Spectrum)
Water, HPLC grade or equivalent (EMD)
Acetonitrile, HPLC grade or equivalent (EMD)
Trifluoroacetic Acid, J. T. Baker
Methanol, HPLC grade or equivalent (EM Science, Cat # MX0488P-1)
Trifluoroacetic Acid, HPLC grade or equivalent (JT Baker, Cat# JT9470-01)

Equipment:

Waters 2695 Alliance HPLC system with photodiode array detector
Waters 2795 Alliance HPLC system with dual wavelength detector
Analytical Balance (Mettler AG285, ID #1028) and (Mettler XS205)
VirTis Lyophilizer AdVantage
Agilent Zorbax SB-C18 5 μm 80 Å 4.6×250 mm column, Cat#880975-902

Example 1

HPLC Procedures

Method 1
Mobile Phase A: 0.1% TFA; H_2O
Mobile Phase B: 0.1% TFA; 50% ACN:50% H_2O
UV: 230 nm
Flow rate: 1.0 mL/min
Column temp.: 30° C.
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Sample temp.: 5° C.
Injection Volume: 10 μL
Sample Concentration: 0.25 mg/mL in MeOH
Gradient: 20% B for 1 min
20-90% B in 23 min
90% B for 6 min
back to 20% B in 1 min
hold at 20% B for 4 min

Run time: 30 min
Post run time: 5 min
Method 2
Mobile Phase A: 0.1% TFA; H_2O:ACN (9:1)
Mobile Phase B: 0.1% TFA; H_2O:ACN (5:5)
UV: 230 nm
Flow rate: 1.0 mL/min
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 10 μL
Sample Concentration: 0.25 mg/mL in MeOH
Gradient: 0% B for 3 min
0-50% B in 13 min
50-70% B in 17 min
70-90% B in 2 min
90% B for 5 min
back to 0% B in 1 min
hold at 0% B for 4 min
Run time: 40 min
Post run time: 5 min
Method 3
Phase A: HPLC grade water with 0.1% TFA (v/v)
Phase B: HPLC grade ACN/water (1:1 v/v) with 0.1% TFA (v/v)
UV: 254 nm
Flow rate: 1.0 mL/min
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 5 μL
Acquisition time: 30 min
Post time: 9 min
Diluent: methanol

Gradient:

Time (min.)	% Phase A	% Phase B
0.0	82	18
7.0	60	40
11.0	60	40
15.0	20	80
30.0	20	80
31.0	82	18

Sample preparation—dissolve the drug product with 200 mL MeOH. Sonicate 6 minutes. The solution can be injected directly into the HPLC (ca. 0.5 mg/mL)
Method 4
Phase A: HPLC grade water with 0.1% TFA (v/v)
Phase B: HPLC grade ACN with 0.1% TFA (v/v)
UV: 254 nm
Flow rate: 1.0 mL/min
Column: Zorbax Bonus RP-C14 5 μm 4.6×150 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 2 μL
Acquisition time: 31 min
Post time: 5 min
Diluent: NMP/0.1% TFA in water (50:50 v/v)

Gradient:

Time (min.)	% Phase A	% Phase B
0.0	93	7
5	93	7
13	73	27
16	73	27
25	10	90
31	10	90

Sample preparation for method 4—dissolve the drug product with a known amount of diluent to prepare a concentration of 4.2 mg/mL for injection directly into the HPLC. It may be necessary to perform a second dilution (the 100 mg/vial dosage form) to obtain a 4.2 mg/mL sample concentration.

Results

The retention times for some Bendamustine impurities using HPLC Method 1 described above are shown in Table 11. An HPLC chromatograph for Ribomustin® using the HPLC procedure described herein is shown in FIG. **6**.

TABLE 11

Retention Time for Bendamustine and some of its Impurities using HPLC Method 1

Sample Name	Retention Time (min)
HP1	14.110
Bendamustine	22.182
BM1 Dimer	24.824
BM1EE	26.968

Although HPLC Method 1 was capable of resolving impurities found in bendamustine it was not capable of separating a potential impurity formed during analysis, the methyl ester of bendamustine (BM1ME). The retention time difference between BM1ME and BM1 Dimer was only 0.3 minutes. In order to resolve BM1 Dimer, another HPLC method (#2) was developed. HPLC method #2 was capable of separating all the impurities but required a longer run time of 45 minutes (Table 12).

TABLE 12

Retention Time for bendamustine and impurities using HPLC Method 2.

Sample Name	Retention Time (min)
HP1	15.694
BM1	25.420
BM1ME	31.065
BM1 Dimer	32.467
BM1EE	36.038

The impurity profile of various lots of Ribomustin using HPLC Method 3 are shown in Table 13.

TABLE 13

Ribomustine Impurity Profile using HPLC Method 3
% Area

Batch	Bendamustine(HCl)	HP1	BM1EE	BM1 Dimer	BM1DCE
03H08	98.14	1.07	0.21	0.34	0.03
03H07	97.67	1.5	0.2	0.33	0.04
02K27	96.93	0.93	0.29	1.18	0.08
03C08	97.61	1.24	0.19	0.46	0.02

Example 2

Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of methanol, ethanol, propanol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions (Table 1) at room temperature. Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

Results summarized in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For all alcohols the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time.

The solubility of bendamustine was also determined in 20% (v/v) TBA containing 25.5 mg/mL mannitol in water, and 30% (v/v) TBA containing 25.5 mg/mL mannitol in water (FIG. **1**). Bendamustine was added to 4 mL of each solution while mixing until it would no longer dissolve. The saturated solutions were allowed to mix for 1 hour at −8° C., 0° C., 5° C., or 25° C. The samples were centrifuged and placed back at the original temperature for a minimum of 30 minutes. The −8° C. sample was placed into an ice bath containing sodium chloride, which lowers the temperature of the ice bath, and the temperature was measured when the sample was pulled for analysis. An aliquot of each sample was taken and prepared for HPLC analysis.

The results of these experiments are shown in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment (FIG. **1**) was based on stability studies described herein.

As indicated in FIG. **1**, the solubility of bendamustine decreased linearly with temperature (25° C. to 0° C.). The solubility of bendamustine was temperature dependant whether it was dissolved in water alone or with an alcohol. The 20% (v/v) TBA may likely be the lower limit required for efficient and robust pharmaceutical manufacturing due to the stability and solubility of bendamustine. A filling solution of 15 mg/mL bendamustine is close to the saturation limit of 17.2 mg/mL bendamustine at 5° C. but higher than the limit at 0° C. The 30% (v/v) TBA is the recommended concentration of TBA for the final formulation and is well within the solubility limit regardless of temperature.

Example 3

Stability

A. Stability in Water

Solutions of bendamustine (15 mg/mL), and mannitol (25.5 mg/mL) were prepared in water at room temperature and immediately placed in an ice bath (to lower the temperature quickly to about 5° C.) for 10 minutes and then refrigerated at 5° C. A sample of each formulation was analyzed by HPLC using the methods described herein after 0, 3, 6 and 24 hours when stored at 5° C.

B. Stability in Alcohols

Solutions containing 15 mg/mL bendamustine, 25.5 mg/mL mannitol, and 1.9%, 5%, 10%, 20% or 30% (v/v) ethyl alcohol in water or 5%, 10%, 20% or 30% (v/v) TBA, methanol, propanol, iso-propanol, or butanol in water were prepared at room temperature, placed into an ice bath for 10 minutes and then refrigerated at 5° C. A sample of each formulation was analyzed by HPLC after 0, 3, 6 and 24 hours when stored at 5° C.

C. Stability Results

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades quickly in water but the stability of bendamustine increases with increasing alcohol concentrations (FIGS. **2**, **3** and **4**). Although alcohols are frequently used in lyophilization to aid in solubility problems, the effect of alcohols on bendamustine stability is unique, unexpected and useful in manufacturing bendamustine with fewer impurities since an aqueous solution can be used while maintaining the stability of bendamustine. TBA was found to be the best stabilizer of the six alcohols tested (FIGS. **2**, **3**, and **4**). All alcohols at 30% (v/v) reduced the formation of impurities HP1 and Dimer at 5° C. for up to 24 hours. With respect to TBA, HP1 reaches only about 0.4% when stored at 5° C. for up to 24 hours. Lower concentrations of alcohol may not be efficient, when formulated at 15 mg/mL bendamustine and stored at 5° C. due to bendamustine precipitation and impurity formation.

Example 4

Formulation Optimization

After the solubility and stability of bendamustine were determined, the formulation was optimized for lyophilization. Since the concentration of bendamustine is higher in a 30% TBA/water saturated solution as compared with other alcohol solutions, it is anticipated that the vial size required to fill 100 mg of bendamustine can be decreased from the current Ribomustin® presentation. Although a saturated solution of bendamustine contains 18 mg/mL at 0° C., a concentration of 15 mg/mL was selected for the formulation to compensate for slight differences in API solubility due to differences in bulk API purity as a result of batch differences. A concentration of 15 mg/mL bendamustine requires 6.67 mL to fill 100 mg of bendamustine HCl per vial.

The surface (sublimation) area to volume ratio is critical to producing a lyophilized product with good appearance that freeze dries quickly. Generally, lyophilized products occupy between 30% to 50% of the vial volume. A 20 mL vial with 6.67 mL contains about 30% of its capacity and has a surface area ratio of 0.796 cm^2/mL.

Mannitol was selected as the bulking agent in order to maintain a formulation similar to Ribomustin®. Studies were performed to evaluate the effect of mannitol on bendamustine solubility and appearance of the product. Mannitol decreases the solubility of bendamustine (at 15 mg/mL) in both ethanol and TBA aqueous solutions. For example, solutions containing 5% and 10% ethanol and TBA without mannitol did not precipitate over 24 hours. However, for samples with mannitol (Table 1) precipitate was observed within 24 hours. There was no precipitate with aqueous solutions containing 30% (v/v) TBA, 15 mg/mL bendamustine, and 25.5 mg/mL mannitol. In order to maintain a well formed cake resistant to breakage during handling, a minimum of 134 mg/vial of mannitol was required with no difference observed in vials up to 200 mg/vial of mannitol.

All alcohols tested increased the stability and solubility of bendamustine. However, a significant mole fraction was required to affect the stability of the filling solution and the ease of manufacturing. Smaller alcohols have the undesirable effect of lowering the freezing point of the bulk solution and thus requiring long lyophilization cycles at lower tempera-

tures. Higher concentrations of methanol and ethanol produced unattractive cakes that were difficult to reconstitute. 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol, or 30% TBA aqueous solutions containing bendamustine (15 mg/mL), mannitol (25.5 mg/mL) were prepared and lyophilized. The lyophilized vials filled from solutions of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol produced either a collapsed cake or a film residue. The only solvent system producing an acceptable cake was 30% TBA. Additionally, reconstitution of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol lyophilized vials were difficult and did not fully dissolve until>45 minutes.

The ability to utilize a smaller vial is constrained by the concentration or solubility of bendamustine in the aqueous/organic solution. At lower concentrations of ethanol, methanol, isopropanol and n-propanol, which produced acceptable cake appearance, a more dilute solution of bendamustine is required due to solubility limitations. To maintain a presentation with 100 mg of bendamustine per vial, a vial larger than 50 mL would be required. Also, stability studies herein indicated that at the lower alcohol concentration, the chemical stability was not sufficient to allow for acceptable filling times.

One of the factors affecting the ease of reconstitution is the porosity of the lyophilate. In general, amorphously precipitated solids with little surface area are more difficult to solubilize. Most lyophilates containing mannitol will reconstitute within 3-5 minutes as long as there is no precipitate formed during lyophilization, frequently caused by evaporation of a liquid (melt back). Based on our experience with several lyophilization solvent systems and not wishing to be bound to any particular theory, the problems associated with Ribomustin® reconstitution may be associated with precipitation caused by melt back during lyophilization. Most organic solvents do not lyophilize efficiently and cause melt back because of their low melting point. TBA (tertiary butyl alcohol) has a high melting point and a similar vapor pressure as compared to water. TBA is removed by sublimation, not evaporation, at about the same rate as water. Lyophilates produced with 30% (v/v) TBA according to the invention reconstitute within 3-10 minutes as compare to commercially available Ribomustin which may take 30-45 minutes.

Based upon the solubility, stability, ease of reconstitution and manufacturing considerations, the following is a preferred pre-lyophilization formulation of the present invention: bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and q.s. using water for Injection. The formulation is then filled at 5° C. using 6.67 mL in an amber 20 mL, 20 mm vial and partially stoppered with a bromobutyl stopper and loaded into a pre-chilled lyophilizer.

Example 5

Impurity Assessment

Major impurities introduced during Ribomustin® manufacturing, compounding, fill, and lyophilization procedure, as determined by HPLC analysis (FIG. **6**), are the hydrolysis product HP1, the Dimer, and the ethyl ester of bendamustine, BM1EE. BM1EE can be formed during drug substance manufacturing, e.g., during recrystalization and/or purification processes. BM1EE is known to be a more potent cytotoxic drug than bendamustine. Experiments were undertaken to determine if the use of a 30% TBA aqueous filling solution would lead to the formation of bendamustine t-butyl ester.

Experiments were performed using traditional Fisher esterfication reaction conditions required for the formation of t-butyl ester of bendamustine. Bendamustine was heated in 60° C. TBA with HCl for 20 hours. No reaction was observed. This result indicated that it would be very difficult to form the tert-butyl ester of bendamustine during the fill/finish process. No new impurities in drug product manufactured from TBA have been observed in stability studies to date.

To aid in the testing of the drug product, synthetic routes using more reactive sources of the t-butyl moiety were developed. Another attempt to make tert-butyl ester was carried out by formation of the acyl chloride of bendamustine. A suspension of bendamustine in methylene chloride was treated with oxalyl chloride and N,N-dimethylformamide. After acyl chloride was formed, the solvent was concentrated. The residue was added to methylene chloride, tert-butanol, triethylamine, and 4-dimethylaminopyridine and the mixture was stirred at room temperature overnight. After adding all solvents and purification, an unknown compound was given. The LC-MS did not match the molecular weight of bendamustine tert-butyl ester and the proton NMR did not showed the peak for tert-butyl. Therefore, this attempt also failed to produce the bendamustine tert-butyl ester. Thus, using TBA as the co-solvent has an additional benefit of not forming the ester from the alcohol.

Example 6

Lyophilization Cycle Development

Numerous lyophilization cycles were performed to evaluate the critical stages of lyophilization and achieve the most efficient drying cycle. Experiments were performed to evaluate the effect of the freezing rate, primary drying temperature, time, and pressure on the product.

A. Freezing Rate

The literature reports that TBA adopts different crystal forms depending on the freeze rate. In some TBA solutions, the slower the product froze, the quicker it dried. Larger crystals formed during slow freezing producing bigger pores allowing more efficient sublimation. However, during studies with bendamustine, the freezing rate was not found to be a critical processing parameter when evaluated at 2 and 8 hours.

B. Primary and Secondary Drying

During the first attempts to lyophilize from 30% TBA solutions, the lyophilized cake fractured and powder was ejected from the vial. These cakes appeared to contain amorphous particles within the lyophilate, an indication of melt back. This phenomenon was reproducible and occurred when the product reached about −10° C. (refer to FIG. **5**) independent of the warming rate. Several variables were tested to determine the cause and solution to the problem of the powder ejection. The pressure was raised from 50 μm to 150 μm during primary drying, but powder ejection was still observed but to a lesser extent. This experiment was then repeated except the freezing rate was extended to 8 hours from 2 hours. This change had no effect.

The length of primary drying was next evaluated. For example, the following very slow drying cycle was evaluated: freezing from +25° C. to −50° C. in eight hours; holding at −50° C. for 5 hours, warming and drying from −50° C. to −25° C. in seven hours; holding for twenty hours at −25° C., warming and drying from −25° C. to −15° C. in two hours and holding for twenty hours at −15° C., warming and drying from −15° C. to 40° C. in six hours and holding for twenty hours at 40° C. while maintaining a chamber pressure of 150 μm throughout drying. No powder ejection (FIG. **5**) was

observed. This cycle resulted in a well-formed cake without fracture that reconstituted readily. Without wishing to be bound to a particular theory, the problems with powder ejection and difficulty with reconstitution may be the result of drying the lyophilate too quickly, thus resulting in strong vapor flow out of the cake as well as melt back. With the use of a less aggressive drying cycle an aesthetic, stable, and easy to reconstitute cake was reproducibly formed. Thus, removing all unbound water and tertiary-butyl alcohol prior to secondary drying may prevent melt back as well as powder ejection. The lyophilization cycle was further optimized under these gentle conditions (FIG. **5**). There were no immediate degradation products as a result of drying at 40° C. for up to 20 hours.

Example 7

Lyophilization Cycle

Step	Description	Time (Hour)	Temperature (° C.)	Pressure (Microns)
1	Hold	0.25	5° C.	—
2	Ramp	8	−50° C.	—
3	Hold	4	−50° C.	—
4	Ramp	3	−20° C.	150
5	Hold	6	−20° C.	150
6	Ramp	1	−15° C.	150
7	Hold	20	−15° C.	150
8	Ramp	0.5	−12° C.	150
9	Hold	15.5	−12 C.	150
10	Ramp	15	35 C.	50
11	Hold	10	35° C.	50
12	Ramp	1	40 C.	50
	Hold	5	40 C.	50
Total		89.25	—	—

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compostions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the spirit and scope of the invention. More specifically, it will be apparent that certain solvents which are both chemically and physiologically related to the solvents disclosed herein may be substituted for the solvents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit and scope of the invention as defined by the appended claims.

All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents, patent applications, and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

What is claimed is:

1. A pharmaceutical composition comprising bendamustine or bendamustine hydrochloride, mannitol, tertiary-butyl alcohol and water.

2. The pharmaceutical composition according to claim **1**, wherein said bendamustine or bendamustine hydrochloride is present at a concentration of about 12 to 17 mg/ml, said mannitol is present at a concentration of about 20-30 mg/ml, and said tertiary-butyl alcohol is present at a concentration of about 10-50% (v/v).

3. The pharmaceutical composition according to claim **2**, wherein said bendamustine or bendamustine hydrochloride is present at a concentration of about 15 mg/ml, said mannitol is present at a concentration of about 25.5. mg/ml, and said tertiary-butyl alcohol is present at a concentration of about 30% (v/v).

4. A lyophilized pharmaceutical composition made from the pharmaceutical composition according to claim **1**.

5. The lyophilized pharmaceutical composition according to claim **4**, wherein said bendamustine or bendamustine hydrochloride is present in said pharmaceutical composition at a concentration of about 12 to 17 mg/ml, said mannitol is present in said pharmaceutical composition at a concentration of about 20-30 mg/ml, and said tertiary-butyl alcohol is present in said pharmaceutical composition at a concentration of about 10-50% (v/v).

6. The lyophilized pharmaceutical composition according to claim **5**, wherein said bendamustine or bendamustine hydrochloride is present in said pharmaceutical composition at a concentration of about 15 mg/ml, said mannitol is present in said pharmaceutical composition at a concentration of about 25.5 mg/ml, and said tertiary-butyl alcohol is present in said pharmaceutical composition at a concentration of about 30% (v/v).

7. The lyophilized pharmaceutical composition according to claim **4** containing not more than about 0.5% bendamustine ethylester.

8. The lyophilized pharmaceutical composition according to claim **5** containing not more than about 0.5% bendamustine ethylester.

9. The lyophilized pharmaceutical composition according to claim **6** containing not more than about 0.5% bendamustine ethylester.

* * * * *

Exhibit C

US008609863B2

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

(10) **Patent No.: US 8,609,863 B2**
(45) **Date of Patent: *Dec. 17, 2013**

(54) **BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS**

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(*) 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.

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(51) **Int. Cl.**
C07D 235/04 (2006.01)

(52) **U.S. Cl.**
USPC **548/304.7**; 34/284

(58) **Field of Classification Search**
USPC 34/284; 548/304.7
See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides pharmaceutical formulations of lyophilized bendamustine suitable for pharmaceutical use. The present invention further provides methods of producing lyophilized bendamustine. The pharmaceutical formulations can be used for any disease that is sensitive to treatment with bendamustine, such as neoplastic diseases.

4 Claims, 6 Drawing Sheets

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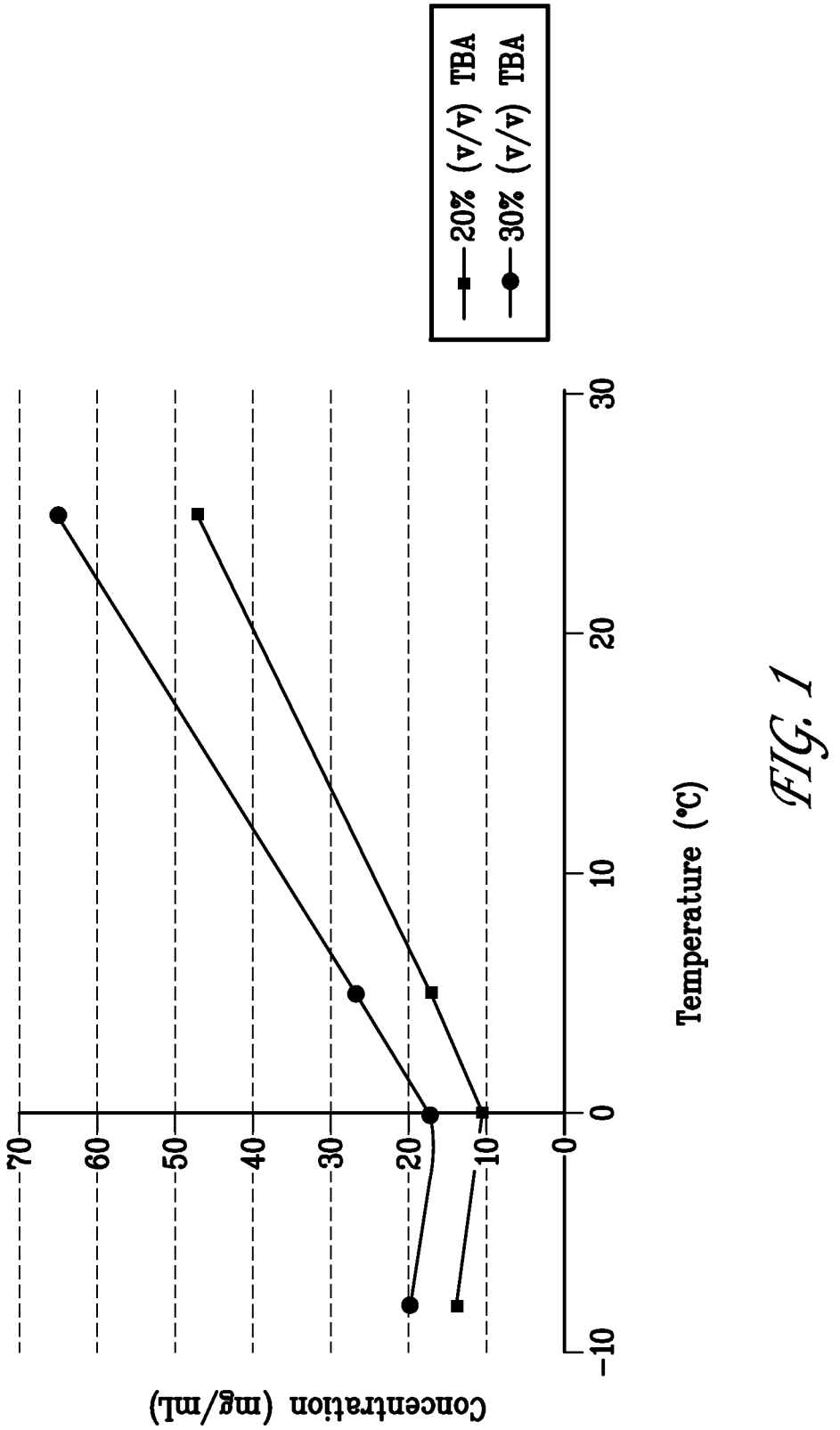

20% (v/v) TBA
30% (v/v) TBA
Concentration (mg/mL)
70
60
50
40
30
20
10
0
Temperature (°C)
30
20
10
0
-10

FIG. 1

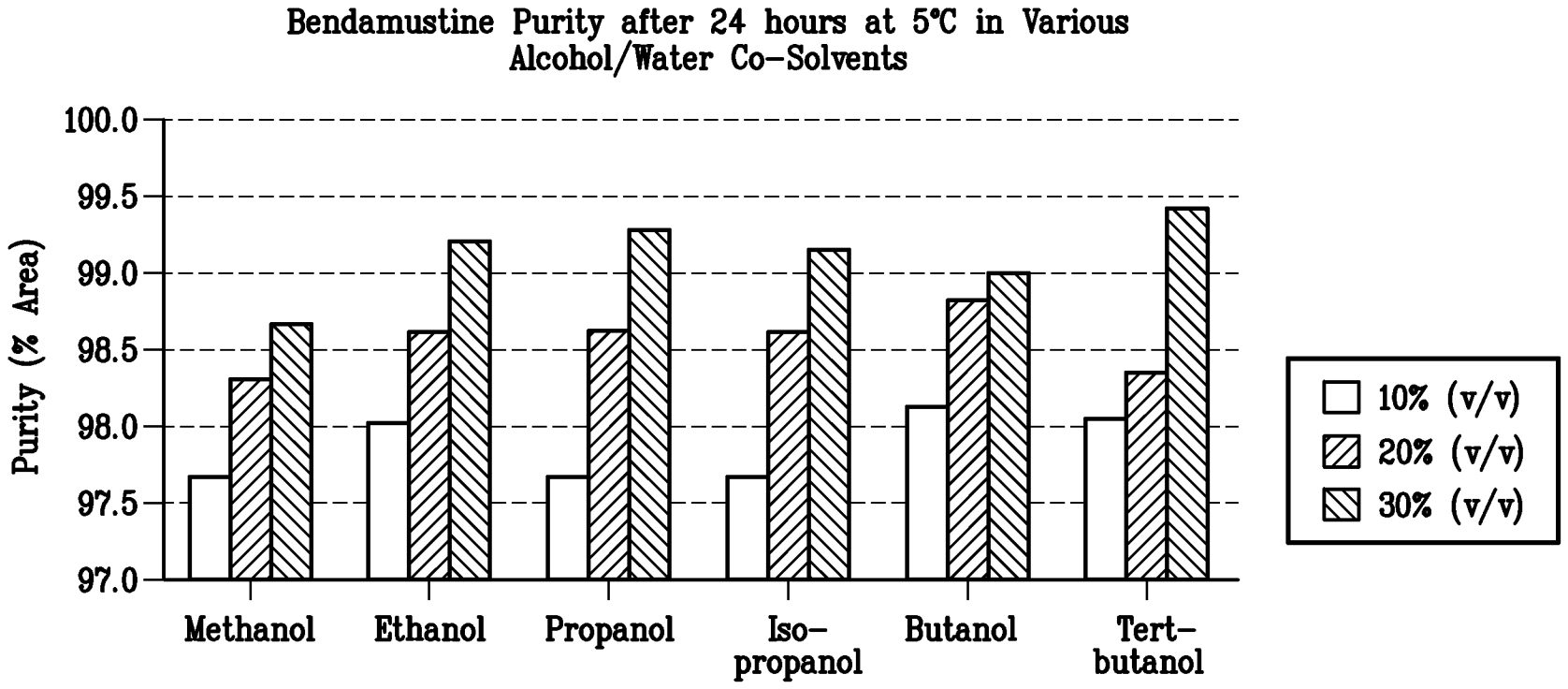

Bendamustine Purity after 24 hours at 5°C in Various Alcohol/Water Co-Solvents
Purity (% Area)
100.0
99.5
99.0
98.5
98.0
97.5
97.0
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol
10% (v/v)
20% (v/v)
30% (v/v)

FIG. 2

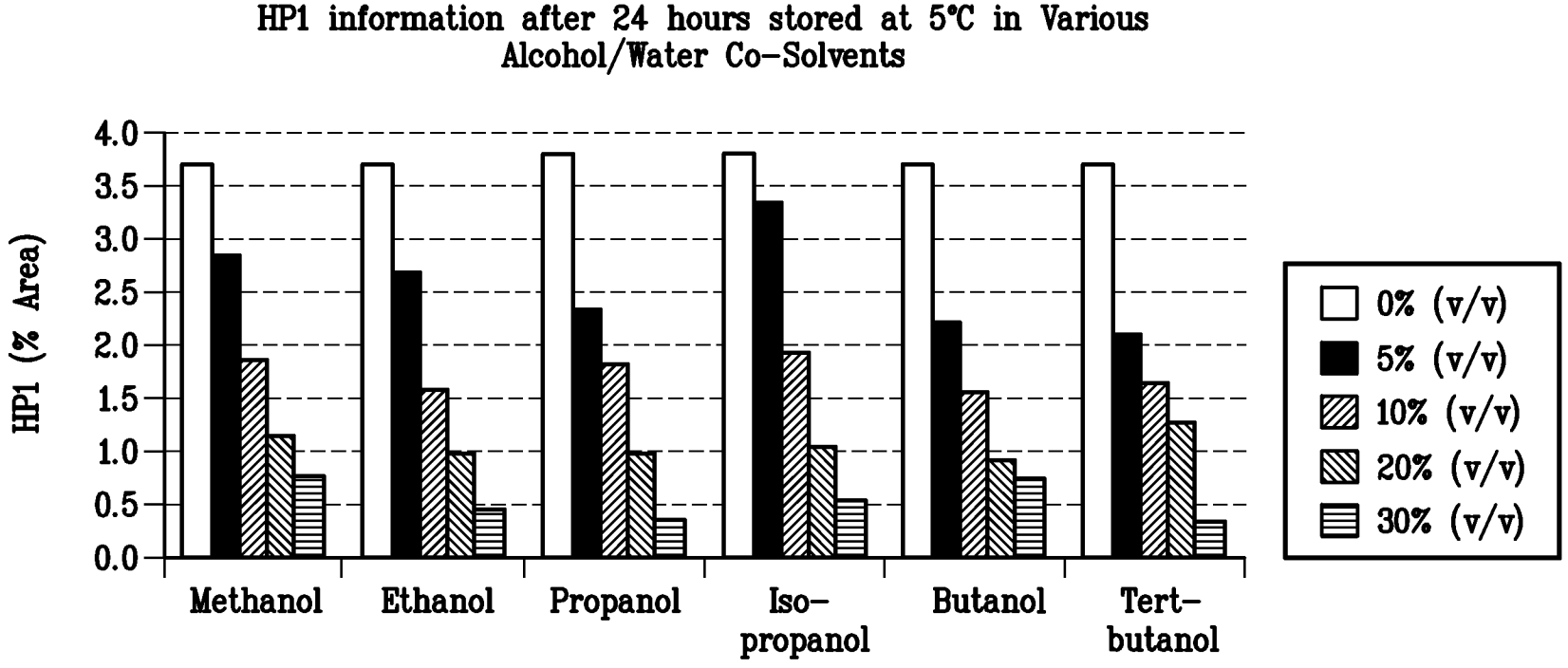

HP1 information after 24 hours stored at 5°C in Various Alcohol/Water Co-Solvents
HP1 (% Area)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol
0% (v/v)
5% (v/v)
10% (v/v)
20% (v/v)
30% (v/v)

FIG. 3

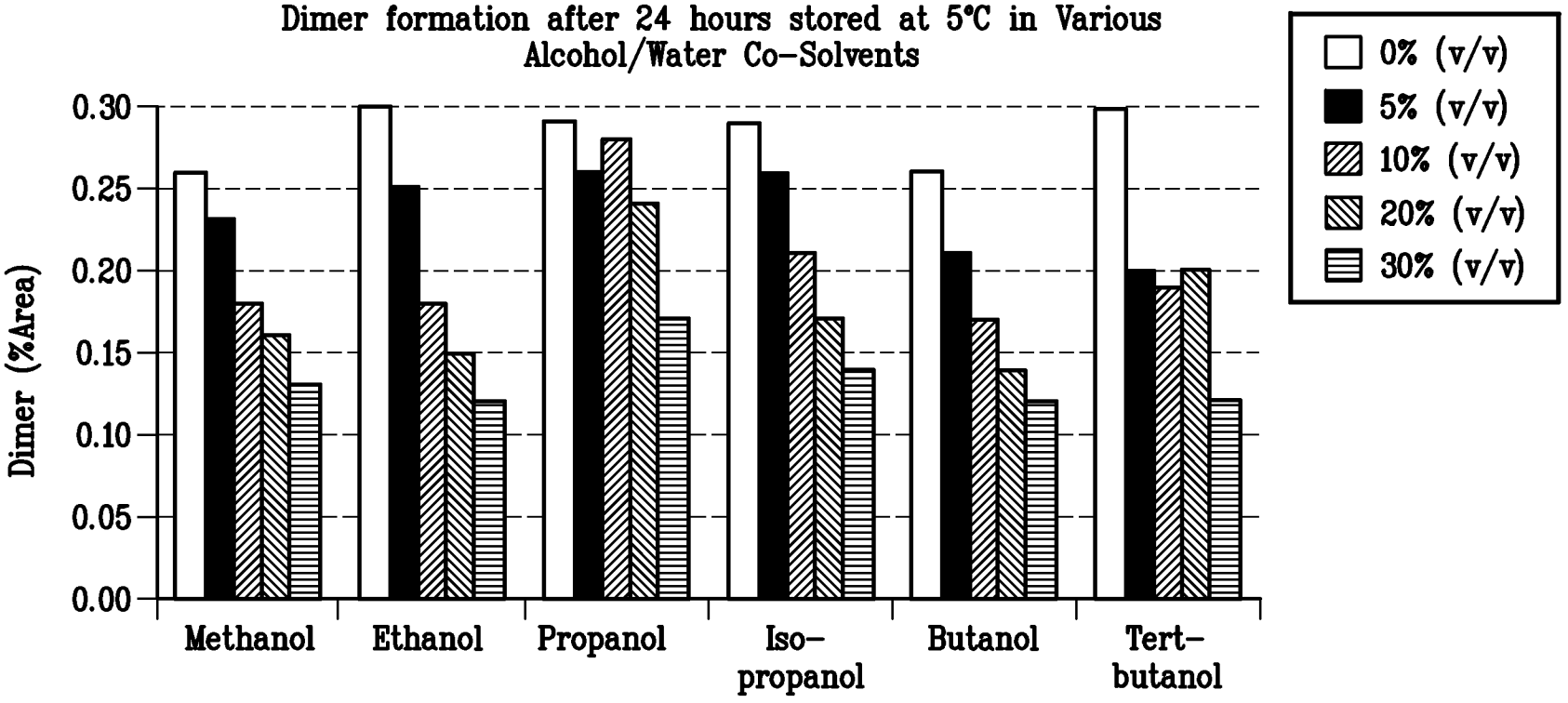

Dimer formation after 24 hours stored at 5°C in Various Alcohol/Water Co-Solvents
Dimer (%Area)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol
0% (v/v)
5% (v/v)
10% (v/v)
20% (v/v)
30% (v/v)

FIG. 4

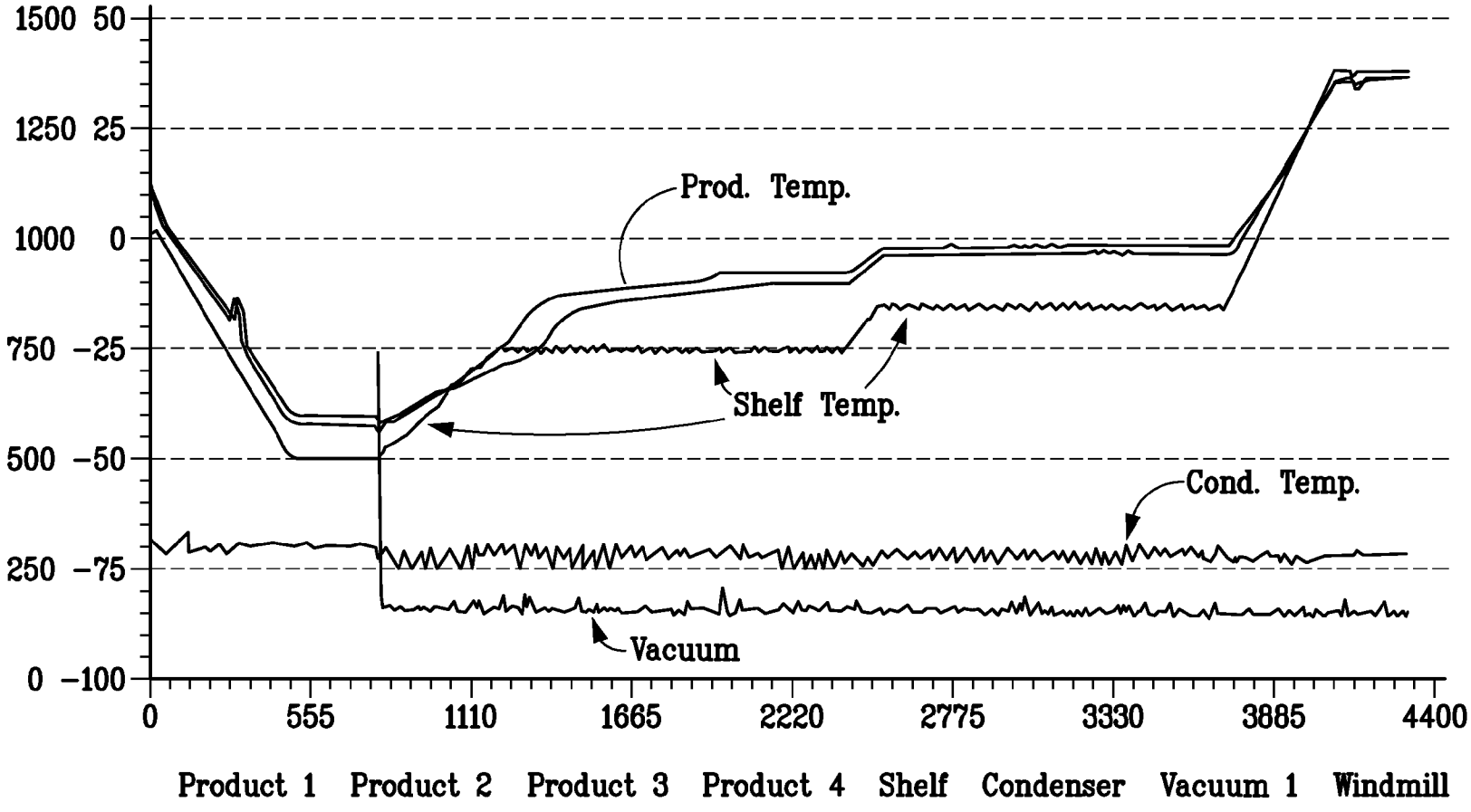

1500 50
1250 25
1000 0
750 -25
500 -50
250 -75
0 -100
Prod. Temp.
Shelf Temp.
Cond. Temp.
Vacuum
0
555
1110
1665
2220
2775
3330
3885
4400
Product 1
Product 2
Product 3
Product 4
Shelf
Condenser
Vacuum 1
Windmill

FIG. 5

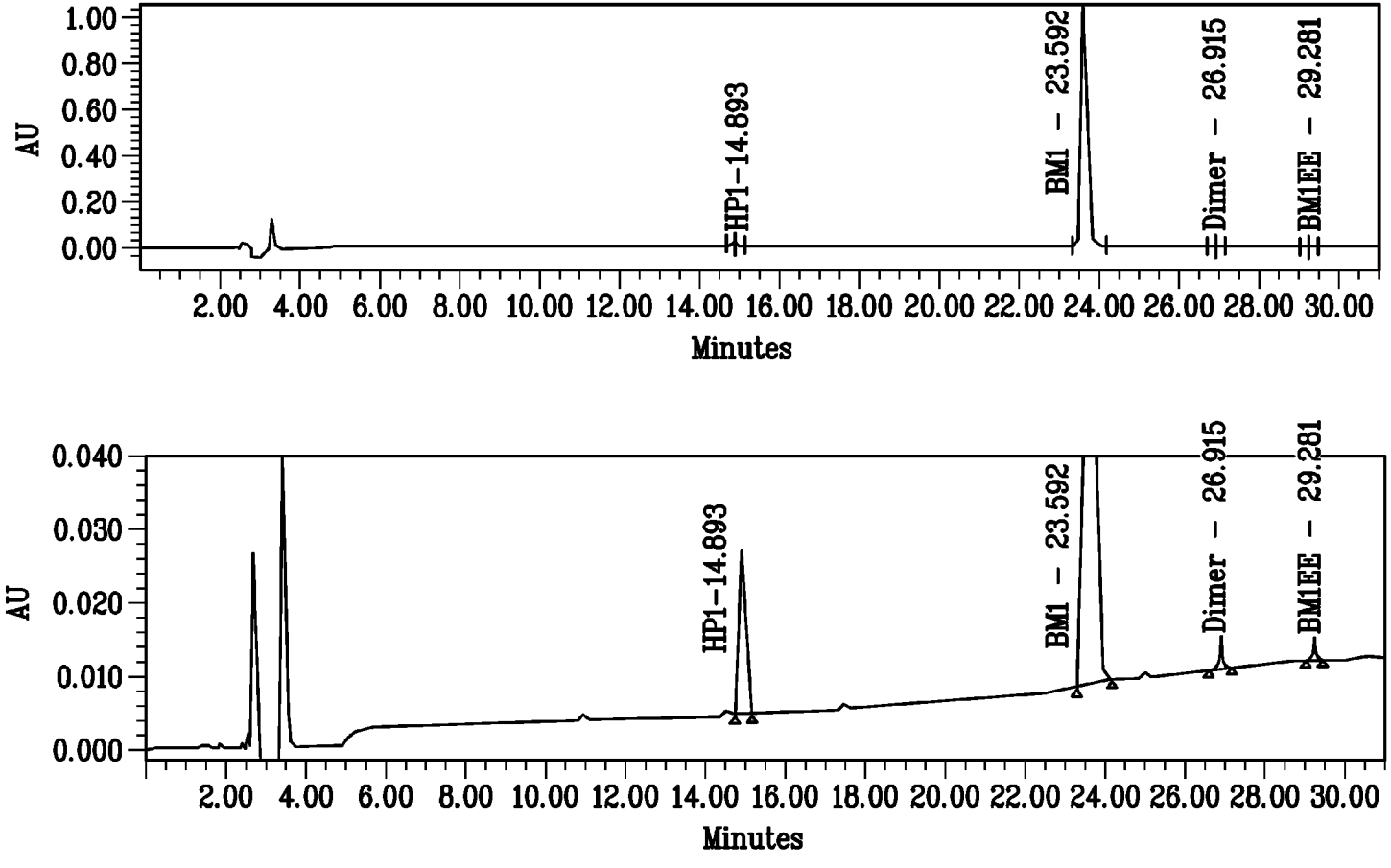

AU
1.00
0.80
0.60
0.40
0.20
0.00
HP1-14.893
BM1 - 23.592
Dimer - 26.915
BM1EE - 29.281
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00
Minutes
AU
0.040
0.030
0.020
0.010
0.000
HP1-14.893
BM1 - 23.592
Dimer - 26.915
BM1EE - 29.281
2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00
Minutes

FIG. 6

BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 13/654,898, filed Oct. 18, 2012, which is a continuation of U.S. application Ser. No. 11/330,868, filed Jan. 12, 2006, which claims the benefit of U.S. Provisional Application No. 60/644,354, filed Jan. 14, 2005, the entireties of which are incorporated herein for all purposes.

FIELD OF THE INVENTION

The present invention pertains to the field of pharmaceutical compositions for the treatment of various disease states, especially neoplastic diseases and autoimmune diseases. Particularly, it relates to pharmaceutical formulations comprising nitrogen mustards, particularly the nitrogen mustard bendamustine, e.g., bendamustine HCl.

BACKGROUND OF THE INVENTION

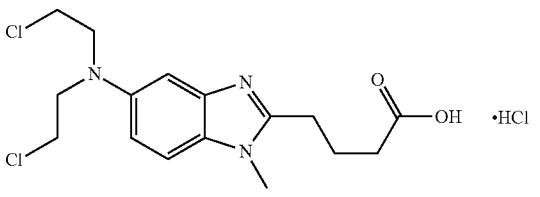
The present invention claims the benefit of and priority to U.S. Ser. No. 60/644,354, filed Jan. 14, 2005, entitled, "Bendamustine Pharmaceutical Compositions," which is incorporated herein by reference in its entirety, including figures and claims.

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

Because of their high reactivity in aqueous solutions, nitrogen mustards are difficult to formulate as pharmaceuticals and are often supplied for administration in a lyophilized form that requires reconstitution, usually in water, by skilled hospital personal prior to administration. Once in aqueous solution, nitrogen mustards are subject to degradation by hydrolysis, thus, the reconstituted product should be administered to a patient as soon as possible after its reconstitution.

Bendamustine, (4-{5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl}butyric acid, is an atypical structure with a benzimidazole ring, whose structure includes an active nitrogen mustard (see Formula I, which shows bendamustine hydrochloride).

Formula I



Bendamustine was initially synthesized in 1963 in the German Democratic Republic (GDR) and was available from 1971 to 1992 in that location under the name Cytostasan®. Since that time, it has been marketed in Germany under the tradename Ribomustin®. It has been widely used in Germany to treat chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer.

Due to its degradation in aqueous solutions (like other nitrogen mustards), bendamustine is supplied as a lyophilized product. The current lyophilized formulation of bendamustine (Ribomustin®) contains bendamustine hydrochloride and mannitol in a sterile lyophilized form as a white powder for intravenous use following reconstitution. The finished lyophilisate is unstable when exposed to light. Therefore, the product is stored in brown or amber-colored glass bottles. The current lyophilized formulation of bendamustine contains degradation products that may occur during manufacturing of the drug substance and/or during the lyophilization process to make the finished drug product.

Currently bendamustine is formulated as a lyophilized powder for injection with 100 mg of drug per 50 mL vial or 25 mg of drug per 20 mL vial. The vials are opened and reconstituted as close to the time of patient administration as possible. The product is reconstituted with 40 mL (for the 100 mg presentation) or 10 mL (for the 25 mg presentation) of Sterile Water for Injection. The reconstituted product is further diluted into 500 mL, q.s., 0.9% Sodium Chloride for Injection. The route of administration is by intravenous infusion over 30 to 60 minutes.

Following reconstitution with 40 mL Sterile Water for Injection, vials of bendamustine are stable for a period of 7 hours under room temperature storage or for 6 days upon storage at 2-8° C. The 500 mL admixture solution must be administered to the patient within 7 hours of vial reconstitution (assuming room temperature storage of the admixture).

The reconstitution of the present bendamustine lyophilized powder is difficult. Reports from the clinic indicate that reconstitution can require at least fifteen minutes and may require as long as thirty minutes. Besides being burdensome and time-consuming for the healthcare professional responsible for reconstituting the product, the lengthy exposure of bendamustine to water during the reconstitution process increases the potential for loss of potency and impurity formation due to the hydrolysis of the product by water.

Thus, a need exists for lyophilized formulations of bendamustine that are easier to reconstitute and which have a better impurity profile than the current lyophilate (lyophilized powder) formulations of bendamustine.

German (GDR) Patent No. 34727 discloses a method of preparing ω-[5-bis-(β-chloroethyl)-amino-benzimidazolyl-(2)]-alkane carboxylic acids substituted in the 1-position.

German (GDR) Patent No. 80967 discloses an injectable preparation of γ-[1-methyl-5-bis-(β-chloroethyl)-amino-benzimaidazolyl-(2)]-butric acid hydrochloride.

German (GDR) Patent No. 159877 discloses a method for preparing 4-[1-methyl-5-bis(2-chloroethyl)amino-benzimidazolyl-2)-butyric acid.

German (GDR) Patent No. 159289 discloses an injectable solution of bendamustine.

Ribomustin® bendamustine Product monograph (updated January 2002) http://www.ribosepharm.de/pdf/ribomustin_bendamustin/productmonograph.pdf provides information about Ribomustin® including product description.

Ni et al. report that the nitrosourea SarCNU was more stable in pure tertiary butanol than in pure acetic acid, dimethyl sulfoxide, methylhydroxy, water or in TBA/water mixtures (Ni et al. (2001) *Intl. J. Phamaceutics* 226:39-46).

Lyophilized cyclophoshamide is known in the art see e.g., U.S. Pat. Nos. 5,418,223; 5,413,995; 5,268,368; 5,227,374; 5,130,305; 4,659,699; 4,537,883; and 5,066,647.

The lyophilized nitrogen mustard Ifosfamide is disclosed in International Publication No. WO 2003/066027; U.S. Pat. Nos. 6,613,927; 5,750,131; 5,972,912; 5,227,373; and 5,204,335.

Teagarden et al. disclose lyophilized formulations of prostaglandin E-1 made by dissolving PGE-1 in a solution of lactose and tertiary butyl alcohol (U.S. Pat. No. 5,770,230).

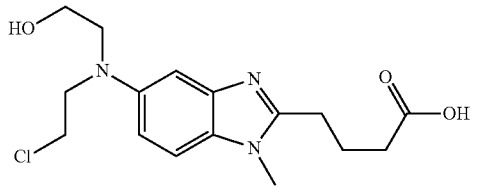
SUMMARY OF THE INVENTION

The present invention is directed to stable pharmaceutical compositions of nitrogen mustards, in particular lyophilized

bendamustine and its use in treatment of various disease states, especially neoplastic diseases and autoimmune diseases.

An embodiment of the invention is a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1, as shown in Formula II,

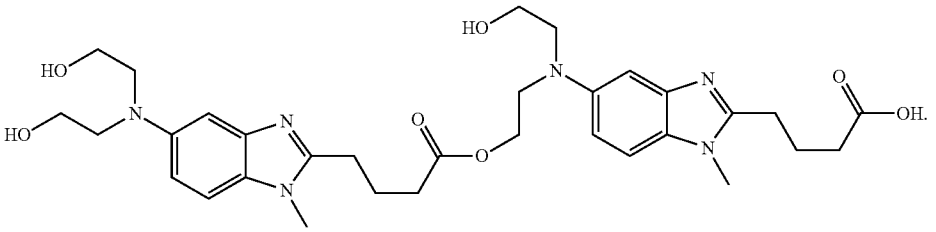
Formula II



at the time of release or where the HP1 is the amount of HP1 present at time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as described herein. In a preferred embodiment is a pharmaceutical composition of bendamustine containing not more than about 0.5% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%.

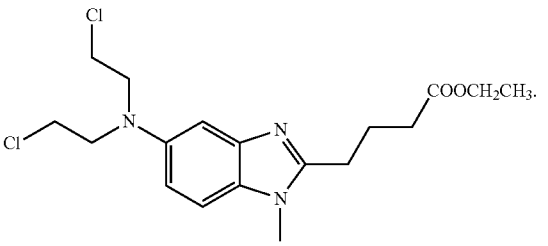
Another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.1% to about 0.3% bendamustine dimer as shown in Formula III at release or at time zero after reconstitution

Formula III



Yet another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5%, preferably 0.15% to about 0.5%, bendamustine ethylester, as shown in Formula IV at release or at time zero after reconstitution

Formula IV



Yet another embodiment of the invention is a lyophilized preparation of bendamustine wherein the concentration of

bendamustine ethylester (Formula IV) is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the lyophilized preparation.

In another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1 at the time of drug product release. In a preferred embodiment is a lyophilized preparation of bendamustine containing not more than about 0.50% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%. An aspect of this embodiment is lyophilized preparations of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 at the time of release of drug product where the lyophilized preparation is packaged in a vial or other pharmaceutically acceptable container.

In yet another aspect of the invention, the lyophilized preparations of bendamustine are stable with respect to the amount of HP1 for at least about 6 months, preferably 12 months, preferably 24 months, to about 36 months or greater when stored at about 2° to about 30°. Preferred temperatures for storage are about 5° C. and about room temperature.

Another embodiment of the invention is a pharmaceutical dosage form that includes a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% HP1, preferably not more than about 0.50%, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%, where the HP1 is the amount of HP1 present at release or at time zero after reconstitution of a lyophilized preparation of bendamustine of the present invention. In preferred aspects of the invention, the dosage form can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

Yet another embodiment of the invention is a pharmaceutical dosage form that includes a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1. Preferred dosage forms can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

In still another embodiment, the invention includes a pharmaceutical composition of bendamustine including bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine), preferably not more than about 0.50%, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not

more than about 0.35%, even more preferably not more than 0.30%, and a trace amount of one or more organic solvents, wherein said HP1 is the amount of HP1 present at release or time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as disclosed herein. In different aspects of this embodiment, the organic solvent is selected from one or more of tertiary butanol, n-propanol, n-butanol, isopropanol, ethanol, methanol, acetone, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, 1-pentanol, methyl acetate, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, and cyclohexane. Preferred organic solvents include one or more of ethanol, methanol, propanol, butanol, isopropanol, and tertiary butanol. A more preferred organic solvent is tertiary butanol, also known as TBA, t-butanol, tert-butyl alcohol or tertiary butyl alcohol.

The present invention involves a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a release specification for bendamustine degradants at less than about 4.0%, preferably about 2.0% to about 4.0%, (area percent bendamustine) or otherwise to achieve the pharmaceutical compositions described herein. An aspect of this embodiment is a method for obtaining agency approval for a bendamustine product which includes setting a release specification for HP1 to be less than or equal to 1.5% (area percent Bendamustine). The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment is a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a shelf-life specification for bendamustine degradants at less than about 7.0%, preferably about 5.0% to about 7.0%, (area percent bendamustine) where the product is stored at about 2° C. to about 30° C. Preferred temperatures for storage are about 5° C. and about room temperature. The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment of the invention is a process for manufacturing a lyophilized preparation of bendamustine which includes controlling for the concentration of bendamustine degradants in the final product, such that the concentration of bendamustine degradants is less than about 4.0%, preferably no more than about 2.0% to about 4.0%, (area percent of bendamustine) at release or otherwise to achieve the pharmaceutical compositions described herein. The bendamustine product herein contains not more than about 0.5% to about 0.9%, preferably about 0.5%, (area percent of bendamustine) HP1 at release.

The present invention discloses a process for manufacturing a lyophilized preparation of bendamustine which comprises controlling for the concentration of bendamustine degradants in the final product, such that, at release, the concentration of HP1 is less than 0.9%, preferably 0.5%, (area percent of bendamustine) and, at the time of product expiration, the concentration of bendamustine degradants is less than about 7.0%, preferably no more than about 5.0% to about 7.0%; wherein said product is stored at about 2° C. to about 30° C.

Another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of HP1 produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% to about 0.9% (area percent of bendamustine) preferably 0.50%, preferably 0.45%, more preferably 0.40%, more preferably 0.35%, even more preferably 0.30%. An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% (area percent bendamustine). An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester (as shown in Formula IV) produced during lyophilization from about 0 to 24 hours is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the pre-lyophilization solution. A preferred organic solvent is tertiary butanol.

The invention also discloses methods for preparing a bendamustine lyophilized preparation that includes dissolving bendamustine in a stabilizing concentration of an alcohol solvent of between about 5% to about 100% (v/v alcohol to form a pre-lyophilization solution; and lyophilizing the pre-lyophilization solution; wherein the bendamustine lyophilized preparation made from such methods contains not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 as shown in Formula II, wherein said HP1 is the amount of HP1 present at release or at time zero after reconstitution of the lyophilized pharmaceutical composition of bendamustine. Other alcohol concentrations include about 5% to about 99.9%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 40%, about 20% to about 35%. Preferred concentrations of alcohol are from about 20% to about 30%. Preferred alcohols include one or more of methanol, ethanol, propanol, iso-propanol, butanol, and tertiary-butanol. A more preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

In a preferred method for preparing a bendamustine lyophilized preparation, lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to a temperature below about −40° C., preferably −50° C., to form a frozen solution; ii) holding the frozen solution at or below −40° C., preferably −50° C., for at least 2 hours; iii) ramping the frozen solution to a primary drying temperature between about −40° C. and about −10° C. to form a dried solution; iv) holding for about 10 to about 70 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vii) holding for about 5 to about 40 hours to form a bendamustine lyophilized preparation. In a more preferred method lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to about −50° C. to form a frozen solution; ii) holding the frozen solution at about −50° C. for at least 2 hours to about 4 hours; iii) ramping to a primary drying temperature between about −20° C. and about −12° C. to form a dried solution; iv)

holding at a primary drying temperature for about 10 to about 48 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vi) holding at a secondary drying temperature for at least 5 hours up to about 20 hours. A preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

Another embodiment of the invention is the lyophilized powder or preparation obtained from the methods of preparing a bendamustine lyophilized preparation disclosed herein.

The invention also involves bendamustine formulations for lyophilization that include an excipient and a stabilizing concentration of an organic solvent. A preferred formulation includes bendamustine at a concentration of about 15 mg/mL, mannitol at a concentration of about 25.5 mg/mL, tertiary-butyl alcohol at a concentration of about 30% (v/v) and water. Included in this embodiment of the invention are the lyophilized preparations made from such bendamustine formulations.

Included in the inventions are methods of treating a medical condition in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition. Some conditions amenable to treatment with the compositions of the invention include chronic lymphocytic leukemia (CLL), Hodgkin's disease, non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), breast cancer, small cell lung cancer, hyperproliferative disorders, and an autoimmune disease. Preferred conditions include NHL, CLL, breast cancer, and MM. Preferred autoimmune diseases include rheumatoid arthritis, multiple sclerosis or lupus.

Included in the inventions are the use of the pharmaceutical compositions or pharmaceutical preparations of the invention in the manufacture of a medicament for the treatment of a medical condition, as defined herein, in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition.

Also included in the invention are methods of treating in which the pharmaceutical compositions of the invention are in combination with one or more anti-neoplastic agents where the antineoplastic agent is given prior, concurrently, or subsequent to the administration of the pharmaceutical composition of the invention. Preferred antineoplastic agents are antibodies specific for CD20.

Another embodiment of the invention is a lyophilization cycle for producing lyophilized bendamustine preparations of the invention. A preferred lyophilization cycle includes a) freezing to about −50° C. over about 8 hours; b) holding at −50° C. for about 4 hours; c) ramping to −25° C. over about 3 hours; d) holding at −10° C. for 30 hours; e) ramping to between about 25° C. and about 40° C. or higher for about 3 hours; f) holding between about 25° C. and about 40° C. for about 25 hours; g) ramping to about 20° C. in 1 hour; h) unloading at about 20° C., at a pressure of 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. An aspect of this cycle involves step (e) which is ramped to about 30-35° C. for 3 hours and then ramped to 40° C. for 5 hours. Another aspect of this embodiment is the lyophilized powered prepared from such lyophilization cycles. A more preferred lyophilization cycle includes i) starting with a shelf temperature of about 5° C. for loading; ii) freezing to about −50° C. over about 8 hours; iii) holding at −50° C. for about 4 hours; iv) ramping to about −20° C. over about 3 hours; v) holding at about −20° C. for 6 hours; ramping to about −15° C. over about 1 hour; vi) holding at −15° C. for about 20 hours; vii) ramping to about −15° C. over about 1 hour; viii) holding at about −15° C. for about 20 hours; ix) ramping to about −12° C. over about 0.5 hours; x) holding at about −12° C. for about 15.5 hours; xi) ramping to between about 25° C. and about 40° C. or higher for about 15 hours; xii) holding between about 25° C. and about 40° C. for about 10 hours; xiii) ramping to about 40° C. over about 1 hour; and xiv) holding at about 40° C. for about 5 hours; unloading at about 5° C., at a pressure of about 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. In a preferred embodiment step (xi) is ramped to about 30-35° C. for about 15 hours.

The invention also encompasses a pharmaceutical dosage form of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1 (area percent of bendamustine) wherein said dosage form comprises a vial or other pharmaceutically acceptable container, wherein said HP1 is the amount of HP1 present pre-reconstitution or at time zero after reconstitution of said dosage form. Preferred concentrations of bendamustine include about 10 to about 500 mg/container, about 100 mg/container, about 5 mg to about 2 g/container and about 170 mg/container.

The present invention also includes pre-lyophilized pharmaceutical compositions of bendamustine. A preferred pre-lyophilized composition includes bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and water.

These and other embodiments of the invention are described hereinbelow or are evident to persons of ordinary skill in the art based on the following disclosures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the solubility of bendamustine at various temperatures for two different solutions of bendamustine in tertiary butanol.

FIG. **2** shows the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the bendamustine peak.

FIG. **3** shows HP1 (Formula II) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **4** shows dimer (Formula III) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **5**-*shows* a lyophilization cycle for bendamustine using a TBA/water co-solvent.

FIG. **6** shows a chromatogram for Ribomustin® using HPLC method No. 1.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the terms "formulate" refers to the preparation of a drug, e.g., bendamustine, in a form suitable for administration to a mammalian patient, preferably a human. Thus, "formulation" can include the addition of pharmaceutically acceptable excipients, diluents, or carriers.

As used herein, the term "lyophilized powder" or "lyophilized preparation" refers to any solid material obtained by

lyophilization, i.e., freeze-drying of an aqueous solution. The aqueous solution may contain a non-aqueous solvent, i.e. a solution composed of aqueous and one or more non-aqueous solvent(s). Preferably, a lyophilized preparation is one in which the solid material is obtained by freeze-drying a solution composed of aqueous and one or more non-aqueous solvents, more preferably the non-aqueous solvent is an alcohol.

By "stable pharmaceutical composition" is meant any pharmaceutical composition having sufficient stability to have utility as a pharmaceutical product. Preferably, a stable pharmaceutical composition has sufficient stability to allow storage at a convenient temperature, preferably between −20° C. and 40° C., more preferably about 2° C. to about 30° C., for a reasonable period of time, e.g., the shelf-life of the product which can be as short as one month but is typically six months or longer, more preferably one year or longer even more preferably twenty-four months or longer, and even more preferably thirty-six months or longer. The shelf-life or expiration can be that amount of time where the active ingredient degrades to a point below 90% purity. For purposes of the present invention stable pharmaceutical composition includes reference to pharmaceutical compositions with specific ranges of impurities as described herein. Preferably, a stable pharmaceutical composition is one which has minimal degradation of the active ingredient, e.g., it retains at least about 85% of un-degraded active, preferably at least about 90%, and more preferably at least about 95%, after storage at 2-30° C. for a 2-3 year period of time.

By "stable lyophilized preparation" is meant any lyophilized preparation having sufficient stability, such characteristics as similarly defined herein for a stable pharmaceutical composition, to have utility as a pharmaceutical product

By "degraded" is meant that the active has undergone a change in chemical structure.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered that will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of neoplasms, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer. Therapeutically effective amount can also mean preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment). Further, therapeutically effective amount can be that amount that increases the life expectancy of a patient afflicted with a terminal disorder. Typical therapeutically effective doses for bendamustine for the treatment of non-Hodgkin's lymphoma can be from about 60-120 mg/m^2 given as a single dose on two consecutive days. The cycle can be repeated about every three to four weeks. For the treatment of chronic lymphocytic leukemia (CLL) bendamustine can be given at about 80-100 mg/m^2 on days 1 and 2. The cycle can be repeated after about 4 weeks. For the treatment of Hodgkin's disease (stages II-IV), bendamustine can be given in the "DBVBe regimen" with daunorubicin 25 mg/m^2 on days 1 and 15, bleomycin 10 mg/m^2 on days 1 and 15, vincristine 1.4 mg/m^2 on days 1 and 15, and bendamustine 50 mg/m^2 on days 1-5 with repetition of the cycle about every 4 weeks. For breast cancer, bendamustine (120 mg/m^2) on days 1 and 8 can be given in combination with methotrexate 40 mg/m^2 on days 1 and 8, and 5-fluorouracil 600 mg/m^2 on days 1 and 8 with repetition of the cycle about every 4 weeks. As a second-line of therapy for breast cancer, bendamustine can be given at about 100-150 mg/m^2 on days 1 and 2 with repetition of the cycle about every 4 weeks.

As used herein "neoplastic" refers to a neoplasm, which is an abnormal growth, such growth occurring because of a proliferation of cells not subject to the usual limitations of growth. As used herein, "anti-neoplastic agent" is any compound, composition, admixture, co-mixture, or blend which inhibits, eliminates, retards, or reverses the neoplastic phenotype of a cell.

As used herein "hyperproliferation" is the overproduction of cells in response to a particular growth factor. "Hyperproliferative disorders" are diseases in which the cells overproduce in response to a particular growth factor. Examples of such "hyperproliferative disorders" include diabetic retinopathy, psoriasis, endometriosis, cancer, macular degenerative disorders and benign growth disorders such as prostate enlargement.

As used herein, the term "vial" refers to any walled container, whether rigid or flexible.

"Controlling" as used herein means putting process controls in place to facilitate achievement of the thing being controlled. For example, in a given case, "controlling" can mean testing samples of each lot or a number of lots regularly or randomly; setting the concentration of degradants as a release specification; selecting process conditions, e.g., use of alcohols and/or other organic solvents in the pre-lyophilization solution or dispersion, so as to assure that the concentration of degradants of the active ingredient is not unacceptably high; etc. Controlling for degradants by setting release specifications for the amount of degradants can be used to facilitate regulatory approval of a pharmaceutical product by a regulatory agency, such as the U.S. Food and Drug Administration and similar agencies in other countries or regions ("agency").

The term "pharmaceutically acceptable" as used herein means that the thing that is pharmaceutically acceptable, e.g., components, including containers, of a pharmaceutical composition, does not cause unacceptable loss of pharmacological activity or unacceptable adverse side effects. Examples of pharmaceutically acceptable components are provided in The United States Pharmacopeia (USP), The National Formulary (NF), adopted at the United States Pharmacopeial Convention, held in Rockville, Md. in 1990 and FDA Inactive Ingredient Guide 1990, 1996 issued by the U.S. Food and Drug Administration (both are hereby incorporated by reference herein, including any drawings). Other grades of solutions or components that meet necessary limits and/or specifications that are outside of the USP/NF may also be used.

The term "pharmaceutical composition" as used herein shall mean a composition that is made under conditions such that it is suitable for administration to humans, e.g., it is made under GMP conditions and contains pharmaceutically acceptable excipients, e.g., without limitation, stabilizers, bulking agents, buffers, carriers, diluents, vehicles, solubilizers, and binders. As used herein pharmaceutical composition includes but is not limited to a pre-lyophilization solution or dispersion as well as a liquid form ready for injection or infusion after reconstitution of a lyophilized preparation.

A "pharmaceutical dosage form" as used herein means the pharmaceutical compositions disclosed herein being in a container and in an amount suitable for reconstitution and administration of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. Preferably, a "pharmaceutical dosage form" as used herein means a lyophilized pharmaceutical composition disclosed herein in a container

and in an amount suitable for reconstitution and delivery of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. The pharmaceutical dosage form can comprise a vial or syringe or other suitable pharmaceutically acceptable container. The pharmaceutical dosage form suitable for injection or infusion use can include sterile aqueous solutions or dispersions or sterile powders comprising an active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol such as glycerol, propylene glycol, or liquid polyethylene glycols and the like, vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The prevention of the growth of microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

As used herein, the term "excipient" means the substances used to formulate active pharmaceutical ingredients (API) into pharmaceutical formulations; in a preferred embodiment, an excipient does not lower or interfere with the primary therapeutic effect of the API. Preferably, an excipient is therapeutically inert. The term "excipient" encompasses carriers, diluents, vehicles, solubilizers, stabilizers, bulking agents, and binders. Excipients can also be those substances present in a pharmaceutical formulation as an indirect or unintended result of the manufacturing process. Preferably, excipients are approved for or considered to be safe for human and animal administration, i.e., GRAS substances (generally regarded as safe). GRAS substances are listed by the Food and Drug administration in the Code of Federal Regulations (CFR) at 21 CFR §182 and 21 CFR §184, incorporated herein by reference. Preferred excipients include, but are not limited to, hexitols, including mannitol and the like.

As used herein "a stabilizing concentration of an organic solvent" or "a stabilizing concentration of an alcohol" means that amount of an organic solvent or alcohol that reduces the level of degradation of bendamustine to achieve a specified level of degradants in the final drug product. For example, with respect to the degradant HP1, a stabilizing concentration of an organic solvent is that amount which results in an HP1 concentration (area percent of bendamustine) of less than about 0.5%, preferably less than 0.45%, preferably less than 0.40%, more preferably less than 0.35%, more preferably less than 0.30%, and even more preferably less than 0.25%. With respect to the overall or total degradant concentration of the final drug product, a stabilizing concentration of an organic solvent is that amount that results in a total degradant concentration (at the time of drug product release) of less than about 7% (area percent bendamustine), preferably less than about 6%, more preferably less than about 5%, and even more preferably less than about 4.0%. By "area percent of bendamustine" is meant the amount of a specified degradant, e.g., HP1, relative to the amount of bendamustine as determined, e.g., by HPLC.

The term "organic solvent" means an organic material, usually a liquid, capable of dissolving other substances.

As used herein, "trace amount of an organic solvent" means an amount of solvent that is equal to or below recommended levels for pharmaceutical products, for example, as recommended by ICH guidelines (International Conferences on Harmonization, Impurities—Guidelines for Residual Solvents. Q3C. Federal Register. 1997; 62(247):67377). The lower limit is the lowest amount that can be detected.

The term "release" or "at release" means the drug product has met the release specifications and can be used for its intended pharmaceutical purpose.

A. General

The invention provides stable, pharmaceutically acceptable compositions prepared from bendamustine. In particular, the invention provides formulations for the lyophilization of bendamustine HCl. The lyophilized powder obtained from such formulations is more easily reconstituted than the presently available lyophilized powder of bendamustine. Further, the lyophilized products of the present invention have a better impurity profile than Ribomustin® with respect to certain impurities, in particular HP1, bendamustine dimer, and bendamustine ethylester, prior to reconstitution, upon storage of the lyophilate, or following reconstitution and admixture.

The present invention further provides formulations of bendamustine useful for treating neoplastic diseases. The formulations described herein can be administered alone or in combination with at least one additional anti-neoplastic agent and/or radioactive therapy.

An aspect of the invention is conditions and means for enhancing the stability of bendamustine prior to and during the lyophilization process, upon shelf storage or upon reconstitution.

Anti-neoplastic agents which may be utilized in combination with the formulations of the invention include those provided in the Merck Index 11, pp 16-17, Merck & Co., Inc. (1989) and The Chemotherapy Source Book (1997). Both books are widely recognized and readily available to the skilled artisan.

There are large numbers of antineoplastic agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for treatment of neoplasia by combination drug chemotherapy. Such antineoplastic agents fall into several major categories, namely, antibiotic-type agents, covalent DNA-binding drugs, antimetabolite agents, hormonal agents, including glucocorticoids such as prednisone and dexamethasone, immunological agents, interferon-type agents, differentiating agents such as the retinoids, pro-apoptotic agents, and a category of miscellaneous agents, including compounds such as antisense, small interfering RNA, and the like. Alternatively, other anti-neoplastic agents, such as metallomatrix proteases (MMP) inhibitors, SOD mimics or $alpha_v$ $beta_3$ inhibitors may be used.

One family of antineoplastic agents which may be used in combination with the compounds of the inventions consists of antimetabolite-type antineoplastic agents. Suitable antimetabolite antineoplastic agents may be selected from the group consisting of alanosine, AG2037 (Pfizer), 5-FU-fibrinogen, acanthifolic acid, aminothiadiazole, brequinar sodium, carmofur, Ciba-Geigy CGP-30694, cyclopentyl cytosine, cytarabine phosphate stearate, cytarabine conjugates, Lilly DATHF, Merrel Dow DDFC, dezaguanine, dideoxycytidine, dideoxyguanosine, didox, Yoshitomi DMDC, doxifluridine, Wellcome EHNA, Merck & Co. EX-015, fazarabine, floxuridine, fludarabine phosphate, 5-fluorouracil, N-(2'-furanidyl)-5-fluorouracil, Daiichi Seiyaku FO-152, isopropyl pyrrolizine, Lilly LY-188011, Lilly LY-264618, methobenzaprim, methotrexate, Wellcome MZPES, norspermidine, NCI NSC-127716, NCI NSC-264880, NCI NSC-39661, NCI NSC-612567, Warner-Lambert PALA, pentostatin, piritrexim, plicamycin, Asahi Chemical PL-AC, Takeda TAC-788, thioguanine, tiazofurin, Erbamont TIF, trimetrexate, tyrosine kinase inhibitors, tyrosine protein kinase inhibitors, Taiho UFT and uricytin.

A second family of antineoplastic agents which may be used in combination with the compounds of the invention consists of covalent DNA-binding agents. Suitable alkylating-type antineoplastic agents may be selected from the group consisting of Shionogi 254-S, aldo-phosphamide analogues, altretamine, anaxirone, Boehringer Mannheim BBR-2207, bestrabucil, budotitane, Wakunaga CA-102, carboplatin, carmustine, Chinoin-139, Chinoin-153, chlorambucil, cisplatin, cyclophosphamide, American Cyanamid CL-286558, Sanofi CY-233, cyplatate, Degussa D-19-384, Sumimoto DACHP(Myr)2, diphenylspiromustine, diplatinum cytostatic, Erba distamycin derivatives, Chugai DWA-2114R, ITI E09, elmustine, Erbamont FCE-24517, estramustine phosphate sodium, fotemustine, Unimed G-6-M, Chinoin GYKI-17230, hepsul-fam, ifosfamide, iproplatin, lomustine, mafosfamide, melphalan, mitolactol, Nippon Kayaku NK-121, NCI NSC-264395, NCI NSC-342215, oxaliplatin, Upjohn PCNU, prednimustine, Proter PTT-119, ranimustine, semustine, SmithKline SK&F-101772, Yakult Honsha SN-22, spiromustine, Tanabe Seiyaku TA-077, tauromustine, temozolomide, teroxirone, tetraplatin and trimelamol.

Another family of antineoplastic agents which may be used in combination with the compounds disclosed herein consists of antibiotic-type antineoplastic agents. Suitable antibiotic-type antineoplastic agents may be selected from the group consisting of Taiho 4181-A, aclarubicin, actinomycin D, actinoplanone, alanosine, Erbamont ADR-456, aeroplysinin derivative, Ajinomoto AN-201-II, Ajinomoto AN-3, Nippon Soda anisomycins, anthracycline, azino-mycin-A, bisucaberin, Bristol-Myers BL-6859, Bristol-Myers BMY-25067, Bristol-Myers BMY-25551, Bristol-Myers BMY-26605, Bristol-Myers BMY-27557, Bristol-Myers BMY-28438, bleomycin sulfate, bryostatin-1, Taiho C-1027, calichemycin, chromoximycin, dactinomycin, daunorubicin, Kyowa Hakko DC-102, Kyowa Hakko DC-79, Kyowa Hakko DC-88A, Kyowa Hakko DC89-A1, Kyowa Hakko DC92-B, ditrisarubicin B, Shionogi DOB-41, doxorubicin, doxorubicin-fibrinogen, elsamicin-A, epirubicin, erbstatin, esorubicin, esperamicin-Al, esperamicin-Alb, Erbamont FCE-21954, Fujisawa FK-973, fostriecin, Fujisawa FR-900482, glidobactin, gregatin-A, grincamycin, herbimycin, idarubicin, illudins, kazusamycin, kesarirhodins, Kyowa Hakko KM-5539, Kirin Brewery KRN-8602, Kyowa Hakko KT-5432, Kyowa Hakko KT-5594, Kyowa Hakko KT-6149, American Cyanamid LL-D49194, Meiji Seika ME 2303, menogaril, mitomycin, mitoxantrone, SmithKline M-TAG, neoenactin, Nippon Kayaku NK-313, Nippon Kayaku NKT-01, SRI International NSC-357704, oxalysine, oxaunomycin, peplomycin, pilatin, pirarubicin, porothramycin, pyrindamycin A, Tobishi RA-I, rapamycin, rhizoxin, rodorubicin, sibanomicin, siwenmycin, Sumitomo SM-5887, Snow Brand SN-706, Snow Brand SN-07, sorangicin-A, sparsomycin, SS Pharmaceutical SS-21020, SS Pharmaceutical SS-7313B, SS Pharmaceutical SS-9816B, steffimycin B, Taiho 4181-2, talisomycin, Takeda TAN-868A, terpentecin, thrazine, tricrozarin A, Upjohn U-73975, Kyowa Hakko UCN-10028A, Fujisawa WF-3405, Yoshitomi Y-25024 and zorubicin.

A fourth family of antineoplastic agents which may be used in combination with the compounds of the invention include a miscellaneous family of antineoplastic agents selected from the group consisting of alpha-carotene, alpha-difluoromethyl-arginine, acitretin, arsenic trioxide, Avastin® (bevacizumab), Biotec AD-5, Kyorin AHC-52, alstonine, amonafide, amphethinile, amsacrine, Angiostat, ankinomycin, anti-neoplaston A10, antineoplaston A2, antineoplaston A3, antineoplaston A5, antineoplaston AS2-1, Henkel APD, aphidicolin glycinate, asparaginase, Avarol, baccharin, batracylin, benfluoron, benzotript, Ipsen-Beaufour BIM-23015, bisantrene, Bristo-Myers BMY-40481, Vestar boron-10, bromofosfamide, Wellcome BW-502, Wellcome BW-773, caracemide, carmethizole hydrochloride, Ajinomoto CDAF, chlorsulfaquinoxalone, Chemes CHX-2053, Chemex CHX-100, Warner-Lambert CI-921, Warner-Lambert CI-937, Warner-Lambert CI-941, Warner-Lambert CI-958, clanfenur, claviridenone, ICN compound 1259, ICN compound 4711, Contracan, Yakult Honsha CPT-11, crisnatol, curaderm, cytochalasin B, cytarabine, cytocytin, Merz D-609, DABIS maleate, dacarbazine, datelliptinium, didemnin-B, dihaematoporphyrin ether, dihydrolenperone, dinaline, distamycin, Toyo Pharmar DM-341, Toyo Pharmar DM-75, Daiichi Seiyaku DN-9693, elliprabin, elliptinium acetate, epothionesTsumura EPMTC, erbitux, ergotamine, erlotnib, etoposide, etretinate, fenretinide, Fujisawa FR-57704, gallium nitrate, genkwadaphnin, Gleevec® (imatnib), Chugai GLA-43, Glaxo GR-63178, gefitinib, grifolan NMF-5N, hexadecylphosphocholine, Green Cross HO-221, homoharringtonine, hydroxyurea, BTG ICRF-187, indanocine, ilmofosine, isoglutamine, isotretinoin, Otsuka JI-36, Ramot K-477, Otsuak K-76COONa, Kureha Chemical K-AM, MECT Corp KI-8110, American Cyanamid L-623, leukoregulin, lonidamine, Lundbeck LU-23-112, Lilly LY-186641, NCI (US) MAP, marycin, mefloquine, Merrel Dow MDL-27048, Medco MEDR-340, merbarone, merocyanine derivatives, methylanilinoacridine, Molecular Genetics MGI-136, minactivin, mitonafide, mitoquidone, mopidamol, motretinide, Zenyaku Kogyo MST-16, N-(retinoyl)amino acids, Nisshin Flour Milling N-021, N-acylated-dehydroalanines, nafazatrom, Taisho NCU-190, nocodazole derivative, Normosang, NCI NSC-145813, NCI NSC-361456, NCI NSC-604782, NCI NSC-95580, octreotide, Ono ONO-112, oquizanocine, Akzo Org-10172, paclitaxel, pancratistatin, pazelliptine, Warner-Lambert PD-111707, Warner-Lambert PD-115934, Warner-Lambert PD-131141, Pierre Fabre PE-1001, ICRT peptide D, piroxantrone, polyhaematoporphyrin, polypreic acid, Efamol porphyrin, probimane, procarbazine, proglumide, Invitron protease nexin I, Tobishi RA-700, razoxane, Sapporo Breweries RBS, restrictin-P, retelliptine, retinoic acid, Rhone-Poulenc RP-49532, Rhone-Poulenc RP-56976, Rituxan® (and other anti CD20 antibodies, e.g. Bexxar®, Zevalin®), SmithKline SK&F-104864, statins (Lipitor® etc.), Sumitomo SM-108, Kuraray SMANCS, SeaPharm SP-10094, spatol, spirocyclopropane derivatives, spirogermanium, Unimed, SS Pharmaceutical SS-554, strypoldinone, Stypoldione, Suntory SUN 0237, Suntory SUN 2071, superoxide dismutase, Thalidomide, Thalidomide analogs, Toyama T-506, Toyama T-680, taxol, Teijin TEI-0303, teniposide, thaliblastine, Eastman Kodak TJB-29, tocotrienol, Topostin, Teijin TT-82, Kyowa Hakko UCN-01, Kyowa Hakko UCN-1028, ukrain, Eastman Kodak USB-006, vinblastine sulfate, vincristine, vindesine, vinestramide, vinorelbine, vintriptol, vinzolidine, withanolides and Yamanouchi YM-534, Zometa®.

Examples of radioprotective agents which may be used in the combination chemotherapy of this invention are AD-5, adchnon, amifostine analogues, detox, dimesna, 1-102, MM-159, N-acylated-dehydroalanines, TGF-Genentech, tiprotimod, amifostine, WR-151327, FUT-187, ketoprofen transdermal, nabumetone, superoxide dismutase (Chiron and Enzon).

Methods for preparation of the antineoplastic agents described above may be found in the literature. Methods for preparation of doxorubicin, for example, are described in U.S. Pat. Nos. 3,590,028 and 4,012,448. Methods for prepar-

ing metallomatrix protease inhibitors are described in EP 780386. Methods for preparing .alpha$_v$ .beta$_3$ inhibitors are described in WO 97/08174.

Preferred anti-neoplastic agents include, without limitation, one or more of daunorubicin, bleomycin, vincristine, doxorubicin, dacarbazine, prednisolone, mitoxantrone, prednisone, methotrexate, 5-fluorouracil, dexamethasone, thalidomide, thalidomide derivatives, 2ME2, Neovastat, R 11 5777, arsenic trioxide, bortezomib, tamoxifen, G3139 (antisense), and SU5416, mitomycin, anti-CD20 antibodies, such as Rituxan® and R-etodolac.

Preferred drug regimens for which the present formulation may be used in conjunction with or as a replacement for one or more of the components includes, without limitation, ABVD (doxorubicin, bleomycin, vincristine, dacarbazine), DBV (daunorubicin, belomycin, vincristine), CVPP (cyclophosphamide, vinblastine, procarbazine, prednisolone), COP (cyclophosphamide, vincristine, prednisolone), CHOP (cyclophosphamide, doxorubicin,

vincristine and prednisone) and CMF (cyclophosphamide, methotrexate, 5-fluorouracil). Additional regimens are given in Table A below.

TABLE A

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
AC	Doxorubicin & Cyclophosphamide	Breast cancer
CFM (CF, FNC)	Cyclophosphamide, Fluorouracil, Mitaxantrone	Breast cancer
CMF	Cyclophosphamide, Methotrexate, Fluorouracil	Breast cancer
NFL	Mitoxantrone, Fluorouracil, Leucovorin	Breast cancer
Sequential Dox-CMF	Doxorubicin	Breast cancer
VATH	Vinblastine, Doxorubicin, Thiotepa, Fluoxymesterone	Breast cancer
EMA-86	Etoposide, Mitoxantrone, Ctyarabine	AML (induction)
7 + 3	Cytarabine WITH Daunorubicin OR Idarobicin OR Mitoxantrone	AML (induction)
5 + 2	Cytarabine WITH Daunorubicin OR Mitoxantrone	AML (induction)
HiDAC	Cytarabine	AML (post-remission)
ABVD	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine	Hodgkin's
ChlVPP	Chlorambucil, Vinblastine, Procarbazine, Prednisone	Hodgkin's
EVA	Etoposide, Vinblastine, Doxorubicin	Hodgkin's
MOPP	Mechlorethamine, Vincristine, Procarbazine, Prednisone	Hodgkin's
MOPP/ABV Hybrid	Mechlorethamine, Vincristine, Procarbazine, Prednisone, Doxorubicin, Bleomycin, Vinblastine	Hodgkin's
MOPP/ABVD	Mechlorethamine, Doxorubicin, Vinblastine, Bleomycin, Etoposide, Prednisone	Hodgkin's
CNOP	Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone	Non-Hodgkin's
COMLA	Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, Cytarabine	Non-Hodgkin's
DHAP	Dexamethasone, Cisplatin, Cytarabine	Non-Hodgkin's

TABLE A-continued

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
ESHAP	Etoposide, Methylprednisilone, Cisplatin, Cytarabine	Non-Hodgkin's
MACOP-B	Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Vincristine, Prednisone, Bleomycin, Septra, Ketoconazole	Non-Hodgkin's
m-BACOD	Methotrexate, Leucovorin, Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, Dexamethasone	Non-Hodgkin's
MINE-ESHAP	Mesna, Ifosfamide, Mitoxantrone, Etoposide	Non-Hodgkin's
NOVP	Mitoxantrone, Vinblastine, Prednisone, Vincristine	Non-Hodgkin's
ProMACE/ cytaBOM	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Cytarabine, Bleomycin, Vincristine, Methotrexate, Leucovorin, Septra	Non-Hodgkin's
M2	Vincristine, Carmustine, Cyclophosphamide, Melphalan, Prednisone	Multiple Myeloma
MP	Melphalan, Prednisone	Multiple Myeloma
VAD	Vincristine, Doxorubicin, Dexamethasone	Multiple Myeloma
VBMCP	Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone	Multiple Myeloma

As described herein, a lyophilized formulation of bendamustine is achieved following removal of an organic solvent in water. The most typical example of the solvent used to prepare this formulation is tertiary butanol (TBA). Other organic solvents can be used including ethanol, n-propanol, n-butanol, isoproponal, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, acetone, 1-pentanol, methyl acetate, methanol, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, cyclohexane. These preceding solvents may be used individually or in combination. Useful solvents must form stable solutions with bendamustine and must not appreciably degrade or deactivate the API. The solubility of bendamustine in the selected solvent must be high enough to form commercially useful concentrations of the drug in solvent. Additionally, the solvent should be capable of being removed easily from an aqueous dispersion or solution of the drug product, e.g., through lyophilization or vacuum drying. Preferably, a solution having a concentration of about 2-80 mg/mL, preferably about 5 to 40 mg/mL, more preferably 5-20 mg/mL and even more preferably 12 to 17 mg/mL bendamustine is used.

A pharmaceutically acceptable lyophilization excipient can be dissolved in the aqueous phase. Examples of excipients useful for the present invention include, without limitation, sodium or potassium phosphate, citric acid, tartaric acid, gelatin, glycine, and carbohydrates such as lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose and hetastarch. Mannitol is a preferred excipient. Other excipients that may be used if desired include antioxidants, such as, without limitation, ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxyanisole, butyl-hydroxytoluene or alpha-tocopherol acetate, or chelators.

A typical formulation and lyophilization cycle useful in accordance with the present invention is provided below. Lyophilization can be carried out using standard equipment as used for lyophilization or vacuum drying. The cycle may be varied depending upon the equipment and facilities used for the fill/finish.

In accordance with a typical embodiment of the present invention, an aqueous pre-lyophilization solution or dispersion is first formulated in a pharmaceutically acceptable compounding vessel. The solution is aseptically filtered into a sterile container, filled into an appropriate sized vial, partially stoppered and loaded into the lyophilizer. Using lyophilization techniques described herein the solution is lyophilized until a moisture content in the range of about 0.1 to about 8.0 percent is achieved. The resulting lyophilization powder is stable as a lyophilized powder for about six months to greater than about 2 years, preferably greater than about 3 years at about 5° C. to about 25° C. and can be readily reconstituted with Sterile Water for Injection, or other suitable carrier, to provide liquid formulations of bendamustine, suitable for internal administration e.g., by parenteral injection. For intravenous administration, the reconstituted liquid formulation, i.e., the pharmaceutical composition, is preferably a solution.

The pre-lyophilization solution or dispersion normally is first formulated in a pharmaceutically acceptable container by: 1) adding an excipient, such as mannitol (about 0 to about 50 mg/mL) with mixing to water (about 65% of the total volume) at ambient temperature, 2) adding an organic solvent (0.5-99.9% v/v), such as TBA to the aqueous solution with mixing at about 20°-35° C., 4) adding bendamustine HCl to the desired concentration with mixing, 5) adding water to achieve the final volume, and 6) cooling the solution to about 1° C. to about 30° C., preferably about 5° C. Although the preceding steps are shown in a certain order, it is understood that one skilled in the art can change the order of the steps and quantities as needed. Quantities can be prepared on a weight basis also.

The pre-lyophilization solution or dispersion can be sterilized prior to lyophilization, sterilization is generally performed by aseptic filtration, e.g., through a 0.22 micron or less filter. Multiple sterilization filters can be used. Sterilization of the solution or dispersion can be achieved by other methods known in the art, e.g., radiation.

In this case, after sterilization, the solution or dispersion is ready for lyophilization. Generally, the filtered solution will be introduced into a sterile receiving vessel, and then transferred to any suitable container or containers in which the formulation may be effectively lyophilized. Usually the formulation is effectively and efficiently lyophilized in the containers in which the product is to be marketed, such as, without limitation, a vial, as described herein and as known in the art.

A typical procedure for use in lyophilizing the pre-lyophilization solutions or dispersions is set forth below. However, a person skilled in the art would understand that modifications to the procedure or process may be made depending on such things as, but not limited to, the pre-lyophilization solution or dispersion and lyophilization equipment.

Initially, the product is placed in a lyophilization chamber under a range of temperatures and then subjected to temperatures well below the product's freezing point, generally for several hours. Preferably, the temperature will be at or below about −40° C. for at least 2 hours. After freezing is complete, the chamber and the condenser are evacuated through vacuum pumps, the condenser surface having been previously chilled by circulating refrigerant. Preferably, the condenser will have been chilled below the freezing point of the solution preferably to about −40°, more preferably to about −50° C. or lower, even more preferably to about −60° C. or lower. Additionally, evacuation of the chamber should continue until a pressure of about 10 to about 600 microns, preferably about 50 to about 150 microns is obtained.

The product composition is then warmed under vacuum in the chamber and condenser. This usually will be carried out by warming the shelves within the lyophilizer on which the product rests during the lyophilization process at a pressure ranging from about 10 to about 600 microns. The warming process will optimally take place very gradually, over the course of several hours. For example, the product temperature should initially be increased from about −30° C. to about −10° C. and maintained for about 10-70 hours. Additionally, the product temperature can be increased from the freezing temperature to about 25° C.-40° C. over a period of 30-192 hours. To prevent powder ejection of the lyophilate from vials, complete removal of the organic solvent and water should be done during the initial drying phase. Complete drying can be confirmed by stabilization of vacuum, condenser temperature and product shelf temperature. After the initial drying, the product temperature should be increased to about 25° C.-40° C. and maintained for about 5-40 hours.

Once the drying cycle is completed, the pressure in the chamber can be slowly released to atmospheric pressure (or slightly below) with sterile, dry-nitrogen gas (or equivalent gas). If the product composition has been lyophilized in containers such as vials, the vials can be stoppered, removed and sealed. Several representative samples can be removed for purposes of performing various physical, chemical, and microbiological tests to analyze the quality of the product.

The lyophilized bendamustine formulation is typically marketed in pharmaceutical dosage form. The pharmaceutical dosage form of the present invention, although typically in the form of a vial, may be any suitable container, such as ampoules, syringes, co-vials, which are capable of maintaining a sterile environment. Such containers can be glass or plastic, provided that the material does not interact with the bendamustine formulation. The closure is typically a stopper, most typically a sterile rubber stopper, preferably a bromobutyl rubber stopper, which affords a hermetic seal.

After lyophilization, the bendamustine lyophilization powder may be filled into containers, such as vials, or alternatively the pre-lyophilization solution can be filled into such vials and lyophilized therein, resulting in vials which directly contain the lyophilized bendamustine formulation. Such vials are, after filling or lyophilization of the solution therein, sealed, as with a stopper, to provide a sealed, sterile, pharmaceutical dosage form. Typically, a vial will contain a lyophilized powder including about 10-500 mg/vial, preferably about 100 mg/vial, bendamustine and about 5 mg-2 g/vial, preferably about 170 mg/vial, mannitol.

The lyophilized formulations of the present invention may be reconstituted with water, preferably Sterile Water for Injection, or other sterile fluid such as co-solvents, to provide an appropriate solution of bendamustine for administration, as through parenteral injection following further dilution into an appropriate intravenous admixture container, for example, normal saline.

B. Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of alcohols commonly used in lyophilization, e.g., methanol, ethanol, propanol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, combined with mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions at

room temperature (see Table 1). Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

The results shown in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For the alcohols tested, the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time. Bendamustine did not precipitate immediately with any alcohol, but crystallized after storage at 5° C. Alcohols varied in their effect on solubility. Without wishing to be bound to any particular theory, smaller alcohols such as methanol and ethanol have less of an effect on solubility as compared with larger alcohols (tertiary-butanol and n-butanol). However, the shape of the alcohol is also important. For example n-propanol was found to be better than iso-propanol in preventing precipitation in this system. The two alcohols with the greatest effect on solubility were n-propanol and tertiary-butanol.

TABLE 1

Bendamustine solubility over a 24 hour period in various alcohols when stored at 5° C.

	Zero Time	3 Hours	6 Hours	24 Hours
Methanol (v/v)				
0% (Water Only)	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	Precipitate
30%	CCS	CCS	CCS	CCS
Ethanol (v/v)				
1.9%	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
n-Propanol (v/v/)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
Iso-propanol (v/v)				
5%	CCS	Precipitate	Precipitate	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
n-Butanol (v/v)				
5%	CCS	CCS	CCS	CCS
10%	CCS	CCS	CCS	CCS
20%	2 layers	2 layers	2 layers	2 layers
30%	2 layers	2 layers	2 layers	2 layers
Tert-Butanol (v/v)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS

CCS stands for clear colorless solution

Experiments to quantitatively determine the solubility of bendamustine at various temperatures for three different solutions are summarized in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment was based on stability studies (results described below). For both solutions tested, the solubility of bendamustine decreased linearly with temperatures from 25° C. to 0° C. This experiment confirmed the data shown in Table 1 and highlights the difference in bendamustine solubility for 20% and 30% TBA solutions.

TABLE 2

Solubility of bendamustine in TBA

	−8° C.	0° C.	5° C.	25° C.
20% (v/v) TBA				
25.5 mg/mL mannitol Water, q.s. to desired volume	14 mg/mL	11 mg/mL	17 mg/mL	47 mg/mL
30% (v/v) TBA				
25.5 mg/mL mannitol Water, q.s. to desired volume	20 mg/mL	18 mg/mL	27 mg/mL	65 mg/mL

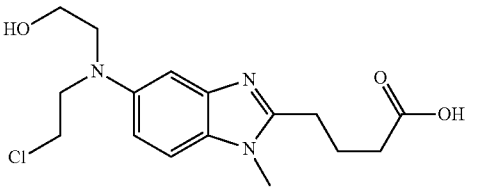
C. Stability

Because of its instability in aqueous solutions due to hydrolysis with water, bendamustine requires lyophilization in order to make a product suitable for pharmaceutical use. However, during the manufacturing of lyophilized drug products, aqueous solutions are commonly needed for filling, prior to lyophilization. Thus, the use of aqueous solutions during the compounding and fill processes for bendamustine and other nitrogen mustards can result in degradation of the drug product. Consequently, the effect of various alcohols on the degradation of bendamustine was evaluated to determine if formulations could be found that would allow longer fill-finish times, provide lyophilate powders that could be reconstituted more quickly than the current Ribomustin® formulation, and/or provide lyophilized preparations of bendamustine with a better impurity profile with respect to certain impurities, e.g., HP1, and BM1 dimer than Ribomustin®.

Preferably, a lyophilized preparation of the invention is stable with respect to HP1, i.e., the amount of HP1 does not increase appreciably (does not exceed the shelf-life specifications), for 6 months, more preferably 12 months, and even more preferably greater than 24 months, e.g., 36 months, when stored at about 2° C. to about 30° C., preferably 5° C.

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades rapidly in water alone and forms predominantly the hydrolysis product, HP1 (monohydroxy bendamustine).

Formula II



Monohydroxy bendamustine (HP1)

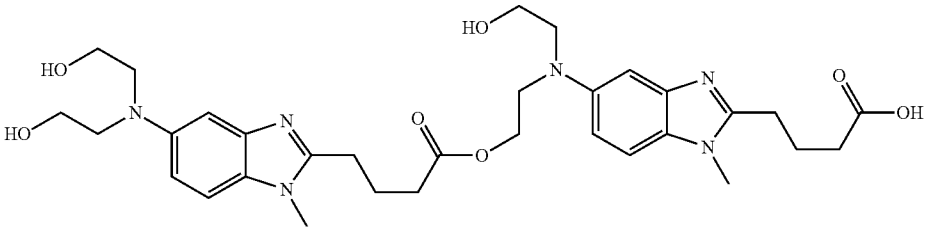
TABLE 3

Stability of bendamustine in water

	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
0% Alcohol, i.e., Water Alone	0 hours	99.11	0.60	0.11
	3 hours	98.83	0.86	0.13
	6 hours	98.44	1.22	0.17
	24 hours	95.67	3.81	0.29

The other major degradant observed during this study and other long term stability studies was the dimer of bendamustine.

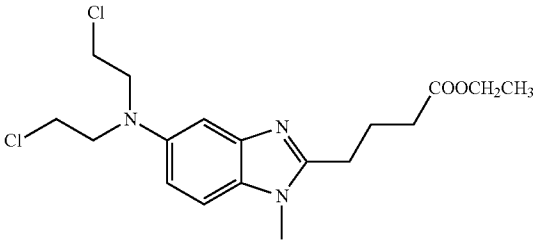
Formula III



Bendamustine Dimer (BM1 Dimer)

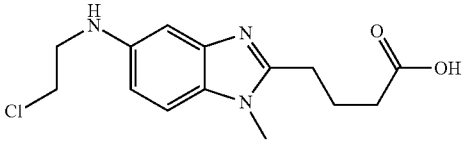
Other degradants contained in the Ribomustin lyophilized product are bendamustine ethylester (BM1EE) (Formula IV) and BM1DCE (Formula V). BM1EE is formed when bendamustine reacts with ethyl alcohol.

Formula IV



Bendamustine ethylester (BM1EE)

Formula V



BM1DCE

FIG. **2** summarizes the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the total peak area. The numerical values for FIG. **2** are provided in Tables 3-9. The purity was highest in solutions containing higher concentration of alcohols, regardless of the alcohol. Of the alcohols evaluated, bendamustine degraded the least in a solution containing about 30% (v/v) TBA. In about 10% and about 20% alcohol solutions, n-butanol was superior in preventing degradation of bendamustine. At 20% and 30% (v/v), n-butanol in water resulted in a biphasic system due to the insolubility of n-butanol in water at these concentrations.

FIGS. **3** and **4** show the amount of degradation of bendamustine as measured by HP1 and dimer formation quantified by HPLC (as described herein). HP1 and dimer formation increased as the amount of alcohol concentration decreased regardless of the alcohol. This increase in impurities occurred with an anticipated time dependence (see Tables 3-9). Tert-butanol and n-butanol appeared superior to other alcohols in preventing degradation of the product. As seen in Table 10, mannitol had no effect on the stabilization of bendamustine with TBA.

TABLE 4

HPLC stability results for the stability of bendamustine in various ethyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

V/V alcohol	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
1.9% Ethanol	0 hours	99.11	0.64	0.12
	3 hours	98.83	0.90	0.14
	6 hours	98.60	1.12	0.15
	24 hours	96.16	3.41	0.27
5% Ethanol	0 hours	99.31	0.44	0.12
	3 hours	99.10	0.64	0.13
	6 hours	98.87	0.86	0.14
	24 hours	96.89	2.68	0.25
10% Ethanol	0 hours	99.44	0.33	0.11
	3 hours	99.28	0.48	0.12
	6 hours	99.10	0.65	0.12
	24 hours	98.03	1.57	0.18
20% Ethanol	0 hours	99.54	0.22	0.10
	3 hours	99.45	0.30	0.11
	6 hours	99.36	0.39	0.11
	24 hours	98.61	0.96	0.15
30% Ethanol	0 hours	99.62	0.15	0.10
	3 hours	99.56	0.21	0.11
	6 hours	99.52	0.24	0.12
	24 hours	99.21	0.45	0.12

TABLE 5

HPLC stability results for bendamustine in various Tert-butanol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Tert-butanol	0 hours	99.34	0.41	0.12
	3 hours	99.10	0.64	0.14
	6 hours	98.85	0.88	0.13
	24 hours	97.58	2.09	0.20
10% Tert-butanol	0 hours	99.46	0.30	0.11
	3 hours	99.26	0.48	0.12
	6 hours	99.05	0.69	0.13
	24 hours	98.04	1.64	0.19
20% Tert-butanol	0 hours	99.59	0.17	0.11
	3 hours	99.48	0.29	0.11
	6 hours	99.35	0.40	0.12
	24 hours	98.35	1.27	0.20
30% Tert-butanol	0 hours	99.63	0.13	0.10
	3 hours	99.60	0.16	0.10
	6 hours	99.58	0.18	0.11
	24 hours	99.42	0.34	0.12

TABLE 6

HPLC stability results for various n-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% n-Propanol	0 hours	99.25	0.43	0.13
	3 hours	99.00	0.66	0.15
	6 hours	98.72	0.94	0.16
	24 hours	97.24	2.33	0.26
10% n-Propanol	0 hours	99.34	0.33	0.15
	3 hours	99.17	0.48	0.14
	6 hours	98.92	0.70	0.16
	24 hours	97.67	1.83	0.28
20% n-Propanol	0 hours	99.45	0.33	0.13
	3 hours	99.42	0.26	0.13
	6 hours	99.29	0.39	0.14
	24 hours	98.60	0.97	0.24
30% n-Propanol	0 hours	99.53	0.15	0.13
	3 hours	99.51	0.15	0.15
	6 hours	99.44	0.20	0.11
	24 hours	99.27	0.36	0.17

TABLE 7

HPLC stability results for bendamustine in various iso-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Iso-propanol	0 hours	99.21	0.48	0.13
	3 hours	98.65	0.72	0.14
	6 hours	98.56	1.02	0.14
	24 hours	96.14	3.35	0.26
10% Iso-propanol	0 hours	99.32	0.37	0.12
	3 hours	99.11	0.55	0.14
	6 hours	98.85	0.75	0.16
	24 hours	97.68	1.92	0.21
20% Iso-propanol	0 hours	99.49	0.21	0.11
	3 hours	99.39	0.31	0.12
	6 hours	99.22	0.42	0.13
	24 hours	98.61	1.04	0.17
30% Iso-propanol	0 hours	99.56	0.15	0.10
	3 hours	99.47	0.20	0.12
	6 hours	99.40	0.24	0.11
	24 hours	99.15	0.52	0.14

TABLE 8

HPLC stability results for bendamustine in various methyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Methanol	0 hours	99.35	0.40	0.12
	3 hours	98.97	0.70	0.14
	6 hours	98.66	0.95	0.14
	24 hours	96.65	2.83	0.23
10% Methanol	0 hours	99.42	0.34	0.11
	3 hours	99.01	0.59	0.12
	6 hours	98.86	0.80	0.12
	24 hours	97.65	1.85	0.18
20% Methanol	0 hours	99.56	0.22	0.11
	3 hours	99.31	0.38	0.11
	6 hours	98.99	0.50	0.12
	24 hours	98.31	1.15	0.16
30% Methanol	0 hours	99.59	0.18	0.10
	3 hours	99.43	0.27	0.11
	6 hours	99.25	0.34	0.11
	24 hours	98.65	0.76	0.13

TABLE 9

HPLC stability results for bendamustine in various n-butyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Butanol	0 hours	99.25	0.49	0.13
	3 hours	98.94	0.73	0.14
	6 hours	98.76	0.91	0.14
	24 hours	97.46	2.20	0.21
10% Butanol	0 hours	99.44	0.30	0.11
	3 hours	99.18	0.49	0.12
	6 hours	99.03	0.64	0.12
	24 hours	98.13	1.55	0.17
20% Butanol[a]	0 hours	99.54	0.23	0.10
	3 hours	99.45	0.31	0.11
	6 hours	99.30	0.40	0.11
	24 hours	98.81	0.91	0.14
30% Butanol[a]	0 hours	99.55	0.24	0.10
	3 hours	99.40	0.29	0.10
	6 hours	99.40	0.37	0.11
	24 hours	99.00	0.74	0.12

[a]Both solutions had 2 layers/phases of liquids in the vial. Solutions were vortexed prior to sample preparation.

The results in Tables 1-9 indicate that the stability of bendamustine HCl with respect to HP1 and dimer improves with increasing alcohol concentration.

TABLE 10

HPLC stability results for bendamustine in TBA with and without mannitol over a 24 hour period.

Sample	Purity (% Area)	HP1 (%)
TBA 20% (v/v) with Mannitol		
0 hours	99.59	0.17
24 hours @ 5° C.	99.35	1.27
TBA 20% (v/v) without Mannitol		
0 hours	100.0	0.00
24 hours @ 5° C.	98.80	1.21

NOTE:
The samples analyzed without mannitol were analyzed by HPLC using a normal phase method while the samples analyzed with mannitol used a reverse phase HPLC method. Slight variability may be seen in other samples analyzed between the two methods.

D. Lyophilization Cycle Development

Different pre-lyophilization formulations were prepared at various concentrations of bendamustine, mannitol, and alcohols in water. The cycle development was changed and optimized at each step for freezing (fast vs. slow), primary drying (both temperature and pressure), and secondary drying as described herein.

Based upon all of the information detailed above on solubility, stability, and ease of lyophilization, preferred formulations include the following:

Ingredients	Concentration
Bendamustine	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Alcohol	about 0.5%-40% (v/v)
Water, q.s. to	desired volume

wherein the alcohol is selected from methanol, n-propanol, or isopropanol

Ingredients	Concentration
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	1-20% (v/v)
Water, q.s. to	desired volume

wherein the alcohol is selected from methanol, n-propanol, or isopropanol

Ingredients	Concentration
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	5-40% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Alcohol	about 5-15% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Alcohol	about 10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Butanol	about 0.5-20% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Butanol	about 10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-100% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99.9% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 90-99% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Tertiary butanol	about 5-80% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Tertiary butanol	about 10-50% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12.5-15 mg/mL
Mannitol	about 0-30 mg/mL
Ethanol	about 20-30% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Tertiary butanol	about 30% (v/v)
Water, q.s. to	desired volume

EXAMPLES

The following Examples are provided to illustrate certain aspects of the present invention and to aid those of skill in the art in practicing the invention. These Examples are in no way to be considered to limit the scope of the invention in any manner.

Materials:

Bendamustine HCl, (Degussa, Lot #s 0206005 and 0206007)

Mannitol, NF or equivalent (Mallinckrodt)

Ethyl Alcohol Dehydrated (200 proof), USP or equivalent (Spectrum)

Tertiary-butyl alcohol, ACS (EM Science)

Methanol (Spectrum and EMD)

Propanol (Spectrum)

Iso-propanol (Spectrum)

Butanol (Spectrum)

Water, HPLC grade or equivalent (EMD)

Acetonitrile, HPLC grade or equivalent (EMD)

Trifluoroacetic Acid, J. T. Baker

Methanol, HPLC grade or equivalent (EM Science, Cat # MX0488P-1)

Trifluoroacetic Acid, HPLC grade or equivalent (JT Baker, Cat# JT9470-01)

Equipment:

Waters 2695 Alliance HPLC system with photodiode array detector

Waters 2795 Alliance HPLC system with dual wavelength detector

Analytical Balance (Mettler AG285, ID #1028) and (Mettler XS205)

VirTis Lyophilizer AdVantage

Agilent Zorbax SB-C18 5 μm 80 Å 4.6×250 mm column, Cat#880975-902

Example 1

HPLC Procedures

Method 1

Mobile Phase A: 0.1% TFA; H_2O

Mobile Phase B: 0.1% TFA; 50% ACN:50% H_2O

UV: 230 nm

Flow rate: 1.0 mL/min

Column temp.: 30° C.

Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm

Sample temp.: 5° C.

Injection Volume: 10 μL

Sample Concentration: 0.25 mg/mL in MeOH

Gradient: 20% B for 1 min

20-90% B in 23 min

90% B for 6 min

back to 20% B in 1 min

hold at 20% B for 4 min

Run time: 30 min

Post run time: 5 min

Method 2

Mobile Phase A: 0.1% TFA; H_2O:ACN (9:1)

Mobile Phase B: 0.1% TFA; H_2O:ACN (5:5)

UV: 230 nm

Flow rate: 1.0 mL/min

Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm

Column temp.: 30° C.

Sample temp.: 5° C.

Injection Volume: 10 μL

Sample Concentration: 0.25 mg/mL in MeOH

Gradient: 0% B for 3 min

0-50% B in 13 min

50-70% B in 17 min

70-90% B in 2 min

90% B for 5 min

back to 0% B in 1 min

hold at 0% B for 4 min

Run time: 40 min

Post run time: 5 min

Method 3

Phase A: HPLC grade water with 0.1% TFA(v/v)

Phase B: HPLC grade ACN/water(1:1 v/v) with 0.1% TFA (v/v)

UV: 254 nm

Flow rate: 1.0 mL/min

Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm

Column temp.: 30° C.

Sample temp.: 5° C.

Injection Volume: 5 μL

Acquisition time: 30 min

Post time: 9 min

Diluent: methanol

Gradient:

Time (min.)	% Phase A	% Phase B
0.0	82	18
7.0	60	40
11.0	60	40
15.0	20	80
30.0	20	80
31.0	82	18

Sample preparation—dissolve the drug product with 200 mL MeOH. Sonicate 6 minutes. The solution can be injected directly into the HPLC (ca. 0.5 mg/mL)

Method 4
Phase A: HPLC grade water with 0.1% TFA(v/v)
Phase B: HPLC grade ACN with 0.1% TFA(v/v)
UV: 254 nm
Flow rate: 1.0 mL/min
Column: Zorbax Bonus RP-C14 5 μm 4.6×150 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 2 μL
Acquisition time: 31 min
Post time: 5 min
Diluent: NMP/0.1% TFA in water (50:50 v/v)
Gradient:

Time (min.)	% Phase A	% Phase B
0.0	93	7
5	93	7
13	73	27
16	73	27
25	10	90
31	10	90

Sample preparation for method 4—dissolve the drug product with a known amount of diluent to prepare a concentration of 4.2 mg/mL for injection directly into the HPLC. It may be necessary to perform a second dilution (the 100 mg/vial dosage form) to obtain a 4.2 mg/mL sample concentration.

Results

The retention times for some Bendamustine impurities using HPLC Method 1 described above are shown in Table 11. An HPLC chromatograph for Ribomustin® using the HPLC procedure described herein is shown in FIG. **6**.

TABLE 11

Retention Time for Bendamustine and some of its Impurities using HPLC Method 1

Sample Name	Retention Time (min)
HP1	14.110
Bendamustine	22.182
BM1 Dimer	24.824
BM1EE	26.968

Although HPLC Method 1 was capable of resolving impurities found in bendamustine it was not capable of separating a potential impurity formed during analysis, the methyl ester of bendamustine (BM1ME). The retention time difference between BM1ME and BM1 Dimer was only 0.3 minutes. In order to resolve BM1 Dimer, another HPLC method (#2) was developed. HPLC method #2 was capable of separating all the impurities but required a longer run time of 45 minutes (Table 12).

TABLE 12

Retention Time for bendamustine and impurities using HPLC Method 2

Sample Name	Retention Time (min)
HP1	15.694
BM1	25.420
BM1ME	31.065
BM1 Dimer	32.467
BM1EE	36.038

The impurity profile of various lots of Ribomustin using HPLC Method 3 are shown in Table 13.

TABLE 13

Ribomustine Impuirty Profile using HPLC Method 3 % Area

Batch	Bendamustine(HCl)	HP1	BM1EE	BM1 Dimer	BM1DCE
03H08	98.14	1.07	0.21	0.34	0.03
03H07	97.67	1.5	0.2	0.33	0.04
02K27	96.93	0.93	0.29	1.18	0.08
03C08	97.61	1.24	0.19	0.46	0.02

Example 2

Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of methanol, ethanol, propanol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions (Table 1) at room temperature. Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

Results summarized in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For all alcohols the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time.

The solubility of bendamustine was also determined in 20% (v/v) TBA containing 25.5 mg/mL mannitol in water, and 30% (v/v) TBA containing 25.5 mg/mL mannitol in water (FIG. **1**). Bendamustine was added to 4 mL of each solution while mixing until it would no longer dissolve. The saturated solutions were allowed to mix for 1 hour at −8° C., 0° C., 5° C., or 25° C. The samples were centrifuged and placed back at the original temperature for a minimum of 30 minutes. The −8° C. sample was placed into an ice bath containing sodium chloride, which lowers the temperature of the ice bath, and the temperature was measured when the sample was pulled for analysis. An aliquot of each sample was taken and prepared for HPLC analysis.

The results of these experiments are shown in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment (FIG. **1**) was based on stability studies described herein.

As indicated in FIG. **1**, the solubility of bendamustine decreased linearly with temperature (25° C. to 0° C.). The solubility of bendamustine was temperature dependant whether it was dissolved in water alone or with an alcohol. The 20% (v/v) TBA may likely be the lower limit required for efficient and robust pharmaceutical manufacturing due to the stability and solubility of bendamustine. A filling solution of 15 mg/mL bendamustine is close to the saturation limit of 17.2 mg/mL bendamustine at 5° C. but higher than the limit at 0° C. The 30% (v/v) TBA is the recommended concentration of TBA for the final formulation and is well within the solubility limit regardless of temperature.

Example 3

Stability

A. Stability in Water

Solutions of bendamustine (15 mg/mL), and mannitol (25.5 mg/mL) were prepared in water at room temperature and immediately placed in an ice bath (to lower the temperature quickly to about 5° C.) for 10 minutes and then refriger-

ated at 5° C. A sample of each formulation was analyzed by HPLC using the methods described herein after 0, 3, 6 and 24 hours when stored at 5° C.

B. Stability in Alcohols

Solutions containing 15 mg/mL bendamustine, 25.5 mg/mL mannitol, and 1.9%, 5%, 10%, 20% or 30% (v/v) ethyl alcohol in water or 5%, 10%, 20% or 30% (v/v) TBA, methanol, propanol, iso-propanol, or butanol in water were prepared at room temperature, placed into an ice bath for 10 minutes and then refrigerated at 5° C. A sample of each formulation was analyzed by HPLC after 0, 3, 6 and 24 hours when stored at 5° C.

C. Stability Results

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades quickly in water but the stability of bendamustine increases with increasing alcohol concentrations (FIGS. **2**, **3** and **4**). Although alcohols are frequently used in lyophilization to aid in solubility problems, the effect of alcohols on bendamustine stability is unique, unexpected and useful in manufacturing bendamustine with fewer impurities since an aqueous solution can be used while maintaining the stability of bendamustine. TBA was found to be the best stabilizer of the six alcohols tested (FIGS. **2**, **3**, and **4**). All alcohols at 30% (v/v) reduced the formation of impurities HP1 and Dimer at 5° C. for up to 24 hours. With respect to TBA, HP1 reaches only about 0.4% when stored at 5° C. for up to 24 hours. Lower concentrations of alcohol may not be efficient, when formulated at 15 mg/mL bendamustine and stored at 5° C. due to bendamustine precipitation and impurity formation.

Example 4

Formulation Optimization

After the solubility and stability of bendamustine were determined, the formulation was optimized for lyophilization. Since the concentration of bendamustine is higher in a 30% TBA/water saturated solution as compared with other alcohol solutions, it is anticipated that the vial size required to fill 100 mg of bendamustine can be decreased from the current Ribomustin® presentation. Although a saturated solution of bendamustine contains 18 mg/mL at 0° C., a concentration of 15 mg/mL was selected for the formulation to compensate for slight differences in API solubility due to differences in bulk API purity as a result of batch differences. A concentration of 15 mg/mL bendamustine requires 6.67 mL to fill 100 mg of bendamustine HCl per vial.

The surface (sublimation) area to volume ratio is critical to producing a lyophilized product with good appearance that freeze dries quickly. Generally, lyophilized products occupy between 30% to 50% of the vial volume. A 20 mL vial with 6.67 mL contains about 30% of its capacity and has a surface area ratio of 0.796 cm^2/mL.

Mannitol was selected as the bulking agent in order to maintain a formulation similar to Ribomustin®. Studies were performed to evaluate the effect of mannitol on bendamustine solubility and appearance of the product. Mannitol decreases the solubility of bendamustine (at 15 mg/mL) in both ethanol and TBA aqueous solutions. For example, solutions containing 5% and 10% ethanol and TBA without mannitol did not precipitate over 24 hours. However, for samples with mannitol (Table 1) precipitate was observed within 24 hours. There was no precipitate with aqueous solutions containing 30% (v/v) TBA, 15 mg/mL bendamustine, and 25.5 mg/mL mannitol. In order to maintain a well formed cake resistant to breakage during handling, a minimum of 134 mg/vial of mannitol was required with no difference observed in vials up to 200 mg/vial of mannitol.

All alcohols tested increased the stability and solubility of bendamustine. However, a significant mole fraction was required to affect the stability of the filling solution and the ease of manufacturing. Smaller alcohols have the undesirable effect of lowering the freezing point of the bulk solution and thus requiring long lyophilization cycles at lower temperatures. Higher concentrations of methanol and ethanol produced unattractive cakes that were difficult to reconstitute. 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol, or 30% TBA aqueous solutions containing bendamustine (15 mg/mL), mannitol (25.5 mg/mL) were prepared and lyophilized. The lyophilized vials filled from solutions of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol produced either a collapsed cake or a film residue. The only solvent system producing an acceptable cake was 30% TBA. Additionally, reconstitution of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol lyophilized vials were difficult and did not fully dissolve until >45 minutes.

The ability to utilize a smaller vial is constrained by the concentration or solubility of bendamustine in the aqueous/organic solution. At lower concentrations of ethanol, methanol, isopropanol and n-propanol, which produced acceptable cake appearance, a more dilute solution of bendamustine is required due to solubility limitations. To maintain a presentation with 100 mg of bendamustine per vial, a vial larger than 50 mL would be required. Also, stability studies herein indicated that at the lower alcohol concentration, the chemical stability was not sufficient to allow for acceptable filling times.

One of the factors affecting the ease of reconstitution is the porosity of the lyophilate. In general, amorphously precipitated solids with little surface area are more difficult to solubilize. Most lyophilates containing mannitol will reconstitute within 3-5 minutes as long as there is no precipitate formed during lyophilization, frequently caused by evaporation of a liquid (melt back). Based on our experience with several lyophilization solvent systems and not wishing to be bound to any particular theory, the problems associated with Ribomustin® reconstitution may be associated with precipitation caused by melt back during lyophilization. Most organic solvents do not lyophilize efficiently and cause melt back because of their low melting point. TBA (tertiary butyl alcohol) has a high melting point and a similar vapor pressure as compared to water. TBA is removed by sublimation, not evaporation, at about the same rate as water. Lyophilates produced with 30% (v/v) TBA according to the invention reconstitute within 3-10 minutes as compare to commercially available Ribomustin which may take 30-45 minutes.

Based upon the solubility, stability, ease of reconstitution and manufacturing considerations, the following is a preferred pre-lyophilization formulation of the present invention: bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and q.s. using water for Injection. The formulation is then filled at 5° C. using 6.67 mL in an amber 20 mL, 20 mm vial and partially stoppered with a bromobutyl stopper and loaded into a pre-chilled lyophilizer.

Example 5

Impurity Assessment

Major impurities introduced during Ribomustin® manufacturing, compounding, fill, and lyophilization procedure, as

determined by HPLC analysis (FIG. **6**), are the hydrolysis product HP1, the Dimer, and the ethyl ester of bendamustine, BM1EE. BM1EE can be formed during drug substance manufacturing, e.g., during recrystallization and/or purification processes. BM1EE is known to be a more potent cytotoxic drug than bendamustine. Experiments were undertaken to determine if the use of a 30% TBA aqueous filling solution would lead to the formation of bendamustine t-butyl ester.

Experiments were performed using traditional Fisher esterfication reaction conditions required for the formation of t-butyl ester of bendamustine. Bendamustine was heated in 60° C. TBA with HCl for 20 hours. No reaction was observed. This result indicated that it would be very difficult to form the tert-butyl ester of bendamustine during the fill/finish process. No new impurities in drug product manufactured from TBA have been observed in stability studies to date.

To aid in the testing of the drug product, synthetic routes using more reactive sources of the t-butyl moiety were developed. Another attempt to make tert-butyl ester was carried out by formation of the acyl chloride of bendamustine. A suspension of bendamustine in methylene chloride was treated with oxalyl chloride and N,N-dimethylformamide. After acyl chloride was formed, the solvent was concentrated. The residue was added to methylene chloride, tert-butanol, triethylamine, and 4-dimethylaminopyridine and the mixture was stirred at room temperature overnight. After adding all solvents and purification, an unknown compound was given. The LC-MS did not match the molecular weight of bendamustine tert-butyl ester and the proton NMR did not showed the peak for tert-butyl. Therefore, this attempt also failed to produce the bendamustine tert-butyl ester. Thus, using TBA as the co-solvent has an additional benefit of not forming the ester from the alcohol.

Example 6

Lyophilization Cycle Development

Numerous lyophilization cycles were performed to evaluate the critical stages of lyophilization and achieve the most efficient drying cycle. Experiments were performed to evaluate the effect of the freezing rate, primary drying temperature, time, and pressure on the product.

A. Freezing Rate

The literature reports that TBA adopts different crystal forms depending on the freeze rate. In some TBA solutions, the slower the product froze, the quicker it dried. Larger crystals formed during slow freezing producing bigger pores allowing more efficient sublimation. However, during studies with bendamustine, the freezing rate was not found to be a critical processing parameter when evaluated at 2 and 8 hours.

B. Primary and Secondary Drying

During the first attempts to lyophilize from 30% TBA solutions, the lyophilized cake fractured and powder was ejected from the vial. These cakes appeared to contain amorphous particles within the lyophilate, an indication of melt back. This phenomenon was reproducible and occurred when the product reached about −10° C. (refer to FIG. **5**) independent of the warming rate. Several variables were tested to determine the cause and solution to the problem of the powder ejection. The pressure was raised from 50 μm to 150 μm during primary drying, but powder ejection was still observed but to a lesser extent. This experiment was then repeated except the freezing rate was extended to 8 hours from 2 hours. This change had no effect.

The length of primary drying was next evaluated. For example, the following very slow drying cycle was evaluated: freezing from +25° C. to −50° C. in eight hours; holding at −50° C. for 5 hours, warming and drying from −50° C. to −25° C. in seven hours; holding for twenty hours at −25° C., warming and drying from −25° C. to −15° C. in two hours and holding for twenty hours at −15° C., warming and drying from −15° C. to 40° C. in six hours and holding for twenty hours at 40° C. while maintaining a chamber pressure of 150 μm throughout drying. No powder ejection (FIG. **5**) was observed. This cycle resulted in a well-formed cake without fracture that reconstituted readily. Without wishing to be bound to a particular theory, the problems with powder ejection and difficulty with reconstitution may be the result of drying the lyophilate too quickly, thus resulting in strong vapor flow out of the cake as well as melt back. With the use of a less aggressive drying cycle an aesthetic, stable, and easy to reconstitute cake was reproducibly formed. Thus, removing all unbound water and tertiary-butyl alcohol prior to secondary drying may prevent melt back as well as powder ejection. The lyophilization cycle was further optimized under these gentle conditions (FIG. **5**). There were no immediate degradation products as a result of drying at 40° C. for up to 20 hours.

Example 7

Lyophilization Cycle

Step	Description	Time (Hour)	Temperature (° C.)	Pressure (Microns)
1	Hold	0.25	5° C.	—
2	Ramp	8	−50° C.	—
3	Hold	4	−50° C.	—
4	Ramp	3	−20° C.	150
5	Hold	6	−20° C.	150
6	Ramp	1	−15° C.	150
7	Hold	20	−15° C.	150
8	Ramp	0.5	−12° C.	150
9	Hold	15.5	−12 C.	150
10	Ramp	15	35 C.	50
11	Hold	10	35° C.	50
12	Ramp	1	40 C.	50
	Hold	5	40 C.	50
Total		89.25	—	—

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the spirit and scope of the invention. More specifically, it will be apparent that certain solvents which are both chemically and physiologically related to the solvents disclosed herein may be substituted for the solvents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit and scope of the invention as defined by the appended claims.

All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All

patents, patent applications, and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

What is claimed is:

1. A stable lyophilized preparation comprising bendamustine hydrochloride, mannitol, and a trace amount of tertiary-butyl alcohol (TBA), wherein the ratio by weight of bendamustine hydrochloride to mannitol is 15:25.5.

2. The stable lyophilized preparation of claim **1** in a vial containing 25 mg bendamustine hydrochloride.

3. The stable lyophilized preparation of claim **1** in a vial containing 100 mg bendamustine hydrochloride.

4. A stable lyophilized preparation comprising bendamustine hydrochloride and mannitol in a ratio by weight of about 15:25.5, wherein the preparation is obtained by a process comprising:

a) preparing a composition comprising bendamustine hydrochloride, mannitol, tertiary-butyl alcohol and water, wherein the bendamustine hydrochloride and mannitol are present in the ratio by weight of about 15:25.5, and

b) lyophilizing the composition from step a) to obtain the preparation.

* * * * *

Exhibit D

US008791270B2

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

(10) **Patent No.: US 8,791,270 B2**
(45) **Date of Patent: Jul. 29, 2014**

(54) **BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS**

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USPC **548/304.7**; 34/284

(58) **Field of Classification Search**

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention provides pharmaceutical formulations of lyophilized bendamustine suitable for pharmaceutical use. The present invention further provides methods of producing lyophilized bendamustine. The pharmaceutical formulations can be used for any disease that is sensitive to treatment with bendamustine, such as neoplastic diseases.

23 Claims, 6 Drawing Sheets

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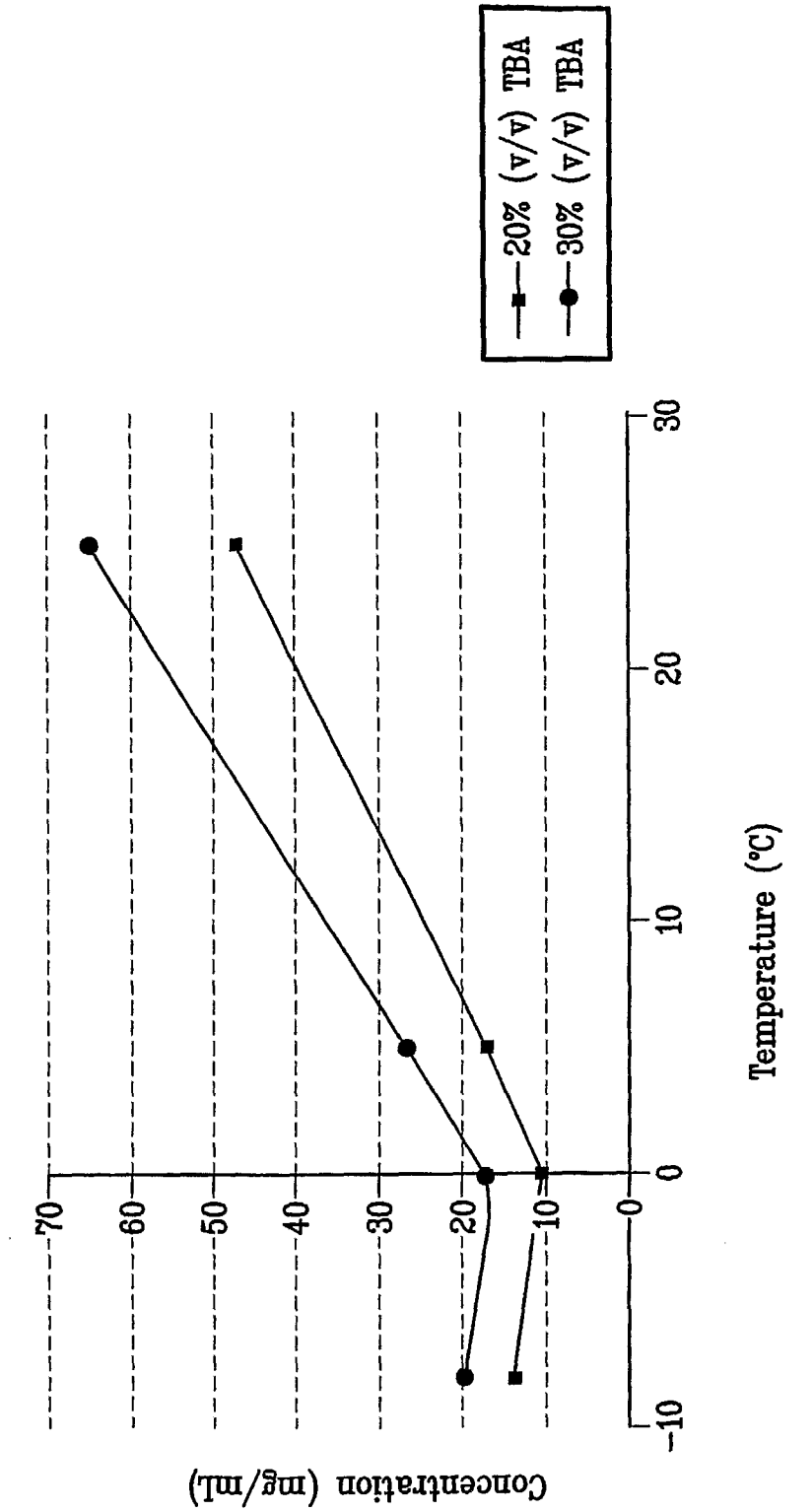

20% (v/v) TBA
30% (v/v) TBA
Concentration (mg/mL)
70
60
50
40
30
20
10
0
Temperature (°C)
30
20
10
0
-10

FIG. 1

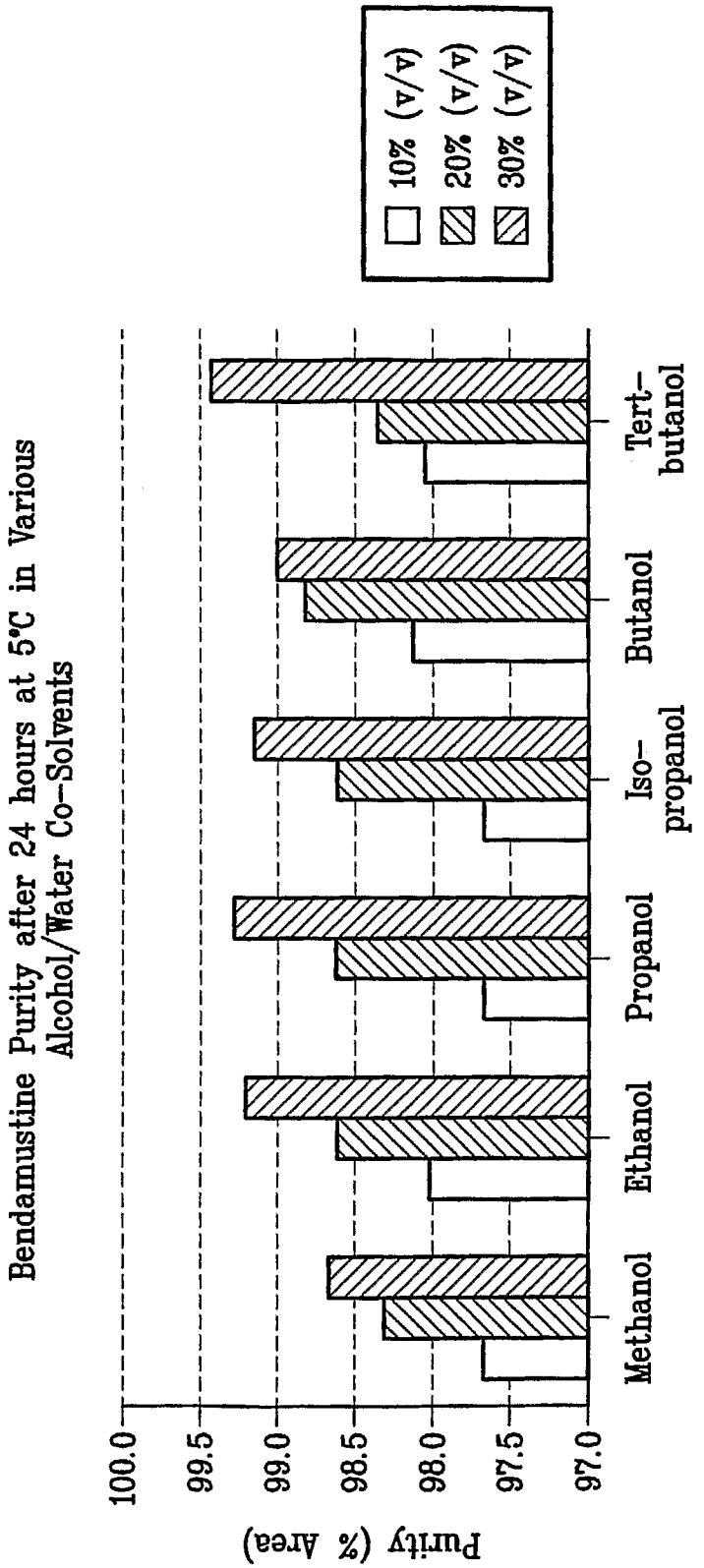

Bendamustine Purity after 24 hours at 5°C in Various Alcohol/Water Co-Solvents
10% (v/v)
20% (v/v)
30% (v/v)
Purity (% Area)
100.0
99.5
99.0
98.5
98.0
97.5
97.0
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol

FIG. 2

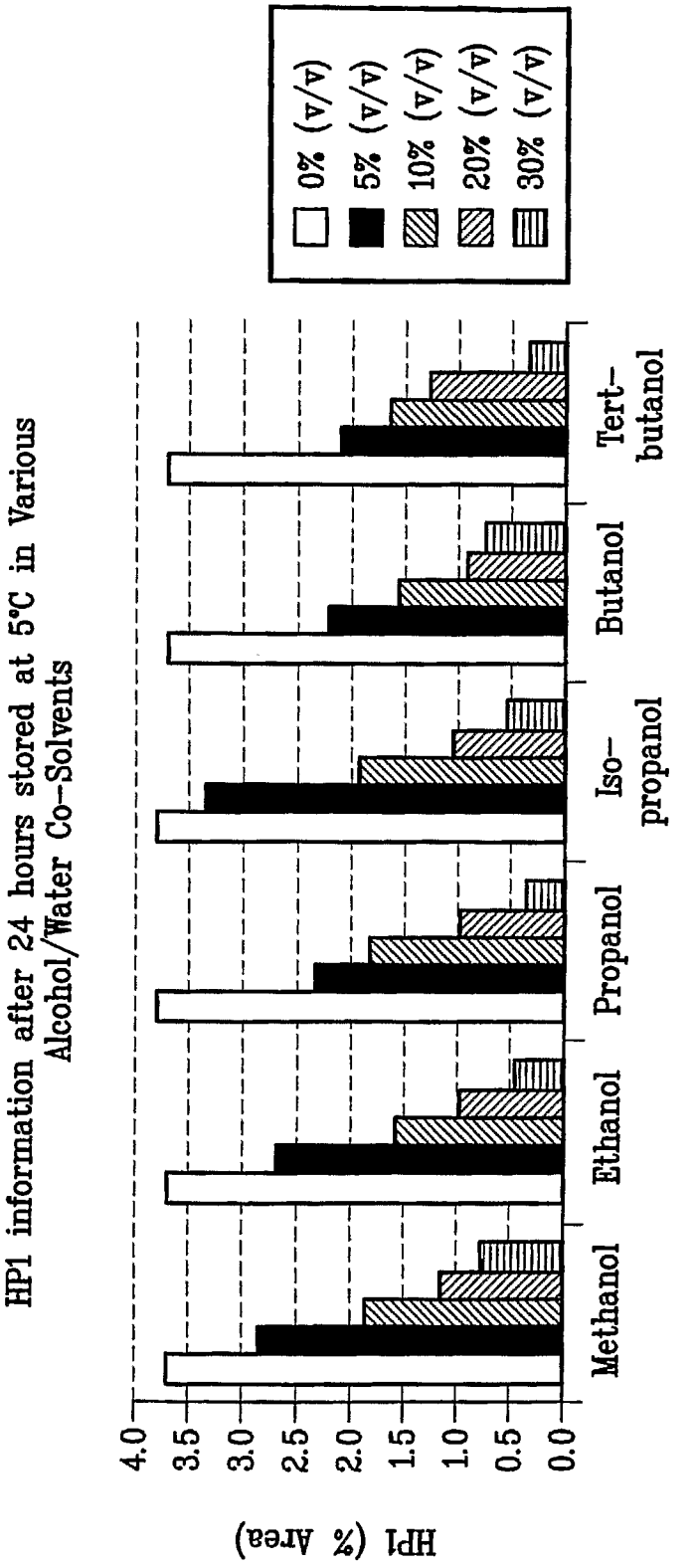

HP1 information after 24 hours stored at 5°C in Various Alcohol/Water Co-Solvents
HP1 (% Area)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0% (v/v)
5% (v/v)
10% (v/v)
20% (v/v)
30% (v/v)
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol

FIG. 3

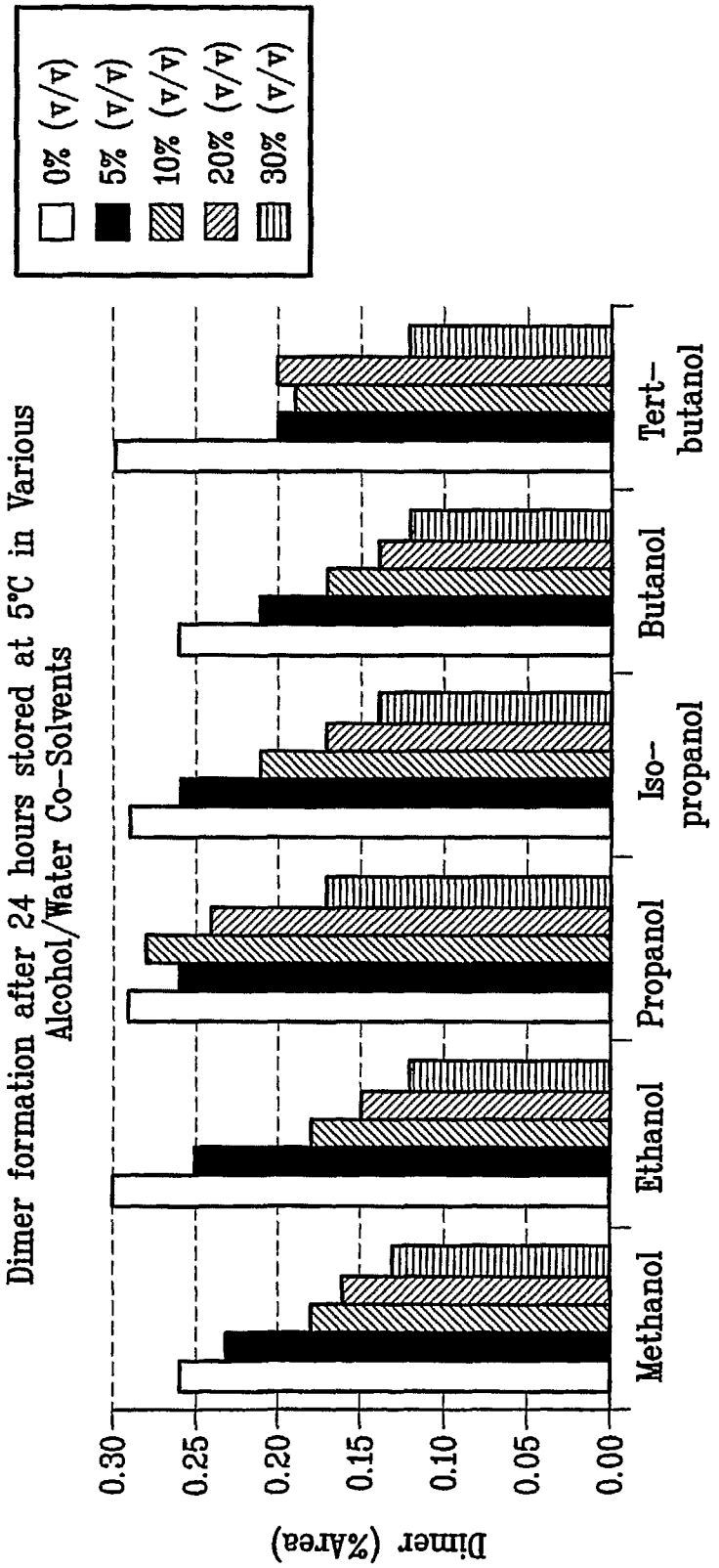

0% (v/v)
5% (v/v)
10% (v/v)
20% (v/v)
30% (v/v)
Dimer formation after 24 hours stored at 5°C in Various Alcohol/Water Co-Solvents
Dimer (%Area)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Methanol
Ethanol
Propanol
Iso-propanol
Butanol
Tert-butanol

FIG. 4

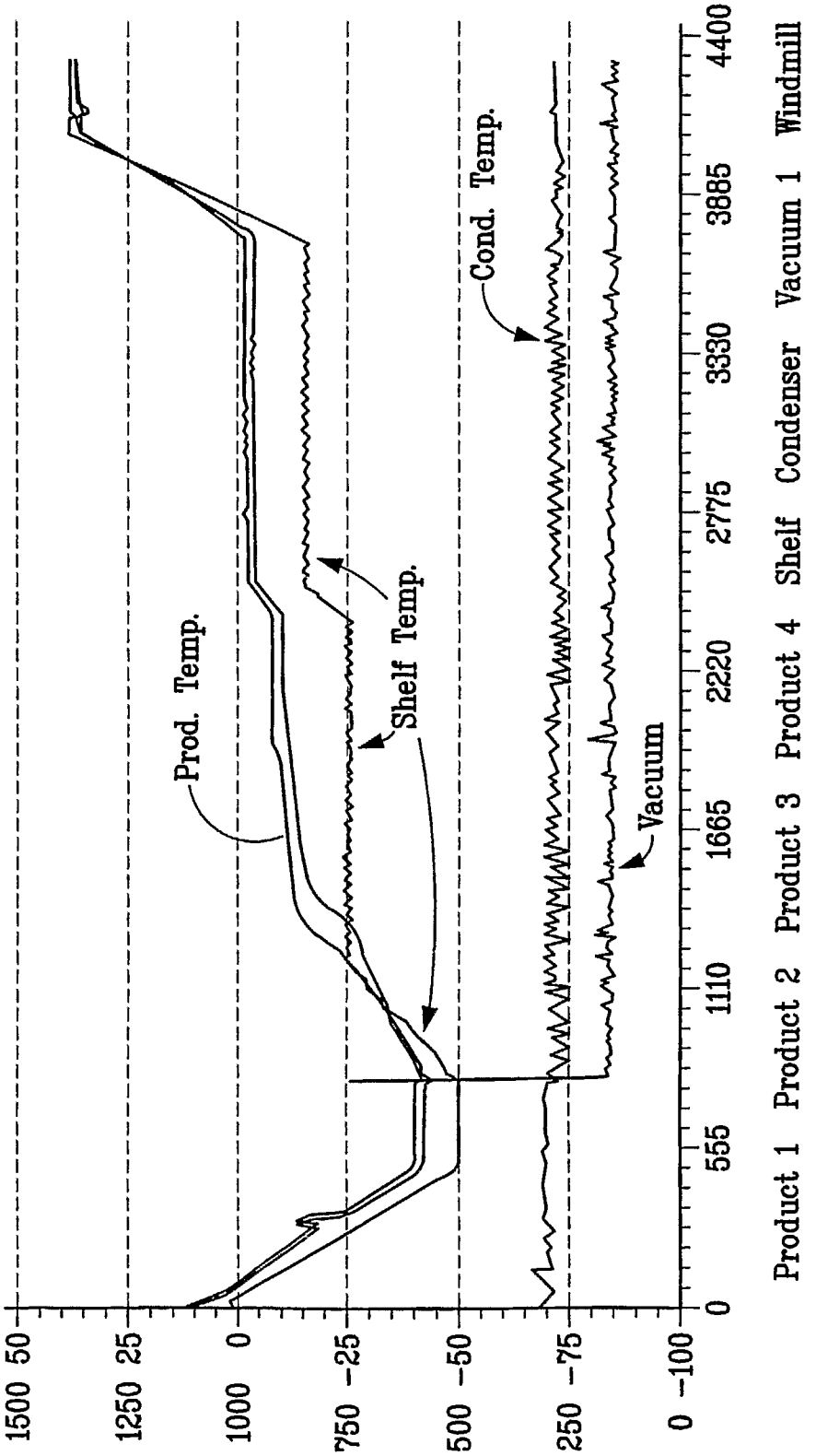

Prod. Temp.
Shelf Temp.
Cond. Temp.
Vacuum
1500 50
1250 25
1000 0
750 -25
500 -50
250 -75
0 -100
0
555
1110
1665
2220
2775
3330
3885
4400
Product 1 Product 2 Product 3 Product 4 Shelf Condenser Vacuum 1 Windmill

FIG. 5

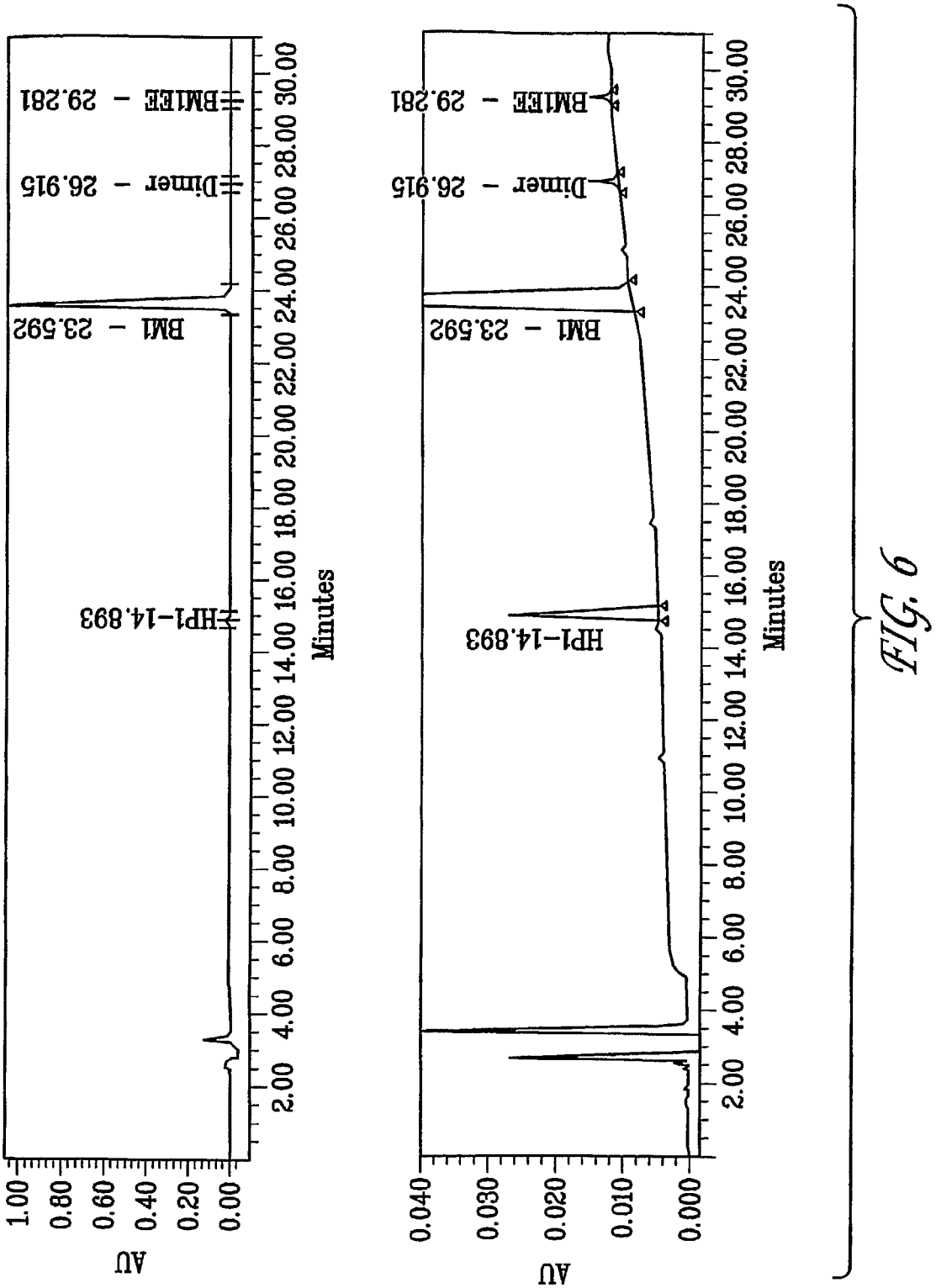

BM1EE - 29.281
Dimer - 26.915
BM1 - 23.592
HP1-14.893
AU
Minutes
1.00
0.80
0.60
0.40
0.20
0.00
0.040
0.030
0.020
0.010
0.000
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00

FIG. 6

BENDAMUSTINE PHARMACEUTICAL COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 13/719,409, filed Dec. 19, 2012, which is a continuation of U.S. application Ser. No. 13/654,898, filed Oct. 18, 2012, now U.S. Pat. No. 8,461,350, which is a continuation of U.S. application Ser. No. 11/330,868, filed Jan. 12, 2006, now U.S. Pat. No. 8,436,190, which claims the benefit of U.S. Provisional Application No. 60/644,354, filed Jan. 14, 2005, the entireties of which are incorporated herein for all purposes.

FIELD OF THE INVENTION

The present invention pertains to the field of pharmaceutical compositions for the treatment of various disease states, especially neoplastic diseases and autoimmune diseases. Particularly, it relates to pharmaceutical formulations comprising nitrogen mustards, particularly the nitrogen mustard bendamustine, e.g., bendamustine HCl.

BACKGROUND OF THE INVENTION

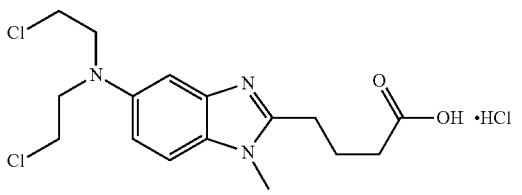
The present invention claims the benefit of and priority to U.S. Ser. No. 60/644,354, filed Jan. 14, 2005, entitled, "Bendamustine Pharmaceutical Compositions," which is incorporated herein by reference in its entirety, including figures and claims.

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

Because of their high reactivity in aqueous solutions, nitrogen mustards are difficult to formulate as pharmaceuticals and are often supplied for administration in a lyophilized form that requires reconstitution, usually in water, by skilled hospital personal prior to administration. Once in aqueous solution, nitrogen mustards are subject to degradation by hydrolysis, thus, the reconstituted product should be administered to a patient as soon as possible after its reconstitution.

Bendamustine, (4-{5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolyl}butyric acid, is an atypical structure with a benzimidazole ring, whose structure includes an active nitrogen mustard (see Formula I, which shows bendamustine hydrochloride).

Formula I



Bendamustine was initially synthesized in 1963 in the German Democratic Republic (GDR) and was available from 1971 to 1992 in that location under the name Cytostasan®. Since that time, it has been marketed in Germany under the tradename Ribomustin®. It has been widely used in Germany to treat chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and breast cancer.

Due to its degradation in aqueous solutions (like other nitrogen mustards), bendamustine is supplied as a lyophilized product. The current lyophilized formulation of bendamustine (Ribomustin®) contains bendamustine hydrochloride and mannitol in a sterile lyophilized form as a white powder for intravenous use following reconstitution. The finished lyophilisate is unstable when exposed to light. Therefore, the product is stored in brown or amber-colored glass bottles. The current lyophilized formulation of bendamustine contains degradation products that may occur during manufacturing of the drug substance and/or during the lyophilization process to make the finished drug product.

Currently bendamustine is formulated as a lyophilized powder for injection with 100 mg of drug per 50 mL vial or 25 mg of drug per 20 mL vial. The vials are opened and reconstituted as close to the time of patient administration as possible. The product is reconstituted with 40 mL (for the 100 mg presentation) or 10 mL (for the 25 mg presentation) of Sterile Water for Injection. The reconstituted product is further diluted into 500 mL, q.s., 0.9% Sodium Chloride for Injection. The route of administration is by intravenous infusion over 30 to 60 minutes.

Following reconstitution with 40 mL Sterile Water for Injection, vials of bendamustine are stable for a period of 7 hours under room temperature storage or for 6 days upon storage at 2-8° C. The 500 mL admixture solution must be administered to the patient within 7 hours of vial reconstitution (assuming room temperature storage of the admixture).

The reconstitution of the present bendamustine lyophilized powder is difficult. Reports from the clinic indicate that reconstitution can require at least fifteen minutes and may require as long as thirty minutes. Besides being burdensome and time-consuming for the healthcare professional responsible for reconstituting the product, the lengthy exposure of bendamustine to water during the reconstitution process increases the potential for loss of potency and impurity formation due to the hydrolysis of the product by water.

Thus, a need exists for lyophilized formulations of bendamustine that are easier to reconstitute and which have a better impurity profile than the current lyophilate (lyophilized powder) formulations of bendamustine.

German (GDR) Patent No. 34727 discloses a method of preparing ω-[5-bis-(β-chloroethyl)-amino-benzimidazolyl-(2)]-alkane carboxylic acids substituted in the 1-position.

German (GDR) Patent No. 80967 discloses an injectable preparation of γ-[1-methyl-5-bis-(β-chloroethyl)-amino-benzimaidazolyl-(2)]-butric acid hydrochloride.

German (GDR) Patent No. 159877 discloses a method for preparing 4-[1-methyl-5-bis(2-chloroethyl)amino-benzimidazolyl-2)-butyric acid.

German (GDR) Patent No. 159289 discloses an injectable solution of bendamustine.

Ribomustin® bendamustine Product monograph (updated 1/2002) http://www.ribosepharm.de/pdf/ribomustin_benda-

mustin/productmonograph.pdf provides information about Ribomustin® including product description.

Ni et al. report that the nitrosourea SarCNU was more stable in pure tertiary butanol than in pure acetic acid, dimethyl sulfoxide, methylhydroxy, water or in TBA/water mixtures (Ni et al. (2001) *Intl. J. Phamaceutics* 226:39-46).

Lyophilized cyclophoshamide is known in the art see e.g., U.S. Pat. Nos. 5,418,223; 5,413,995; 5,268,368; 5,227,374; 5,130,305; 4,659,699; 4,537,883; and 5,066,647.

The lyophilized nitrogen mustard Ifosfamide is disclosed in International Publication No. WO 2003/066027; U.S. Pat. Nos. 6,613,927; 5,750,131; 5,972,912; 5,227,373; and 5,204,335.

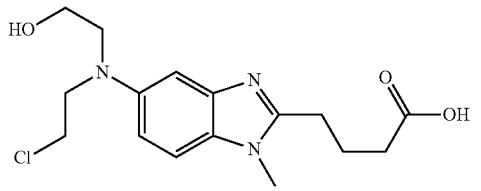
Teagarden et al. disclose lyophilized formulations of prostaglandin E-1 made by dissolving PGE-1 in a solution of lactose and tertiary butyl alcohol (U.S. Pat. No. 5,770,230).

SUMMARY OF THE INVENTION

The present invention is directed to stable pharmaceutical compositions of nitrogen mustards, in particular lyophilized bendamustine and its use in treatment of various disease states, especially neoplastic diseases and autoimmune diseases.

An embodiment of the invention is a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1, as shown in Formula II,

Formula II

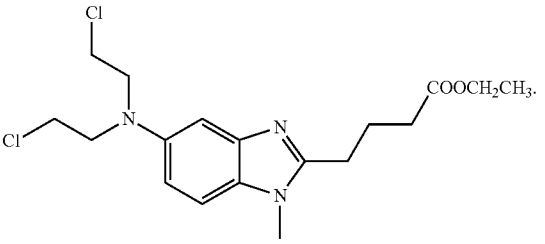


at the time of release or where the HP1 is the amount of HP1 present at time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as described herein. In a preferred embodiment is a pharmaceutical composition of bendamustine containing not more than about 0.5% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%.

Another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.1% to about 0.3% bendamustine dimer as shown in Formula III at release or at time zero after reconstitution

Yet another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5%, preferably 0.15% to about 0.5%, bendamustine ethylester, as shown in Formula IV at release or at time zero after reconstitution

Formula IV



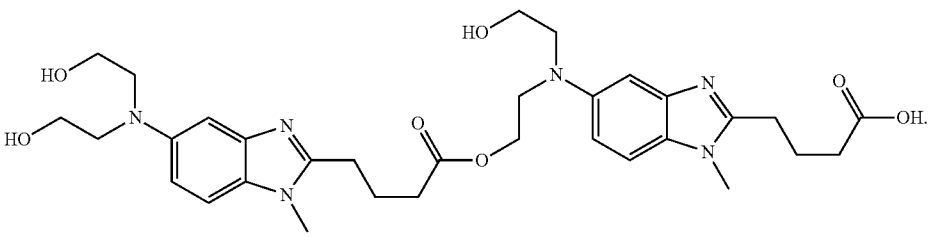
Yet another embodiment of the invention is a lyophilized preparation of bendamustine wherein the concentration of bendamustine ethylester (Formula IV) is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the lyophilized preparation.

In another embodiment of the invention is a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine) HP1 at the time of drug product release. In a preferred embodiment is a lyophilized preparation of bendamustine containing not more than about 0.50% (area percent of bendamustine) HP1, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%. An aspect of this embodiment is lyophilized preparations of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 at the time of release of drug product where the lyophilized preparation is packaged in a vial or other pharmaceutically acceptable container.

In yet another aspect of the invention, the lyophilized preparations of bendamustine are stable with respect to the amount of HP1 for at least about 6 months, preferably 12 months, preferably 24 months, to about 36 months or greater when stored at about 2° to about 30°. Preferred temperatures for storage are about 5° C. and about room temperature.

Another embodiment of the invention is a pharmaceutical dosage form that includes a pharmaceutical composition of bendamustine containing not more than about 0.5% to about 0.9% HP1, preferably not more than about 0.50%, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%, where the HP1 is

Formula III



the amount of HP1 present at release or at time zero after reconstitution of a lyophilized preparation of bendamustine of the present invention. In preferred aspects of the invention, the dosage form can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

Yet another embodiment of the invention is a pharmaceutical dosage form that includes a lyophilized preparation of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1. Preferred dosage forms can be about 5 to about 500 mg of bendamustine, about 10 to about 300 mg of bendamustine, about 25 mg of bendamustine, about 100 mg of bendamustine, and about 200 mg of bendamustine.

In still another embodiment, the invention includes a pharmaceutical composition of bendamustine including bendamustine containing not more than about 0.5% to about 0.9% (area percent of bendamustine), preferably not more than about 0.50%, preferably not more than about 0.45%, more preferably not more than about 0.40%, more preferably not more than about 0.35%, even more preferably not more than 0.30%, and a trace amount of one or more organic solvents, wherein said HP1 is the amount of HP1 present at release or time zero after reconstitution of a lyophilized pharmaceutical composition of bendamustine as disclosed herein. In different aspects of this embodiment, the organic solvent is selected from one or more of tertiary butanol, n-propanol, n-butanol, isopropanol, ethanol, methanol, acetone, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, 1-pentanol, methyl acetate, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, and cyclohexane. Preferred organic solvents include one or more of ethanol, methanol, propanol, butanol, isopropanol, and tertiary butanol. A more preferred organic solvent is tertiary butanol, also known as TBA, t-butanol, tert-butyl alcohol or tertiary butyl alcohol.

The present invention involves a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a release specification for bendamustine degradants at less than about 4.0%, preferably about 2.0% to about 4.0%, (area percent bendamustine) or otherwise to achieve the pharmaceutical compositions described herein. An aspect of this embodiment is a method for obtaining agency approval for a bendamustine product which includes setting a release specification for HP1 to be less than or equal to 1.5% (area percent Bendamustine). The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment is a method for obtaining agency approval for a bendamustine product, the improvement which includes setting a shelf-life specification for bendamustine degradants at less than about 7.0%, preferably about 5.0% to about 7.0%, (area percent bendamustine) where the product is stored at about 2° C. to about 30° C. Preferred temperatures for storage are about 5° C. and about room temperature. The bendamustine product herein contains not more than about 0.5% (area percent of bendamustine) HP1 at release.

Another embodiment of the invention is a process for manufacturing a lyophilized preparation of bendamustine which includes controlling for the concentration of bendamustine degradants in the final product, such that the concentration of bendamustine degradants is less than about 4.0%, preferably no more than about 2.0% to about 4.0%, (area percent of bendamustine) at release or otherwise to achieve the pharmaceutical compositions described herein. The bendamustine product herein contains not more than about 0.5% to about 0.9%, preferably about 0.5%, (area percent of bendamustine) HP1 at release.

The present invention discloses a process for manufacturing a lyophilized preparation of bendamustine which comprises controlling for the concentration of bendamustine degradants in the final product, such that, at release, the concentration of HP1 is less than 0.9%, preferably 0.5%, (area percent of bendamustine) and, at the time of product expiration, the concentration of bendamustine degradants is less than about 7.0%, preferably no more than about 5.0% to about 7.0%; wherein said product is stored at about 2° C. to about 30° C.

Another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of HP1 produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% to about 0.9% (area percent of bendamustine) preferably 0.50%, preferably 0.45%, more preferably 0.40%, more preferably 0.35%, even more preferably 0.30%. An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester produced during lyophilization from about 0 to 24 hours does not exceed about 0.5% (area percent bendamustine). An aspect of this embodiment is the lyophilized powder produced from the pre-lyophilization solution or dispersion.

Still another embodiment of the invention is a bendamustine pre-lyophilization solution or dispersion comprising one or more organic solvents where the solution or dispersions include at least one stabilizing concentration of an organic solvent which reduces the level of degradation of bendamustine so that the amount of bendamustine ethylester (as shown in Formula IV) produced during lyophilization from about 0 to 24 hours is no more than 0.2%, preferably 0.1%, greater than the concentration of bendamustine ethylester as found in the drug substance used to make the pre-lyophilization solution. A preferred organic solvent is tertiary butanol.

The invention also discloses methods for preparing a bendamustine lyophilized preparation that includes dissolving bendamustine in a stabilizing concentration of an alcohol solvent of between about 5% to about 100% (v/v alcohol to form a pre-lyophilization solution; and lyophilizing the pre-lyophilization solution; wherein the bendamustine lyophilized preparation made from such methods contains not more than about 0.5% to about 0.9%, preferably 0.5%, (area percent of bendamustine) HP1 as shown in Formula II, wherein said HP1 is the amount of HP1 present at release or at time zero after reconstitution of the lyophilized pharmaceutical composition of bendamustine. Other alcohol concentrations include about 5% to about 99.9%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 40%, about 20% to about 35%. Preferred concentrations of alcohol are from about 20% to about 30%. Preferred alcohols include one or more of methanol, ethanol, propanol, iso-propanol, butanol, and tertiary-butanol. A more preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an

excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

In a preferred method for preparing a bendamustine lyophilized preparation, lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to a temperature below about −40° C., preferably −50° C., to form a frozen solution; ii) holding the frozen solution at or below −40° C., preferably −50° C., for at least 2 hours; iii) ramping the frozen solution to a primary drying temperature between about −40° C. and about −10° C. to form a dried solution; iv) holding for about 10 to about 70 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vii) holding for about 5 to about 40 hours to form a bendamustine lyophilized preparation. In a more preferred method lyophilizing the pre-lyophilization solution comprises i) freezing the pre-lyophilization solution to about −50° C. to form a frozen solution; ii) holding the frozen solution at about −50° C. for at least 2 hours to about 4 hours; iii) ramping to a primary drying temperature between about −20° C. and about −12° C. to form a dried solution; iv) holding at a primary drying temperature for about 10 to about 48 hours; v) ramping the dried solution to a secondary drying temperature between about 25° C. and about 40° C.; and vi) holding at a secondary drying temperature for at least 5 hours up to about 20 hours. A preferred alcohol is tertiary-butanol. A preferred concentration of tertiary-butanol is about 20% to about 30%, preferably about 30%. An aspect of this embodiment is the addition of an excipient before lyophilization. A preferred excipient is mannitol. Preferred pre-lyophilized concentrations of bendamustine are from about 2 mg/mL to about 50 mg/mL.

Another embodiment of the invention is the lyophilized powder or preparation obtained from the methods of preparing a bendamustine lyophilized preparation disclosed herein.

The invention also involves bendamustine formulations for lyophilization that include an excipient and a stabilizing concentration of an organic solvent. A preferred formulation includes bendamustine at a concentration of about 15 mg/mL, mannitol at a concentration of about 25.5 mg/mL, tertiary-butyl alcohol at a concentration of about 30% (v/v) and water. Included in this embodiment of the invention are the lyophilized preparations made from such bendamustine formulations.

Included in the inventions are methods of treating a medical condition in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition. Some conditions amenable to treatment with the compositions of the invention include chronic lymphocytic leukemia (CLL), Hodgkin's disease, non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), breast cancer, small cell lung cancer, hyperproliferative disorders, and an autoimmune disease. Preferred conditions include NHL, CLL, breast cancer, and MM. Preferred autoimmune diseases include rheumatoid arthritis, multiple sclerosis or lupus.

Included in the inventions are the use of the pharmaceutical compositions or pharmaceutical preparations of the invention in the manufacture of a medicament for the treatment of a medical condition, as defined herein, in a patient that involve administering a therapeutically effective amount of a pharmaceutical composition of the invention where the condition is amenable to treatment with said pharmaceutical composition.

Also included in the invention are methods of treating in which the pharmaceutical compositions of the invention are in combination with one or more anti-neoplastic agents where the antineoplastic agent is given prior, concurrently, or subsequent to the administration of the pharmaceutical composition of the invention. Preferred antineoplastic agents are antibodies specific for CD20.

Another embodiment of the invention is a lyophilization cycle for producing lyophilized bendamustine preparations of the invention. A preferred lyophilization cycle includes a) freezing to about −50° C. over about 8 hours; b) holding at −50° C. for about 4 hours; c) ramping to −25° C. over about 3 hours; d) holding at −10° C. for 30 hours; e) ramping to between about 25° C. and about 40° C. or higher for about 3 hours; f) holding between about 25° C. and about 40° C. for about 25 hours; g) ramping to about 20° C. in 1 hour; h) unloading at about 20° C., at a pressure of 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. An aspect of this cycle involves step (e) which is ramped to about 30-35° C. for 3 hours and then ramped to 40° C. for 5 hours. Another aspect of this embodiment is the lyophilized powered prepared from such lyophilization cycles. A more preferred lyophilization cycle includes i) starting with a shelf temperature of about 5° C. for loading; ii) freezing to about −50° C. over about 8 hours; iii) holding at −50° C. for about 4 hours; iv) ramping to about −20° C. over about 3 hours; v) holding at about −20° C. for 6 hours; ramping to about −15° C. over about 1 hour; vi) holding at −15° C. for about 20 hours; vii) ramping to about −15° C. over about 1 hour; viii) holding at about −15° C. for about 20 hours; ix) ramping to about −12° C. over about 0.5 hours; x) holding at about −12° C. for about 15.5 hours; xi) ramping to between about 25° C. and about 40° C. or higher for about 15 hours; xii) holding between about 25° C. and about 40° C. for about 10 hours; xiii) ramping to about 40° C. over about 1 hour; and xiv) holding at about 40° C. for about 5 hours; unloading at about 5° C., at a pressure of about 13.5 psi in a pharmaceutically acceptable container that is hermetically sealed; wherein the pressure is about 150 microns throughout primary drying and 50 microns throughout secondary drying. In a preferred embodiment step (xi) is ramped to about 30-35° C. for about 15 hours.

The invention also encompasses a pharmaceutical dosage form of bendamustine containing not more than about 0.5% to about 0.9%, preferably 0.5%, HP1 (area percent of bendamustine) wherein said dosage form comprises a vial or other pharmaceutically acceptable container, wherein said HP1 is the amount of HP1 present pre-reconstitution or at time zero after reconstitution of said dosage form. Preferred concentrations of bendamustine include about 10 to about 500 mg/container, about 100 mg/container, about 5 mg to about 2 g/container and about 170 mg/container.

The present invention also includes pre-lyophilized pharmaceutical compositions of bendamustine. A preferred prelyophilized composition includes bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and water.

These and other embodiments of the invention are described hereinbelow or are evident to persons of ordinary skill in the art based on the following disclosures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the solubility of bendamustine at various temperatures for two different solutions of bendamustine in tertiary butanol.

FIG. **2** shows the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the bendamustine peak.

FIG. **3** shows HP1 (Formula II) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **4** shows dimer (Formula III) formation after 24 hours in various alcohol/water co-solvents at 5° C.

FIG. **5**—shows a lyophilization cycle for bendamustine using a TBA/water co-solvent.

FIG. **6** shows a chromatogram for Ribomustin® using HPLC method No. 1.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the terms "formulate" refers to the preparation of a drug, e.g., bendamustine, in a form suitable for administration to a mammalian patient, preferably a human. Thus, "formulation" can include the addition of pharmaceutically acceptable excipients, diluents, or carriers.

As used herein, the term "lyophilized powder" or "lyophilized preparation" refers to any solid material obtained by lyophilization, i.e., freeze-drying of an aqueous solution. The aqueous solution may contain a non-aqueous solvent, i.e. a solution composed of aqueous and one or more non-aqueous solvent(s). Preferably, a lyophilized preparation is one in which the solid material is obtained by freeze-drying a solution composed of aqueous and one or more non-aqueous solvents, more preferably the non-aqueous solvent is an alcohol.

By "stable pharmaceutical composition" is meant any pharmaceutical composition having sufficient stability to have utility as a pharmaceutical product. Preferably, a stable pharmaceutical composition has sufficient stability to allow storage at a convenient temperature, preferably between −20° C. and 40° C., more preferably about 2° C. to about 30° C., for a reasonable period of time, e.g., the shelf-life of the product which can be as short as one month but is typically six months or longer, more preferably one year or longer even more preferably twenty-four months or longer, and even more preferably thirty-six months or longer. The shelf-life or expiration can be that amount of time where the active ingredient degrades to a point below 90% purity. For purposes of the present invention stable pharmaceutical composition includes reference to pharmaceutical compositions with specific ranges of impurities as described herein. Preferably, a stable pharmaceutical composition is one which has minimal degradation of the active ingredient, e.g., it retains at least about 85% of un-degraded active, preferably at least about 90%, and more preferably at least about 95%, after storage at 2-30° C. for a 2-3 year period of time.

By "stable lyophilized preparation" is meant any lyophilized preparation having sufficient stability, such characteristics as similarly defined herein for a stable pharmaceutical composition, to have utility as a pharmaceutical product

By "degraded" is meant that the active has undergone a change in chemical structure.

The term "therapeutically effective amount" as used herein refers to that amount of the compound being administered that will relieve to some extent one or more of the symptoms of the disorder being treated. In reference to the treatment of neoplasms, a therapeutically effective amount refers to that amount which has the effect of (1) reducing the size of the tumor, (2) inhibiting (that is, slowing to some extent, preferably stopping) tumor metastasis, (3) inhibiting to some extent (that is, slowing to some extent, preferably stopping) tumor growth, and/or, (4) relieving to some extent (or, preferably, eliminating) one or more symptoms associated with the cancer. Therapeutically effective amount can also mean preventing the disease from occurring in an animal that may be predisposed to the disease but does not yet experience or exhibit symptoms of the disease (prophylactic treatment). Further, therapeutically effective amount can be that amount that increases the life expectancy of a patient afflicted with a terminal disorder. Typical therapeutically effective doses for bendamustine for the treatment of non-Hodgkin's lymphoma can be from about 60-120 mg/m^2 given as a single dose on two consecutive days. The cycle can be repeated about every three to four weeks. For the treatment of chronic lymphocytic leukemia (CLL) bendamustine can be given at about 80-100 mg/m^2 on days 1 and 2. The cycle can be repeated after about 4 weeks. For the treatment of Hodgkin's disease (stages II-IV), bendamustine can be given in the "DBVBe regimen" with daunorubicin 25 mg/m^2 on days 1 and 15, bleomycin 10 mg/m^2 on days 1 and 15, vincristine 1.4 mg/m^2 on days 1 and 15, and bendamustine 50 mg/m^2 on days 1-5 with repetition of the cycle about every 4 weeks. For breast cancer, bendamustine (120 mg/m^2) on days 1 and 8 can be given in combination with methotrexate 40 mg/m^2 on days 1 and 8, and 5-fluorouracil 600 mg/m^2 on days 1 and 8 with repetition of the cycle about every 4 weeks. As a second-line of therapy for breast cancer, bendamustine can be given at about 100-150 mg/m^2 on days 1 and 2 with repetition of the cycle about every 4 weeks.

As used herein "neoplastic" refers to a neoplasm, which is an abnormal growth, such growth occurring because of a proliferation of cells not subject to the usual limitations of growth. As used herein, "anti-neoplastic agent" is any compound, composition, admixture, co-mixture, or blend which inhibits, eliminates, retards, or reverses the neoplastic phenotype of a cell.

As used herein "hyperproliferation" is the overproduction of cells in response to a particular growth factor. "Hyperproliferative disorders" are diseases in which the cells overproduce in response to a particular growth factor. Examples of such "hyperproliferative disorders" include diabetic retinopathy, psoriasis, endometriosis, cancer, macular degenerative disorders and benign growth disorders such as prostate enlargement.

As used herein, the term "vial" refers to any walled container, whether rigid or flexible.

"Controlling" as used herein means putting process controls in place to facilitate achievement of the thing being controlled. For example, in a given case, "controlling" can mean testing samples of each lot or a number of lots regularly or randomly; setting the concentration of degradants as a release specification; selecting process conditions, e.g., use of alcohols and/or other organic solvents in the pre-lyophilization solution or dispersion, so as to assure that the concentration of degradants of the active ingredient is not unacceptably high; etc. Controlling for degradants by setting release specifications for the amount of degradants can be used to facilitate regulatory approval of a pharmaceutical product by a regulatory agency, such as the U.S. Food and Drug Administration and similar agencies in other countries or regions ("agency").

The term "pharmaceutically acceptable" as used herein means that the thing that is pharmaceutically acceptable, e.g., components, including containers, of a pharmaceutical composition, does not cause unacceptable loss of pharmacological activity or unacceptable adverse side effects. Examples of pharmaceutically acceptable components are provided in The United States Pharmacopeia (USP), The National Formulary (NF), adopted at the United States Pharmacopeial Convention, held in Rockville, Md. in 1990 and FDA Inactive Ingre-

dient Guide 1990, 1996 issued by the U.S. Food and Drug Administration (both are hereby incorporated by reference herein, including any drawings). Other grades of solutions or components that meet necessary limits and/or specifications that are outside of the USP/NF may also be used.

The term "pharmaceutical composition" as used herein shall mean a composition that is made under conditions such that it is suitable for administration to humans, e.g., it is made under GMP conditions and contains pharmaceutically acceptable excipients, e.g., without limitation, stabilizers, bulking agents, buffers, carriers, diluents, vehicles, solubilizers, and binders. As used herein pharmaceutical composition includes but is not limited to a pre-lyophilization solution or dispersion as well as a liquid form ready for injection or infusion after reconstitution of a lyophilized preparation.

A "pharmaceutical dosage form" as used herein means the pharmaceutical compositions disclosed herein being in a container and in an amount suitable for reconstitution and administration of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. Preferably, a "pharmaceutical dosage form" as used herein means a lyophilized pharmaceutical composition disclosed herein in a container and in an amount suitable for reconstitution and delivery of one or more doses, typically about 1-2, 1-3, 1-4, 1-5, 1-6, 1-10, or about 1-20 doses. The pharmaceutical dosage form can comprise a vial or syringe or other suitable pharmaceutically acceptable container. The pharmaceutical dosage form suitable for injection or infusion use can include sterile aqueous solutions or dispersions or sterile powders comprising an active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol such as glycerol, propylene glycol, or liquid polyethylene glycols and the like, vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The prevention of the growth of microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

As used herein, the term "excipient" means the substances used to formulate active pharmaceutical ingredients (API) into pharmaceutical formulations; in a preferred embodiment, an excipient does not lower or interfere with the primary therapeutic effect of the API. Preferably, an excipient is therapeutically inert. The term "excipient" encompasses carriers, diluents, vehicles, solubilizers, stabilizers, bulking agents, and binders. Excipients can also be those substances present in a pharmaceutical formulation as an indirect or unintended result of the manufacturing process. Preferably, excipients are approved for or considered to be safe for human and animal administration, i.e., GRAS substances (generally regarded as safe). GRAS substances are listed by the Food and Drug administration in the Code of Federal Regulations (CFR) at 21 CFR §182 and 21 CFR §184, incorporated herein by reference. Preferred excipients include, but are not limited to, hexitols, including mannitol and the like.

As used herein "a stabilizing concentration of an organic solvent" or "a stabilizing concentration of an alcohol" means that amount of an organic solvent or alcohol that reduces the level of degradation of bendamustine to achieve a specified level of degradants in the final drug product. For example, with respect to the degradant HP1, a stabilizing concentration of an organic solvent is that amount which results in an HP1 concentration (area percent of bendamustine) of less than about 0.5%, preferably less than 0.45%, preferably less than 0.40%, more preferably less than 0.35%, more preferably less than 0.30%, and even more preferably less than 0.25%. With respect to the overall or total degradant concentration of the final drug product, a stabilizing concentration of an organic solvent is that amount that results in a total degradant concentration (at the time of drug product release) of less than about 7% (area percent bendamustine), preferably less than about 6%, more preferably less than about 5%, and even more preferably less than about 4.0%. By "area percent of bendamustine" is meant the amount of a specified degradant, e.g., HP1, relative to the amount of bendamustine as determined, e.g., by HPLC.

The term "organic solvent" means an organic material, usually a liquid, capable of dissolving other substances.

As used herein, "trace amount of an organic solvent" means an amount of solvent that is equal to or below recommended levels for pharmaceutical products, for example, as recommended by ICH guidelines (International Conferences on Harmonization, Impurities—Guidelines for Residual Solvents. Q3C. Federal Register. 1997; 62(247):67377). The lower limit is the lowest amount that can be detected.

The term "release" or "at release" means the drug product has met the release specifications and can be used for its intended pharmaceutical purpose.

A. General

The invention provides stable, pharmaceutically acceptable compositions prepared from bendamustine. In particular, the invention provides formulations for the lyophilization of bendamustine HCl. The lyophilized powder obtained from such formulations is more easily reconstituted than the presently available lyophilized powder of bendamustine. Further, the lyophilized products of the present invention have a better impurity profile than Ribomustin® with respect to certain impurities, in particular HP1, bendamustine dimer, and bendamustine ethylester, prior to reconstitution, upon storage of the lyophilate, or following reconstitution and admixture.

The present invention further provides formulations of bendamustine useful for treating neoplastic diseases. The formulations described herein can be administered alone or in combination with at least one additional anti-neoplastic agent and/or radioactive therapy.

An aspect of the invention is conditions and means for enhancing the stability of bendamustine prior to and during the lyophilization process, upon shelf storage or upon reconstitution.

Anti-neoplastic agents which may be utilized in combination with the formulations of the invention include those provided in the Merck Index 11, pp 16-17, Merck & Co., Inc. (1989) and The Chemotherapy Source Book (1997). Both books are widely recognized and readily available to the skilled artisan.

There are large numbers of antineoplastic agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for treatment of neoplasia by combination drug chemotherapy. Such antineoplastic agents fall into several major categories, namely, antibiotic-type agents, covalent DNA-binding drugs, antimetabolite agents, hormonal agents, including glucocorticoids such as prednisone and dexamethasone, immunological agents, interferon-type agents, differentiating agents such as the retinoids, pro-apoptotic agents, and a category of miscellaneous agents, including compounds such as antisense, small interfering RNA, and the like. Alternatively, other antineoplastic agents, such as metallomatrix proteases (MMP) inhibitors, SOD mimics or $alpha_v$ $beta_3$ inhibitors may be used.

One family of antineoplastic agents which may be used in combination with the compounds of the inventions consists of antimetabolite-type antineoplastic agents. Suitable antimetabolite antineoplastic agents may be selected from the group consisting of alanosine, AG2037 (Pfizer), 5-FU-fibrinogen, acanthifolic acid, aminothiadiazole, brequinar sodium, carmofur, Ciba-Geigy CGP-30694, cyclopentyl cytosine, cytarabine phosphate stearate, cytarabine conjugates, Lilly DATHF, Merrel Dow DDFC, dezaguanine, dideoxycytidine, dideoxyguanosine, didox, Yoshitomi DMDC, doxifluridine, Wellcome EHNA, Merck & Co. EX-015, fazarabine, floxuridine, fludarabine phosphate, 5-fluorouracil, N-(2'-furanidyl)-5-fluorouracil, Daiichi Seiyaku FO-152, isopropyl pyrrolizine, Lilly LY-188011, Lilly LY-264618, methobenzaprim, methotrexate, Wellcome MZPES, norspermidine, NCI NSC-127716, NCI NSC-264880, NCI NSC-39661, NCI NSC-612567, Warner-Lambert PALA, pentostatin, piritrexim, plicamycin, Asahi Chemical PL-AC, Takeda TAC-788, thioguanine, tiazofurin, Erbamont TIF, trimetrexate, tyrosine kinase inhibitors, tyrosine protein kinase inhibitors, Taiho UFT and uricytin.

A second family of antineoplastic agents which may be used in combination with the compounds of the invention consists of covalent DNA-binding agents. Suitable alkylating-type antineoplastic agents may be selected from the group consisting of Shionogi 254-S, aldo-phosphamide analogues, altretamine, anaxirone, Boehringer Mannheim BBR-2207, bestrabucil, budotitane, Wakunaga CA-102, carboplatin, carmustine, Chinoin-139, Chinoin-153, chlorambucil, cisplatin, cyclophosphamide, American Cyanamid CL-286558, Sanofi CY-233, cyplatate, Degussa D-19-384, Sumimoto DACHP(Myr)2, diphenylspiromustine, diplatinum cytostatic, Erba distamycin derivatives, Chugai DWA-2114R, ITI E09, elmustine, Erbamont FCE-24517, estramustine phosphate sodium, fotemustine, Unimed G-6-M, Chinoin GYKI-17230, hepsul-fam, ifosfamide, iproplatin, lomustine, mafosfamide, melphalan, mitolactol, Nippon Kayaku NK-121, NCI NSC-264395, NCI NSC-342215, oxaliplatin, Upjohn PCNU, prednimustine, Proter PTT-119, ranimustine, semustine, SmithKline SK&F-101772, Yakult Honsha SN-22, spiromustine, Tanabe Seiyaku TA-077, tauromustine, temozolomide, teroxirone, tetraplatin and trimelamol.

Another family of antineoplastic agents which may be used in combination with the compounds disclosed herein consists of antibiotic-type antineoplastic agents. Suitable antibiotic-type antineoplastic agents may be selected from the group consisting of Taiho 4181-A, aclarubicin, actinomycin D, actinoplanone, alanosine, Erbamont ADR-456, aeroplysinin derivative, Ajinomoto AN-201-II, Ajinomoto AN-3, Nippon Soda anisomycins, anthracycline, azino-mycin-A, bisucaberin, Bristol-Myers BL-6859, Bristol-Myers BMY-25067, Bristol-Myers BMY-25551, Bristol-Myers BMY-26605, Bristol-Myers BMY-27557, Bristol-Myers BMY-28438, bleomycin sulfate, bryostatin-1, Taiho C-1027, calichemycin, chromoximycin, dactinomycin, daunorubicin, Kyowa Hakko DC-102, Kyowa Hakko DC-79, Kyowa Hakko DC-88A, Kyowa Hakko DC89-A1, Kyowa Hakko DC92-B, ditrisarubicin B, Shionogi DOB-41, doxorubicin, doxorubicin-fibrinogen, elsamicin-A, epirubicin, erbstatin, esorubicin, esperamicin-A1, esperamicin-A1b, Erbamont FCE-21954, Fujisawa FK-973, fostriecin, Fujisawa FR-900482, glidobactin, gregatin-A, grincamycin, herbimycin, idarubicin, illudins, kazusamycin, kesarirhodins, Kyowa Hakko KM-5539, Kirin Brewery KRN-8602, Kyowa Hakko KT-5432, Kyowa Hakko KT-5594, Kyowa Hakko KT-6149, American Cyanamid LL-D49194, Meiji Seika ME 2303, menogaril, mitomycin, mitoxantrone, SmithKline M-TAG, neoenactin, Nippon Kayaku NK-313, Nippon Kayaku NKT-01, SRI International NSC-357704, oxalysine, oxaunomycin, peplomycin, pilatin, pirarubicin, porothramycin, pyrindamycin A, Tobishi RA-I, rapamycin, rhizoxin, rodorubicin, sibanomicin, siwenmycin, Sumitomo SM-5887, Snow Brand SN-706, Snow Brand SN-07, sorangicin-A, sparsomycin, SS Pharmaceutical SS-21020, SS Pharmaceutical SS-7313B, SS Pharmaceutical SS-9816B, steffimycin B, Taiho 4181-2, talisomycin, Takeda TAN-868A, terpentecin, thrazine, tricrozarin A, Upjohn U-73975, Kyowa Hakko UCN-10028A, Fujisawa WF-3405, Yoshitomi Y-25024 and zorubicin.

A fourth family of antineoplastic agents which may be used in combination with the compounds of the invention include a miscellaneous family of antineoplastic agents selected from the group consisting of alpha-carotene, alpha-difluoromethyl-arginine, acitretin, arsenic trioxide, Avastin® (bevacizumab), Biotec AD-5, Kyorin AHC-52, alstonine, amonafide, amphethinile, amsacrine, Angiostat, ankinomycin, anti-neoplaston A10, antineoplaston A2, antineoplaston A3, antineoplaston A5, antineoplaston AS2-1, Henkel APD, aphidicolin glycinate, asparaginase, Avarol, baccharin, batracylin, benfluoron, benzotript, Ipsen-Beaufour BIM-23015, bisantrene, Bristo-Myers BMY-40481, Vestar boron-10, bromofosfamide, Wellcome BW-502, Wellcome BW-773, caracemide, carmethizole hydrochloride, Ajinomoto CDAF, chlorsulfaquinoxalone, Chemes CHX-2053, Chemex CHX-100, Warner-Lambert CI-921, Warner-Lambert CI-937, Warner-Lambert CI-941, Warner-Lambert CI-958, clanfenur, claviridenone, ICN compound 1259, ICN compound 4711, Contracan, Yakult Honsha CPT-11, crisnatol, curaderm, cytochalasin B, cytarabine, cytocytin, Merz D-609, DABIS maleate, dacarbazine, datelliptinium, didemnin-B, dihaematoporphyrin ether, dihydrolenperone, dinaline, distamycin, Toyo Pharmar DM-341, Toyo Pharmar DM-75, Daiichi Seiyaku DN-9693, elliprabin, elliptinium acetate, epothionesTsumura EPMTC, erbitux, ergotamine, erlotnib, etoposide, etretinate, fenretinide, Fujisawa FR-57704, gallium nitrate, genkwadaphnin, Gleevec® (imatnib), Chugai GLA-43, Glaxo GR-63178, gefitinib, grifolan NMF-5N, hexadecylphosphocholine, Green Cross HO-221, homoharringtonine, hydroxyurea, BTG ICRF-187, indanocine, ilmofosine, isoglutamine, isotretinoin, Otsuka JI-36, Ramot K-477, Otsuak K-76COONa, Kureha Chemical K-AM, MECT Corp KI-8110, American Cyanamid L-623, leukoregulin, lonidamine, Lundbeck LU-23-112, Lilly LY-186641, NCI (US) MAP, marycin, mefloquine, Merrel Dow MDL-27048, Medco MEDR-340, merbarone, merocyanine derivatives, methylanilinoacridine, Molecular Genetics MGI-136, minactivin, mitonafide, mitoquidone, mopidamol, motretinide, Zenyaku Kogyo MST-16, N-(retinoyl)amino acids, Nisshin Flour Milling N-021, N-acylated-dehydroalanines, nafazatrom, Taisho NCU-190, nocodazole derivative, Normosang, NCI NSC-145813, NCI NSC-361456, NCI NSC-604782, NCI NSC-95580, octreotide, Ono ONO-112, oquizanocine, Akzo Org-10172, paclitaxel, pancratistatin, pazelliptine, Warner-Lambert PD-111707, Warner-Lambert PD-115934, Warner-Lambert PD-131141, Pierre Fabre PE-1001, ICRT peptide D, piroxantrone, polyhaematoporphyrin, polypreic acid, Efamol porphyrin, probimane, procarbazine, proglumide, Invitron protease nexin I, Tobishi RA-700, razoxane, Sapporo Breweries RBS, restrictin-P, retelliptine, retinoic acid, Rhone-Poulenc RP-49532, Rhone-Poulenc RP-56976, Rituxan® (and other anti CD20 antibodies, e.g. Bexxar®, Zevalin®), SmithKline SK&F-104864, statins (Lipitor® etc.), Sumitomo SM-108, Kuraray SMANCS, SeaPharm SP-10094, spatol, spirocyclopropane

derivatives, spirogermanium, Unimed, SS Pharmaceutical SS-554, strypoldinone, Stypoldione, Suntory SUN 0237, Suntory SUN 2071, superoxide dismutase, Thalidomide, Thalidomide analogs, Toyama T-506, Toyama T-680, taxol, Teijin TEI-0303, teniposide, thaliblastine, Eastman Kodak TJB-29, tocotrienol, Topostin, Teijin TT-82, Kyowa Hakko UCN-01, Kyowa Hakko UCN-1028, ukrain, Eastman Kodak USB-006, vinblastine sulfate, vincristine, vindesine, vinestramide, vinorelbine, vintriptol, vinzolidine, withanolides and Yamanouchi YM-534, Zometa®.

Examples of radioprotective agents which may be used in the combination chemotherapy of this invention are AD-5, adchnon, amifostine analogues, detox, dimesna, 1-102, MM-159, N-acylated-dehydroalanines, TGF-Genentech, tiprotimod, amifostine, WR-151327, FUT-187, ketoprofen transdermal, nabumetone, superoxide dismutase (Chiron and Enzon).

Methods for preparation of the antineoplastic agents described above may be found in the literature. Methods for preparation of doxorubicin, for example, are described in U.S. Pat. Nos. 3,590,028 and 4,012,448. Methods for preparing metallomatrix protease inhibitors are described in EP 780386. Methods for preparing .alpha$_v$ .beta$_3$ inhibitors are described in WO 97/08174.

Preferred anti-neoplastic agents include, without limitation, one or more of daunorubicin, bleomycin, vincristine, doxorubicin, dacarbazine, prednisolone, mitoxantrone, prednisone, methotrexate, 5-fluorouracil, dexamethasone, thalidomide, thalidomide derivatives, 2ME2, Neovastat, R 11 5777, arsenic trioxide, bortezomib, tamoxifen, G3139 (antisense), and SU5416, mitomycin, anti-CD20 antibodies, such as Rituxan® and R-etodolac.

Preferred drug regimens for which the present formulation may be used in conjunction with or as a replacement for one or more of the components includes, without limitation, ABVD (doxorubicin, bleomycin, vincristine, dacarbazine), DBV (daunorubicin, belomycin, vincristine), CVPP (cyclophosphamide, vinblastine, procarbazine, prednisolone), COP (cyclophosphamide, vincristine, prednisolone), CHOP (cyclophosphamide, doxorubicin,

vincristine and prednisone) and CMF (cyclophosphamide, methotrexate, 5-fluorouracil). Additional regimens are given in Table A below.

TABLE A

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
AC	Doxorubicin & Cyclophosphamide	Breast cancer
CFM (CF, FNC)	Cyclophosphamide, Fluorouracil, Mitaxantrone	Breast cancer
CMF	Cyclophosphamide, Methotrexate, Fluorouracil	Breast cancer
NFL	Mitoxantrone, Fluorouracil, Leucovorin	Breast cancer
Sequential Dox-CMF	Doxorubicin	Breast cancer
VATH	Vinblastine, Doxorubicin, Thiotepa, Fluoxymesterone	Breast cancer
EMA-86	Etoposide, Mitoxantrone, Ctyarabine	AML (induction)
7 + 3	Cytarabine WITH Daunorubicin OR Idarobicin OR Mitoxantrone	AML (induction)
5 + 2	Cytarabine WITH Daunorubicin OR Mitoxantrone	AML (induction)
HiDAC	Cytarabine	AML (post-remission)

TABLE A-continued

Cancer Therapeutic Regimens

Abbreviation	Drugs Used	Disease
ABVD	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine	Hodgkin's
ChlVPP	Chlorambucil, Vinblastine, Procarbazine, Prednisone	Hodgkin's
EVA	Etoposide, Vinblastine, Doxorubicin	Hodgkin's
MOPP	Mechlorethamine, Vincristine, Procarbazine, Prednisone	Hodgkin's
MOPP/ABV Hybrid	Mechlorethamine, Vincristine, Procarbazine, Prednisone, Doxorubicin, Bleomycin, Vinblastine	Hodgkin's
MOPP/ABVD	Mechlorethamine, Doxorubicin, Vinblastine, Bleomycin, Etoposide, Prednisone	Hodgkin's
CNOP	Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone	Non-Hodgkin's
COMLA	Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, Cytarabine	Non-Hodgkin's
DHAP	Dexamethasone, Cisplatin, Cytarabine	Non-Hodgkin's
ESHAP	Etoposide, Methylprednisilone, Cisplatin, Cytarabine	Non-Hodgkin's
MACOP-B	Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Vincristine, Prednisone, Bleomycin, Septra, Ketoconazole	Non-Hodgkin's
m-BACOD	Methotrexate, Leucovorin, Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, Dexamethasone	Non-Hodgkin's
MINE-ESHAP	Mesna, Ifosfamide, Mitoxantrone, Etoposide	Non-Hodgkin's
NOVP	Mitoxantrone, Vinblastine, Prednisone, Vincristine	Non-Hodgkin's
ProMACE/ cytaBOM	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Cytarabine, Bleomycin, Vincristine, Methotrexate, Leucovorin, Septra	Non-Hodgkin's
M2	Vincristine, Carmustine, Cyclophosphamide, Melphalan, Prednisone	Multiple Myeloma
MP	Melphalan, Prednisone	Multiple Myeloma
VAD	Vincristine, Doxorubicin, Dexamethasone	Multiple Myeloma
VBMCP	Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone	Multiple Myeloma

As described herein, a lyophilized formulation of bendamustine is achieved following removal of an organic solvent in water. The most typical example of the solvent used to prepare this formulation is tertiary butanol (TBA). Other organic solvents can be used including ethanol, n-propanol, n-butanol, isoproponal, ethyl acetate, dimethyl carbonate, acetonitrile, dichloromethane, methyl ethyl ketone, methyl isobutyl ketone, acetone, 1-pentanol, methyl acetate, methanol, carbon tetrachloride, dimethyl sulfoxide, hexafluoroacetone, chlorobutanol, dimethyl sulfone, acetic acid, cyclohexane. These preceding solvents may be used individually or in combination. Useful solvents must form stable solutions with bendamustine and must not appreciably degrade or deactivate the API. The solubility of bendamustine in the

selected solvent must be high enough to form commercially useful concentrations of the drug in solvent. Additionally, the solvent should be capable of being removed easily from an aqueous dispersion or solution of the drug product, e.g., through lyophilization or vacuum drying. Preferably, a solution having a concentration of about 2-80 mg/mL, preferably about 5 to 40 mg/mL, more preferably 5-20 mg/mL and even more preferably 12 to 17 mg/mL bendamustine is used.

A pharmaceutically acceptable lyophilization excipient can be dissolved in the aqueous phase. Examples of excipients useful for the present invention include, without limitation, sodium or potassium phosphate, citric acid, tartaric acid, gelatin, glycine, and carbohydrates such as lactose, sucrose, maltose, glycerin, dextrose, dextran, trehalose and hetastarch. Mannitol is a preferred excipient. Other excipients that may be used if desired include antioxidants, such as, without limitation, ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxyanisole, butyl-hydroxytoluene or alpha-tocopherol acetate, or chelators.

A typical formulation and lyophilization cycle useful in accordance with the present invention is provided below. Lyophilization can be carried out using standard equipment as used for lyophilization or vacuum drying. The cycle may be varied depending upon the equipment and facilities used for the fill/finish.

In accordance with a typical embodiment of the present invention, an aqueous pre-lyophilization solution or dispersion is first formulated in a pharmaceutically acceptable compounding vessel. The solution is aseptically filtered into a sterile container, filled into an appropriate sized vial, partially stoppered and loaded into the lyophilizer. Using lyophilization techniques described herein the solution is lyophilized until a moisture content in the range of about 0.1 to about 8.0 percent is achieved. The resulting lyophilization powder is stable as a lyophilized powder for about six months to greater than about 2 years, preferably greater than about 3 years at about 5° C. to about 25° C. and can be readily reconstituted with Sterile Water for Injection, or other suitable carrier, to provide liquid formulations of bendamustine, suitable for internal administration e.g., by parenteral injection. For intravenous administration, the reconstituted liquid formulation, i.e., the pharmaceutical composition, is preferably a solution.

The pre-lyophilization solution or dispersion normally is first formulated in a pharmaceutically acceptable container by: 1) adding an excipient, such as mannitol (about 0 to about 50 mg/mL) with mixing to water (about 65% of the total volume) at ambient temperature, 2) adding an organic solvent (0.5-99.9% v/v), such as TBA to the aqueous solution with mixing at about 20°-35° C., 4) adding bendamustine HCl to the desired concentration with mixing, 5) adding water to achieve the final volume, and 6) cooling the solution to about 1° C. to about 30° C., preferably about 5° C. Although the preceding steps are shown in a certain order, it is understood that one skilled in the art can change the order of the steps and quantities as needed. Quantities can be prepared on a weight basis also.

The pre-lyophilization solution or dispersion can be sterilized prior to lyophilization, sterilization is generally performed by aseptic filtration, e.g., through a 0.22 micron or less filter. Multiple sterilization filters can be used. Sterilization of the solution or dispersion can be achieved by other methods known in the art, e.g., radiation.

In this case, after sterilization, the solution or dispersion is ready for lyophilization. Generally, the filtered solution will be introduced into a sterile receiving vessel, and then transferred to any suitable container or containers in which the formulation may be effectively lyophilized. Usually the formulation is effectively and efficiently lyophilized in the containers in which the product is to be marketed, such as, without limitation, a vial, as described herein and as known in the art.

A typical procedure for use in lyophilizing the pre-lyophilization solutions or dispersions is set forth below. However, a person skilled in the art would understand that modifications to the procedure or process may be made depending on such things as, but not limited to, the pre-lyophilization solution or dispersion and lyophilization equipment.

Initially, the product is placed in a lyophilization chamber under a range of temperatures and then subjected to temperatures well below the product's freezing point, generally for several hours. Preferably, the temperature will be at or below about −40° C. for at least 2 hours. After freezing is complete, the chamber and the condenser are evacuated through vacuum pumps, the condenser surface having been previously chilled by circulating refrigerant. Preferably, the condenser will have been chilled below the freezing point of the solution preferably to about −40°, more preferably to about −50° C. or lower, even more preferably to about −60° C. or lower. Additionally, evacuation of the chamber should continue until a pressure of about 10 to about 600 microns, preferably about 50 to about 150 microns is obtained.

The product composition is then warmed under vacuum in the chamber and condenser. This usually will be carried out by warming the shelves within the lyophilizer on which the product rests during the lyophilization process at a pressure ranging from about 10 to about 600 microns. The warming process will optimally take place very gradually, over the course of several hours. For example, the product temperature should initially be increased from about −30° C. to about −10° C. and maintained for about 10-70 hours. Additionally, the product temperature can be increased from the freezing temperature to about 25° C.-40° C. over a period of 30-192 hours. To prevent powder ejection of the lyophilate from vials, complete removal of the organic solvent and water should be done during the initial drying phase. Complete drying can be confirmed by stabilization of vacuum, condenser temperature and product shelf temperature. After the initial drying, the product temperature should be increased to about 25° C.-40° C. and maintained for about 5-40 hours.

Once the drying cycle is completed, the pressure in the chamber can be slowly released to atmospheric pressure (or slightly below) with sterile, dry-nitrogen gas (or equivalent gas). If the product composition has been lyophilized in containers such as vials, the vials can be stoppered, removed and sealed. Several representative samples can be removed for purposes of performing various physical, chemical, and microbiological tests to analyze the quality of the product.

The lyophilized bendamustine formulation is typically marketed in pharmaceutical dosage form. The pharmaceutical dosage form of the present invention, although typically in the form of a vial, may be any suitable container, such as ampoules, syringes, co-vials, which are capable of maintaining a sterile environment. Such containers can be glass or plastic, provided that the material does not interact with the bendamustine formulation. The closure is typically a stopper, most typically a sterile rubber stopper, preferably a bromobutyl rubber stopper, which affords a hermetic seal.

After lyophilization, the bendamustine lyophilization powder may be filled into containers, such as vials, or alternatively the pre-lyophilization solution can be filled into such vials and lyophilized therein, resulting in vials which directly contain the lyophilized bendamustine formulation. Such vials are, after filling or lyophilization of the solution therein, sealed, as with a stopper, to provide a sealed, sterile, pharma-

ceutical dosage form. Typically, a vial will contain a lyophilized powder including about 10-500 mg/vial, preferably about 100 mg/vial, bendamustine and about 5 mg-2 g/vial, preferably about 170 mg/vial, mannitol.

The lyophilized formulations of the present invention may be reconstituted with water, preferably Sterile Water for Injection, or other sterile fluid such as co-solvents, to provide an appropriate solution of bendamustine for administration, as through parenteral injection following further dilution into an appropriate intravenous admixture container, for example, normal saline.

B. Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of alcohols commonly used in lyophilization, e.g., methanol, ethanol, propanol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, combined with mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions at room temperature (see Table 1). Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

The results shown in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For the alcohols tested, the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time. Bendamustine did not precipitate immediately with any alcohol, but crystallized after storage at 5° C. Alcohols varied in their effect on solubility. Without wishing to be bound to any particular theory, smaller alcohols such as methanol and ethanol have less of an effect on solubility as compared with larger alcohols (tertiary-butanol and n-butanol). However, the shape of the alcohol is also important. For example n-propanol was found to be better than iso-propanol in preventing precipitation in this system. The two alcohols with the greatest effect on solubility were n-propanol and tertiary-butanol.

TABLE 1

Bendamustine solubility over a 24 hour period in various alcohols when stored at 5° C.

	Zero Time	3 Hours	6 Hours	24 Hours
NMethanol (v/v)				
0% (Water Only)	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	Precipitate
30%	CCS	CCS	CCS	CCS
Ethanol (v/v)				
1.9%	CCS	CCS	Precipitate	Precipitate
5%	CCS	CCS	Precipitate	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
n-Propanol (v/v/)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS
Iso-propanol (v/v)				
5%	CCS	Precipitate	Precipitate	Precipitate
10%	CCS	CCS	CCS	CCS
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS

TABLE 1-continued

Bendamustine solubility over a 24 hour period in various alcohols when stored at 5° C.

	Zero Time	3 Hours	6 Hours	24 Hours
n-Butanol (v/v)				
5%	CCS	CCS	CCS	CCS
10%	CCS	CCS	CCS	CCS
20%	2 layers	2 layers	2 layers	2 layers
30%	2 layers	2 layers	2 layers	2 layers
Tert-Butanol (v/v)				
5%	CCS	CCS	CCS	Precipitate
10%	CCS	CCS	CCS	Precipitate
20%	CCS	CCS	CCS	CCS
30%	CCS	CCS	CCS	CCS

CCS stands for clear colorless solution

Experiments to quantitatively determine the solubility of bendamustine at various temperatures for three different solutions are summarized in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment was based on stability studies (results described below). For both solutions tested, the solubility of bendamustine decreased linearly with temperatures from 25° C. to 0° C. This experiment confirmed the data shown in Table 1 and highlights the difference in bendamustine solubility for 20% and 30% TBA solutions.

TABLE 2

Solubility of bendamustine in TBA

	−8° C.	0° C.	5° C.	25° C.
20% (v/v) TBA 25.5 mg/mL mannitol Water, q.s. to desired volume	14 mg/mL	11 mg/mL	17 mg/mL	47 mg/mL
30% (v/v) TBA 25.5 mg/mL mannitol Water, q.s. to desired volume	20 mg/mL	18 mg/mL	27 mg/mL	65 mg/mL

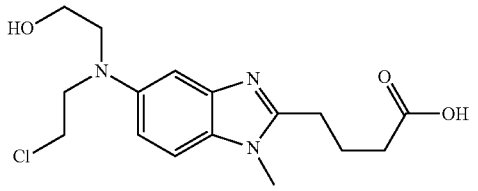
C. Stability

Because of its instability in aqueous solutions due to hydrolysis with water, bendamustine requires lyophilization in order to make a product suitable for pharmaceutical use. However, during the manufacturing of lyophilized drug products, aqueous solutions are commonly needed for filling, prior to lyophilization. Thus, the use of aqueous solutions during the compounding and fill processes for bendamustine and other nitrogen mustards can result in degradation of the drug product. Consequently, the effect of various alcohols on the degradation of bendamustine was evaluated to determine if formulations could be found that would allow longer fill-finish times, provide lyophilate powders that could be reconstituted more quickly than the current Ribomustin® formulation, and/or provide lyophilized preparations of bendamustine with a better impurity profile with respect to certain impurities, e.g., HP1, and BM1 dimer than Ribomustin®.

Preferably, a lyophilized preparation of the invention is stable with respect to HP1, i.e., the amount of HP1 does not increase appreciably (does not exceed the shelf-life specifications), for 6 months, more preferably 12 months, and even more preferably greater than 24 months, e.g., 36 months, when stored at about 2° C. to about 30° C., preferably 5° C.

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades rapidly in water alone and forms predominantly the hydrolysis product, HP1 (monohydroxy bendamustine).

Formula II



Monohydroxy bendamustine (HP1)

TABLE 3

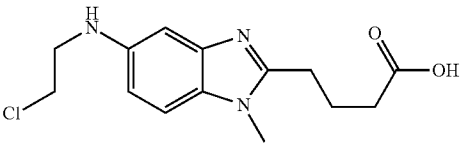
Stability of bendamustine in water

	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
0% Alcohol, i.e., Water Alone	0 hours	99.11	0.60	0.11
	3 hours	98.83	0.86	0.13
	6 hours	98.44	1.22	0.17
	24 hours	95.67	3.81	0.29

The other major degradant observed during this study and other long term stability studies was the dimer of bendamustine.

-continued

Formula V

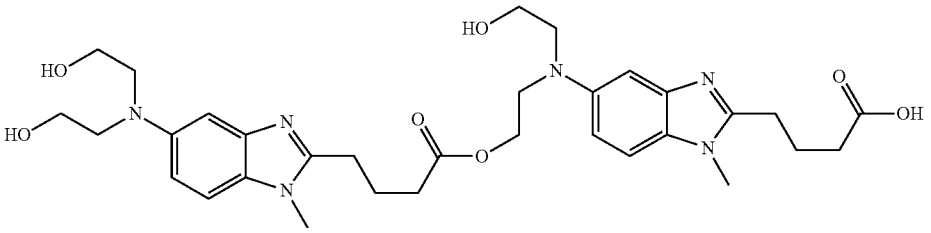


BM1DCE

FIG. **2** summarizes the purity results of an HPLC analysis after incubating bendamustine in various alcohols for 24 hours at 5° C. Results are presented as the area percent of the total peak area. The numerical values for FIG. **2** are provided in Tables 3-9. The purity was highest in solutions containing higher concentration of alcohols, regardless of the alcohol. Of the alcohols evaluated, bendamustine degraded the least in a solution containing about 30% (v/v) TBA. In about 10% and about 20% alcohol solutions, n-butanol was superior in preventing degradation of bendamustine. At 20% and 30% (v/v), n-butanol in water resulted in a biphasic system due to the insolubility of n-butanol in water at these concentrations.

FIGS. **3** and **4** show the amount of degradation of bendamustine as measured by HP1 and dimer formation quantified by HPLC (as described herein). HP1 and dimer formation increased as the amount of alcohol concentration decreased regardless of the alcohol. This increase in impurities occurred with an anticipated time dependence (see Tables 3-9). Tert-butanol and n-butanol appeared superior to other alcohols in preventing degradation of the product. As seen in Table 10, mannitol had no effect on the stabilization of bendamustine with TBA.

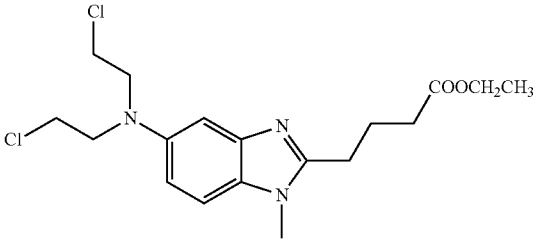
Formula III



Bendamustine Dimer (BM1 Dimer)

Other degradants contained in the Ribomustin lyophilized product are bendamustine ethylester (BM1EE) (Formula IV) and BM1DCE (Formula V). BM is formed when bendamustine reacts with ethyl alcohol.

Formula IV



Bendamustine ethylester (BM1EE)

TABLE 4

HPLC stability results for the stability of bendamustine in various ethyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

V/V alcohol	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
1.9% Ethanol	0 hours	99.11	0.64	0.12
	3 hours	98.83	0.90	0.14
	6 hours	98.60	1.12	0.15
	24 hours	96.16	3.41	0.27
5% Ethanol	0 hours	99.31	0.44	0.12
	3 hours	99.10	0.64	0.13
	6 hours	98.87	0.86	0.14
	24 hours	96.89	2.68	0.25
10% Ethanol	0 hours	99.44	0.33	0.11
	3 hours	99.28	0.48	0.12
	6 hours	99.10	0.65	0.12
	24 hours	98.03	1.57	0.18

TABLE 4-continued

HPLC stability results for the stability of bendamustine in various ethyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

V/V alcohol	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
20% Ethanol	0 hours	99.54	0.22	0.10
	3 hours	99.45	0.30	0.11
	6 hours	99.36	0.39	0.11
	24 hours	98.61	0.96	0.15
30% Ethanol	0 hours	99.62	0.15	0.10
	3 hours	99.56	0.21	0.11
	6 hours	99.52	0.24	0.12
	24 hours	99.21	0.45	0.12

TABLE 5

HPLC stability results for bendamustine in various Tert-butanol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Tert-butanol	0 hours	99.34	0.41	0.12
	3 hours	99.10	0.64	0.14
	6 hours	98.85	0.88	0.13
	24 hours	97.58	2.09	0.20
10% Tert-butanol	0 hours	99.46	0.30	0.11
	3 hours	99.26	0.48	0.12
	6 hours	99.05	0.69	0.13
	24 hours	98.04	1.64	0.19
20% Tert-butanol	0 hours	99.59	0.17	0.11
	3 hours	99.48	0.29	0.11
	6 hours	99.35	0.40	0.12
	24 hours	98.35	1.27	0.20
30% Tert-butanol	0 hours	99.63	0.13	0.10
	3 hours	99.60	0.16	0.10
	6 hours	99.58	0.18	0.11
	24 hours	99.42	0.34	0.12

TABLE 6

HPLC stability results for various n-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% n-Propanol	0 hours	99.25	0.43	0.13
	3 hours	99.00	0.66	0.15
	6 hours	98.72	0.94	0.16
	24 hours	97.24	2.33	0.26
10% n-Propanol	0 hours	99.34	0.33	0.15
	3 hours	99.17	0.48	0.14
	6 hours	98.92	0.70	0.16
	24 hours	97.67	1.83	0.28
20% n-Propanol	0 hours	99.45	0.33	0.13
	3 hours	99.42	0.26	0.13
	6 hours	99.29	0.39	0.14
	24 hours	98.60	0.97	0.24
30% n-Propanol	0 hours	99.53	0.15	0.13
	3 hours	99.51	0.15	0.15
	6 hours	99.44	0.20	0.11
	24 hours	99.27	0.36	0.17

TABLE 7

HPLC stability results for bendamustine in various iso-propyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Iso-propanol	0 hours	99.21	0.48	0.13
	3 hours	98.65	0.72	0.14
	6 hours	98.56	1.02	0.14
	24 hours	96.14	3.35	0.26
10% Iso-propanol	0 hours	99.32	0.37	0.12
	3 hours	99.11	0.55	0.14
	6 hours	98.85	0.75	0.16
	24 hours	97.68	1.92	0.21
20% Iso-propanol	0 hours	99.49	0.21	0.11
	3 hours	99.39	0.31	0.12
	6 hours	99.22	0.42	0.13
	24 hours	98.61	1.04	0.17
30% Iso-propanol	0 hours	99.56	0.15	0.10
	3 hours	99.47	0.20	0.12
	6 hours	99.40	0.24	0.11
	24 hours	99.15	0.52	0.14

TABLE 8

HPLC stability results for bendamustine in various methyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Methanol	0 hours	99.35	0.40	0.12
	3 hours	98.97	0.70	0.14
	6 hours	98.66	0.95	0.14
	24 hours	96.65	2.83	0.23
10% Methanol	0 hours	99.42	0.34	0.11
	3 hours	99.01	0.59	0.12
	6 hours	98.86	0.80	0.12
	24 hours	97.65	1.85	0.18
20% Methanol	0 hours	99.56	0.22	0.11
	3 hours	99.31	0.38	0.11
	6 hours	98.99	0.50	0.12
	24 hours	98.31	1.15	0.16
30% Methanol	0 hours	99.59	0.18	0.10
	3 hours	99.43	0.27	0.11
	6 hours	99.25	0.34	0.11
	24 hours	98.65	0.76	0.13

TABLE 9

HPLC stability results for bendamustine in various n-butyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
5% Butanol	0 hours	99.25	0.49	0.13
	3 hours	98.94	0.73	0.14
	6 hours	98.76	0.91	0.14
	24 hours	97.46	2.20	0.21
10% Butanol	0 hours	99.44	0.30	0.11
	3 hours	99.18	0.49	0.12
	6 hours	99.03	0.64	0.12
	24 hours	98.13	1.55	0.17
20% Butanol[a]	0 hours	99.54	0.23	0.10
	3 hours	99.45	0.31	0.11
	6 hours	99.30	0.40	0.11
	24 hours	98.81	0.91	0.14

TABLE 9-continued

HPLC stability results for bendamustine in various n-butyl alcohol concentrations over a 24 hour period. HP1 and Dimer were impurities that increased in this study.

Concentration alcohol (v/v)	Hold Time	Purity (% Area)	HP1 (%)	Dimer (%)
30% Butanol[a]	0 hours	99.55	0.24	0.10
	3 hours	99.40	0.29	0.10
	6 hours	99.40	0.37	0.11
	24 hours	99.00	0.74	0.12

[a]Both solutions had 2 layers/phases of liquids in the vial. Solutions were vortexed prior to sample preparation.

The results in Tables 1-9 indicate that the stability of bendamustine HCl with respect to HP1 and dimer improves with increasing alcohol concentration.

TABLE 10

HPLC stability results for bendamustine in TBA with and without mannitol over a 24 hour period.

Sample	Purity (% Area)	HP1 (%)
TBA 20% (v/v) with Mannitol		
0 hours	99.59	0.17
24 hours @ 5° C.	99.35	1.27
TBA 20% (v/v) without Mannitol		
0 hours	100.0	0.00
24 hours @ 5° C.	98.80	1.21

NOTE:
The samples analyzed without mannitol were analyzed by HPLC using a normal phase method while the samples analyzed with mannitol used a reverse phase HPLC method. Slight variability may be seen in other samples analyzed between the two methods.

D. Lyophilization Cycle Development

Different pre-lyophilization formulations were prepared at various concentrations of bendamustine, mannitol, and alcohols in water. The cycle development was changed and optimized at each step for freezing (fast vs. slow), primary drying (both temperature and pressure), and secondary drying as described herein.

Based upon all of the information detailed above on solubility, stability, and ease of lyophilization, preferred formulations include the following:

Ingredients	Concentration
Bendamustine	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Alcohol	about 0.5%-40% (v/v)
Water, q.s. to	desired volume

wherein the alcohol is selected from methanol, n-propanol, or isopropanol

Ingredients	Concentration
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	1-20% (v/v)
Water, q.s. to	desired volume

wherein the alcohol is selected from methanol, n-propanol, or isopropanol

Ingredients	Concentration
Bendamustine	about 5-20 mg/mL
Mannitol	10-30 mg/mL
Alcohol	5-40% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Alcohol	about 5-15% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Alcohol	about 10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-40 mg/mL
Mannitol	about 0-50 mg/mL
Butanol	about 0.5-20% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Butanol	about 1-10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Butanol	about 10% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-100% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99.9% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 0.5-99% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 2-50 mg/mL
Mannitol	about 0-50 mg/mL
Tertiary butanol	about 90-99% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 5-20 mg/mL
Mannitol	about 10-30 mg/mL
Tertiary butanol	about 5-80% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12-17 mg/mL
Mannitol	about 20-30 mg/mL
Tertiary butanol	about 10-50% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 12.5-15 mg/mL
Mannitol	about 0-30 mg/mL
Ethanol	about 20-30% (v/v)
Water, q.s. to	desired volume

Ingredients	Concentration
Bendamustine HCl	about 15 mg/mL
Mannitol	about 25.5 mg/mL
Tertiary butanol	about 30% (v/v)
Water, q.s. to	desired volume

EXAMPLES

The following Examples are provided to illustrate certain aspects of the present invention and to aid those of skill in the art in practicing the invention. These Examples are in no way to be considered to limit the scope of the invention in any manner.

Materials:
Bendamustine HCl, (Degussa, Lot #s 0206005 and 0206007)
Mannitol, NF or equivalent (Mallinckrodt)
Ethyl Alcohol Dehydrated (200 proof), USP or equivalent (Spectrum)
Tertiary-butyl alcohol, ACS (EM Science)
Methanol (Spectrum and EMD)
Propanol (Spectrum)
Iso-propanol (Spectrum)
Butanol (Spectrum)
Water, HPLC grade or equivalent (EMD)
Acetonitrile, HPLC grade or equivalent (EMD)
Trifluoroacetic Acid, J. T. Baker
Methanol, HPLC grade or equivalent (EM Science, Cat #MX0488P-1)
Trifluoroacetic Acid, HPLC grade or equivalent (JT Baker, Cat#JT9470-01)
Equipment:
Waters 2695 Alliance HPLC system with photodiode array detector
Waters 2795 Alliance HPLC system with dual wavelength detector
Analytical Balance (Mettler AG285, ID #1028) and (Mettler X5205)
VirTis Lyophilizer AdVantage
Agilent Zorbax SB-C18 5 μm 80 Å 4.6×250 mm column, Cat#880975-902

Example 1

HPLC Procedures

Method 1
Mobile Phase A: 0.1% TFA; H_2O
Mobile Phase B: 0.1% TFA; 50% ACN:50% H_2O
UV: 230 nm
Flow rate: 1.0 mL/min
Column temp.: 30° C.
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Sample temp.: 5° C.
Injection Volume: 10 μL
Sample Concentration:0.25 mg/mL in MeOH
Gradient: 20% B for 1 min
20-90% B in 23 min
90% B for 6 min
back to 20% B in 1 min
hold at 20% B for 4 min
Run time: 30 min
Post run time: 5 min
Method 2
Mobile Phase A: 0.1% TFA; H_2O:ACN (9:1)
Mobile Phase B: 0.1% TFA; H_2O:ACN (5:5)
UV: 230 nm
Flow rate: 1.0 mL/min
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 10 μL
Sample Concentration:0.25 mg/mL in MeOH
Gradient: 0% B for 3 min
0-50% B in 13 min
50-70% B in 17 min
70-90% B in 2 min
90% B for 5 min
back to 0% B in 1 min
hold at 0% B for 4 min

Run time: 40 min
Post run time: 5 min
Method 3
Phase A: HPLC grade water with 0.1% TFA(v/v)
Phase B: HPLC grade ACN/water(1:1v/v) with 0.1% TFA(v/v)
UV: 254 nm
Flow rate: 1.0 mL/min
Column: Zorbax SB-C18 5 μm 80 Å 4.6×250 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 5 μL
Acquisition time: 30 min
Post time: 9 min
Diluent: methanol
Gradient:

Time (min.)	% Phase A	% Phase B
0.0	82	18
7.0	60	40
11.0	60	40
15.0	20	80
30.0	20	80
31.0	82	18

Sample preparation—dissolve the drug product with 200 mL MeOH. Sonicate 6 minutes. The solution can be injected directly into the HPLC (ca. 0.5 mg/mL)

Method 4
Phase A: HPLC grade water with 0.1% TFA(v/v)
Phase B: HPLC grade ACN with 0.1% TFA(v/v)
UV: 254 nm
Flow rate: 1.0 mL/min
Column: Zorbax Bonus RP-C14 5 μm 4.6×150 mm
Column temp.: 30° C.
Sample temp.: 5° C.
Injection Volume: 2 μL
Acquisition time:31 min
Post time: 5 min
Diluent: NMP/0.1% TFA in water (50:50 v/v)
Gradient:

Time (min.)	% Phase A	% Phase B
0.0	93	7
5	93	7
13	73	27
16	73	27
25	10	90
31	10	90

Sample preparation for method 4—dissolve the drug product with a known amount of diluent to prepare a concentration of 4.2 mg/mL for injection directly into the HPLC. It may be necessary to perform a second dilution (the 100 mg/vial dosage form) to obtain a 4.2 mg/mL sample concentration.

Results

The retention times for some Bendamustine impurities using HPLC Method 1 described above are shown in Table 11. An HPLC chromatograph for Ribomustin® using the HPLC procedure described herein is shown in FIG. **6**.

TABLE 11

Retention Time for Bendamustine and some of its Impurities using HPLC Method 1

Sample Name	Retention Time (min)
HP1	14.110
Bendamustine	22.182
BM1 Dimer	24.824
BM1EE	26.968

Although HPLC Method 1 was capable of resolving impurities found in bendamustine it was not capable of separating a potential impurity formed during analysis, the methyl ester of bendamustine (BM1ME). The retention time difference between BM1ME and BM1 Dimer was only 0.3 minutes. In order to resolve BM1 Dimer, another HPLC method (#2) was developed. HPLC method #2 was capable of separating all the impurities but required a longer run time of 45 minutes (Table 12).

TABLE 12

Retention Time for bendamustine and impurities using HPLC Method 2.

Sample Name	Retention Time (min)
HP1	15.694
BM1	25.420
BM1ME	31.065
BM1 Dimer	32.467
BM1EE	36.038

The impurity profile of various lots of Ribomustin using HPLC Method 3 are shown in Table 13.

TABLE 13

Ribomustine Impuirty Profile using HPLC Method 3
% Area

Batch	Bendamustine (HCl)	HP1	BM1EE	BM1 Dimer	BM1DCE
03H08	98.14	1.07	0.21	0.34	0.03
03H07	97.67	1.5	0.2	0.33	0.04
02K27	96.93	0.93	0.29	1.18	0.08
03C08	97.61	1.24	0.19	0.46	0.02

Example 2

Solubility

The solubility of bendamustine HCl (bendamustine) in water (alone) and with varying amounts of methanol, ethanol, propanol, isopropanol, butanol and tertiary-butyl alcohol (TBA) was determined by visual inspection. Amounts of bendamustine at 15 mg/mL, mannitol at 25.5 mg/mL were prepared in 10 mL of the indicated alcohol solutions (Table 1) at room temperature. Samples were then refrigerated at 5° C. and inspected after 0, 3, 6 and 24 hours for particulates and/or precipitates.

Results summarized in Table 1 indicate that bendamustine solubility is dependant on temperature and the amount of alcohol in aqueous solutions. For all alcohols the solubility of bendamustine increased as the concentration of alcohol increased. The formation of a precipitant was also dependent on the temperature and time.

The solubility of bendamustine was also determined in 20% (v/v) TBA containing 25.5 mg/mL mannitol in water,

and 30% (v/v) TBA containing 25.5 mg/mL mannitol in water (FIG. **1**). Bendamustine was added to 4 mL of each solution while mixing until it would no longer dissolve. The saturated solutions were allowed to mix for 1 hour at −8° C., 0° C., 5° C., or 25° C. The samples were centrifuged and placed back at the original temperature for a minimum of 30 minutes. The −8° C. sample was placed into an ice bath containing sodium chloride, which lowers the temperature of the ice bath, and the temperature was measured when the sample was pulled for analysis. An aliquot of each sample was taken and prepared for HPLC analysis.

The results of these experiments are shown in FIG. **1** and Table 2. The amount of TBA, 20% (v/v) and 30% (v/v), used in the experiment (FIG. **1**) was based on stability studies described herein.

As indicated in FIG. **1**, the solubility of bendamustine decreased linearly with temperature (25° C. to 0° C.). The solubility of bendamustine was temperature dependant whether it was dissolved in water alone or with an alcohol. The 20% (v/v) TBA may likely be the lower limit required for efficient and robust pharmaceutical manufacturing due to the stability and solubility of bendamustine. A filling solution of 15 mg/mL bendamustine is close to the saturation limit of 17.2 mg/mL bendamustine at 5° C. but higher than the limit at 0° C. The 30% (v/v) TBA is the recommended concentration of TBA for the final formulation and is well within the solubility limit regardless of temperature.

Example 3

Stability

A. Stability in Water

Solutions of bendamustine (15 mg/mL), and mannitol (25.5 mg/mL) were prepared in water at room temperature and immediately placed in an ice bath (to lower the temperature quickly to about 5° C.) for 10 minutes and then refrigerated at 5° C. A sample of each formulation was analyzed by HPLC using the methods described herein after 0, 3, 6 and 24 hours when stored at 5° C.

B. Stability in Alcohols

Solutions containing 15 mg/mL bendamustine, 25.5 mg/mL mannitol, and 1.9%, 5%, 10%, 20% or 30% (v/v) ethyl alcohol in water or 5%, 10%, 20% or 30% (v/v) TBA, methanol, propanol, iso-propanol, or butanol in water were prepared at room temperature, placed into an ice bath for 10 minutes and then refrigerated at 5° C. A sample of each formulation was analyzed by HPLC after 0, 3, 6 and 24 hours when stored at 5° C.

C. Stability Results

Table 3 shows the stability results of bendamustine in water with no addition of alcohol over a 24 hour period at 5° C. Bendamustine degrades quickly in water but the stability of bendamustine increases with increasing alcohol concentrations (FIGS. **2**, **3** and **4**). Although alcohols are frequently used in lyophilization to aid in solubility problems, the effect of alcohols on bendamustine stability is unique, unexpected and useful in manufacturing bendamustine with fewer impurities since an aqueous solution can be used while maintaining the stability of bendamustine. TBA was found to be the best stabilizer of the six alcohols tested (FIGS. **2**, **3**, and **4**). All alcohols at 30% (v/v) reduced the formation of impurities HP1 and Dimer at 5° C. for up to 24 hours. With respect to TBA, HP1 reaches only about 0.4% when stored at 5° C. for up to 24 hours. Lower concentrations of alcohol may not be efficient, when formulated at 15 mg/mL bendamustine and stored at 5° C. due to bendamustine precipitation and impurity formation.

Example 4

Formulation Optimization

After the solubility and stability of bendamustine were determined, the formulation was optimized for lyophilization. Since the concentration of bendamustine is higher in a 30% TBA/water saturated solution as compared with other alcohol solutions, it is anticipated that the vial size required to fill 100 mg of bendamustine can be decreased from the current Ribomustin® presentation. Although a saturated solution of bendamustine contains 18 mg/mL at 0° C., a concentration of 15 mg/mL was selected for the formulation to compensate for slight differences in API solubility due to differences in bulk API purity as a result of batch differences. A concentration of 15 mg/mL bendamustine requires 6.67 mL to fill 100 mg of bendamustine HCl per vial.

The surface (sublimation) area to volume ratio is critical to producing a lyophilized product with good appearance that freeze dries quickly. Generally, lyophilized products occupy between 30% to 50% of the vial volume. A 20 mL vial with 6.67 mL contains about 30% of its capacity and has a surface area ratio of 0.796 cm^2/mL.

Mannitol was selected as the bulking agent in order to maintain a formulation similar to Ribomustin®. Studies were performed to evaluate the effect of mannitol on bendamustine solubility and appearance of the product. Mannitol decreases the solubility of bendamustine (at 15 mg/mL) in both ethanol and TBA aqueous solutions. For example, solutions containing 5% and 10% ethanol and TBA without mannitol did not precipitate over 24 hours. However, for samples with mannitol (Table 1) precipitate was observed within 24 hours. There was no precipitate with aqueous solutions containing 30% (v/v) TBA, 15 mg/mL bendamustine, and 25.5 mg/mL mannitol. In order to maintain a well formed cake resistant to breakage during handling, a minimum of 134 mg/vial of mannitol was required with no difference observed in vials up to 200 mg/vial of mannitol.

All alcohols tested increased the stability and solubility of bendamustine. However, a significant mole fraction was required to affect the stability of the filling solution and the ease of manufacturing. Smaller alcohols have the undesirable effect of lowering the freezing point of the bulk solution and thus requiring long lyophilization cycles at lower temperatures. Higher concentrations of methanol and ethanol produced unattractive cakes that were difficult to reconstitute. 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol, or 30% TBA aqueous solutions containing bendamustine (15 mg/mL), mannitol (25.5 mg/mL) were prepared and lyophilized. The lyophilized vials filled from solutions of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol produced either a collapsed cake or a film residue. The only solvent system producing an acceptable cake was 30% TBA. Additionally, reconstitution of 10% ethanol, 20% ethanol, 10% iso-propanol, 20% iso-propanol lyophilized vials were difficult and did not fully dissolve until >45 minutes.

The ability to utilize a smaller vial is constrained by the concentration or solubility of bendamustine in the aqueous/organic solution. At lower concentrations of ethanol, methanol, isopropanol and n-propanol, which produced acceptable cake appearance, a more dilute solution of bendamustine is required due to solubility limitations. To maintain a presentation with 100 mg of bendamustine per vial, a vial larger than

50 mL would be required. Also, stability studies herein indicated that at the lower alcohol concentration, the chemical stability was not sufficient to allow for acceptable filling times.

One of the factors affecting the ease of reconstitution is the porosity of the lyophilate. In general, amorphously precipitated solids with little surface area are more difficult to solubilize. Most lyophilates containing mannitol will reconstitute within 3-5 minutes as long as there is no precipitate formed during lyophilization, frequently caused by evaporation of a liquid (melt back). Based on our experience with several lyophilization solvent systems and not wishing to be bound to any particular theory, the problems associated with Ribomustin® reconstitution may be associated with precipitation caused by melt back during lyophilization. Most organic solvents do not lyophilize efficiently and cause melt back because of their low melting point. TBA (tertiary butyl alcohol) has a high melting point and a similar vapor pressure as compared to water. TBA is removed by sublimation, not evaporation, at about the same rate as water. Lyophilates produced with 30% (v/v) TBA according to the invention reconstitute within 3-10 minutes as compare to commercially available Ribomustin which may take 30-45 minutes.

Based upon the solubility, stability, ease of reconstitution and manufacturing considerations, the following is a preferred pre-lyophilization formulation of the present invention: bendamustine HCl about 15 mg/mL, mannitol about 25.5 mg/mL, about 30% (v/v) tertiary-butyl alcohol, and q.s. using water for Injection. The formulation is then filled at 5° C. using 6.67 mL in an amber 20 mL, 20 mm vial and partially stoppered with a bromobutyl stopper and loaded into a pre-chilled lyophilizer.

Example 5

Impurity Assessment

Major impurities introduced during Ribomustin® manufacturing, compounding, fill, and lyophilization procedure, as determined by HPLC analysis (FIG. **6**), are the hydrolysis product HP1, the Dimer, and the ethyl ester of bendamustine, BM1EE. BM1EE can be formed during drug substance manufacturing, e.g., during recrystallization and/or purification processes. BM1EE is known to be a more potent cytotoxic drug than bendamustine. Experiments were undertaken to determine if the use of a 30% TBA aqueous filling solution would lead to the formation of bendamustine t-butyl ester.

Experiments were performed using traditional Fisher esterfication reaction conditions required for the formation of t-butyl ester of bendamustine. Bendamustine was heated in 60° C. TBA with HCl for 20 hours. No reaction was observed. This result indicated that it would be very difficult to form the tert-butyl ester of bendamustine during the fill/finish process. No new impurities in drug product manufactured from TBA have been observed in stability studies to date.

To aid in the testing of the drug product, synthetic routes using more reactive sources of the t-butyl moiety were developed. Another attempt to make tert-butyl ester was carried out by formation of the acyl chloride of bendamustine. A suspension of bendamustine in methylene chloride was treated with oxalyl chloride and N,N-dimethylformamide. After acyl chloride was formed, the solvent was concentrated. The residue was added to methylene chloride, tert-butanol, triethylamine, and 4-dimethylaminopyridine and the mixture was stirred at room temperature overnight. After adding all solvents and purification, an unknown compound was given. The LC-MS did not match the molecular weight of bendamustine tert-butyl ester and the proton NMR did not showed the peak for tert-butyl. Therefore, this attempt also failed to produce the bendamustine tert-butyl ester. Thus, using TBA as the co-solvent has an additional benefit of not forming the ester from the alcohol.

Example 6

Lyophilization Cycle Development

Numerous lyophilization cycles were performed to evaluate the critical stages of lyophilization and achieve the most efficient drying cycle. Experiments were performed to evaluate the effect of the freezing rate, primary drying temperature, time, and pressure on the product.

A. Freezing Rate

The literature reports that TBA adopts different crystal forms depending on the freeze rate. In some TBA solutions, the slower the product froze, the quicker it dried. Larger crystals formed during slow freezing producing bigger pores allowing more efficient sublimation. However, during studies with bendamustine, the freezing rate was not found to be a critical processing parameter when evaluated at 2 and 8 hours.

B. Primary and Secondary Drying

During the first attempts to lyophilize from 30% TBA solutions, the lyophilized cake fractured and powder was ejected from the vial. These cakes appeared to contain amorphous particles within the lyophilate, an indication of melt back. This phenomenon was reproducible and occurred when the product reached about −10° C. (refer to FIG. **5**) independent of the warming rate. Several variables were tested to determine the cause and solution to the problem of the powder ejection. The pressure was raised from 50 μm to 150 μm during primary drying, but powder ejection was still observed but to a lesser extent. This experiment was then repeated except the freezing rate was extended to 8 hours from 2 hours. This change had no effect.

The length of primary drying was next evaluated. For example, the following very slow drying cycle was evaluated: freezing from +25° C. to −50° C. in eight hours; holding at −50° C. for 5 hours, warming and drying from −50° C. to −25° C. in seven hours; holding for twenty hours at −25° C., warming and drying from −25° C. to −15° C. in two hours and holding for twenty hours at −15° C., warming and drying from −15° C. to 40° C. in six hours and holding for twenty hours at 40° C. while maintaining a chamber pressure of 150 μm throughout drying. No powder ejection (FIG. **5**) was observed. This cycle resulted in a well-formed cake without fracture that reconstituted readily. Without wishing to be bound to a particular theory, the problems with powder ejection and difficulty with reconstitution may be the result of drying the lyophilate too quickly, thus resulting in strong vapor flow out of the cake as well as melt back. With the use of a less aggressive drying cycle an aesthetic, stable, and easy to reconstitute cake was reproducibly formed. Thus, removing all unbound water and tertiary-butyl alcohol prior to secondary drying may prevent melt back as well as powder ejection. The lyophilization cycle was further optimized under these gentle conditions (FIG. **5**). There were no immediate degradation products as a result of drying at 40° C. for up to 20 hours.

Example 7

Lyophilization Cycle

Step	Description	Time (Hour)	Temperature (° C.)	Pressure (Microns)
1	Hold	0.25	5° C.	—
2	Ramp	8	−50° C.	—
3	Hold	4	−50° C.	—
4	Ramp	3	−20° C.	150
5	Hold	6	−20° C.	150
6	Ramp	1	−15° C.	150
7	Hold	20	−15° C.	150
8	Ramp	0.5	−12° C.	150
9	Hold	15.5	−12 C.	150
10	Ramp	15	35 C.	50
11	Hold	10	35° C.	50
12	Ramp	1	40 C.	50
	Hold	5	40 C.	50
Total		89.25	—	—

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the spirit and scope of the invention. More specifically, it will be apparent that certain solvents which are both chemically and physiologically related to the solvents disclosed herein may be substituted for the solvents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit and scope of the invention as defined by the appended claims.

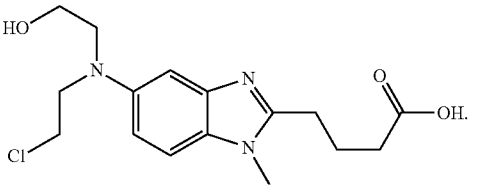
All patents, patent applications, and publications mentioned in the specification are indicative of the levels of those of ordinary skill in the art to which the invention pertains. All patents, patent applications, and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element(s) not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

What is claimed is:

1. A pharmaceutical composition that has been reconstituted from a lyophilized preparation of bendamustine or bendamustine hydrochloride, said composition containing not more than about 0.9% (area percent of bendamustine) of HP1:

(HP1)



2. The pharmaceutical composition of claim **1**, wherein the amount of HP1 is measured at time zero after reconstitution of said lyophilized preparation.

3. The pharmaceutical composition of claim **1**, wherein the amount of HP1 is not more than 0.5% (area percent of bendamustine).

4. The pharmaceutical composition of claim **2**, wherein the amount of HP1 is not more than 0.5% (area percent of bendamustine).

5. The pharmaceutical composition of claim **1**, wherein the amount of HP1 is not more than 0.4% (area percent of bendamustine).

6. The pharmaceutical composition of claim **2**, wherein the amount of HP1 is not more than 0.4% (area percent of bendamustine).

7. A pharmaceutical composition of bendamustine hydrochloride, containing less than or equal to 4.0% (area percent of bendamustine) of bendamustine degradants.

8. The pharmaceutical composition of claim **7**, containing between about 2.0% and 4.0% (area percent of bendamustine) of bendamustine degradants.

9. The pharmaceutical composition of claim **8**, wherein the pharmaceutical composition has been reconstituted from a lyophilized preparation of bendamustine hydrochloride.

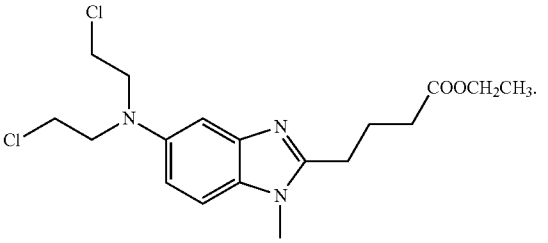
10. The pharmaceutical composition of claim **9**, containing not more than about 0.9% (area percent of bendamustine) of HP1 at time zero after reconstitution.

11. The pharmaceutical composition of claim **9**, containing not more than about 0.5% (area percent of bendamustine) of HP1 at time zero after reconstitution.

12. The pharmaceutical composition of claim **9**, containing not more than about 0.4% (area percent of bendamustine) of HP1 at time zero after reconstitution.

13. The pharmaceutical composition of claim **10**, containing not more than about 0.5% (area percent of bendamustine) of a compound of Formula IV at time zero after reconstitution:

Formula IV



14. The pharmaceutical composition of claim **7**, wherein the pharmaceutical composition is a lyophilized composition.

15. The pharmaceutical composition of claim **8**, wherein the pharmaceutical composition is a lyophilized composition.

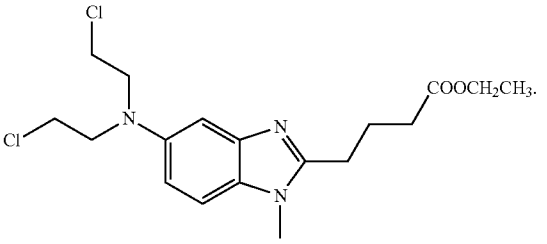
16. The pharmaceutical composition of claim **7**, containing not more than about 0.9% (area percent of bendamustine) of HP1.

17. The pharmaceutical composition of claim **7**, containing not more than about 0.5% (area percent of bendamustine) of HP1.

18. The pharmaceutical composition of claim **7**, containing not more than about 0.4% (area percent of bendamustine) of HP1.

19. The pharmaceutical composition of claim **7**, containing not more than about 0.5% (area percent of bendamustine) of a compound of Formula IV:

Formula IV



20. A method of treating cancer in a patient comprising administering to the patient a pharmaceutical composition of bendamustine hydrochloride according to claim **7**.

21. The method according to claim **20**, wherein the cancer is chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, or breast cancer.

22. The method according to claim **20**, wherein the cancer is chronic lymphocytic leukemia.

23. The method according to claim **20**, wherein the cancer is non-Hodgkin's lymphoma.

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