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(54) Title: IMPROVED METHODS FOR THE STERILIZATION OF BENDAMUSTINE

(57) Abstract: The present application is directed to methods of sterilizing bendamustine and its pharmaceutically acceptable salt forms. Preferred sterilization methods include dry heat sterilization, gamma irradiation, and e beam radiation. Sterile pharmaceutical compositions are also described.

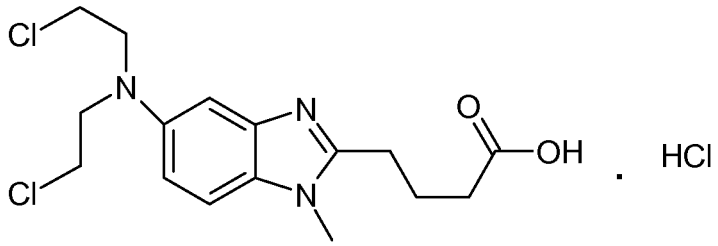
IMPROVED METHODS FOR THE STERILIZATION OF BENDAMUSTINE

TECHNICAL FIELD

The invention is directed to methods of sterilizing bendamustine, or a pharmaceutically acceptable salt thereof. Preferred methods include dry heat, gamma irradiation, and e-beam radiation.

BACKGROUND

Bendamustine, a formulation of which is distributed in the United States as its hydrochloride salt under the trade name TREANDA (Cephalon, Inc., Frazer, PA):



is a nitrogen mustard approved in the United States and elsewhere for the treatment of chronic lymphocytic leukemia (CLL) and B-cell non-Hodgkin's lymphoma (NHL). Bendamustine was first synthesized in the German Democratic Republic in 1963 and received its first marketing approval 1971 in Germany for the treatment of indolent NHL, multiple myeloma, and CLL.

The bis-chloroethylamine moiety makes bendamustine light-sensitive and highly unstable in water. In addition, bendamustine HCl is heat-sensitive, charring when heated to 160 °C and melting when heated to 170 °C. Bendamustine has only ever been commercially available as a sterile pharmaceutical salt composition in a lyophilized form, packaged in amber bottles. Lyophilization is a costly process and is only used for otherwise unstable pharmaceutical compositions or to improve the dissolution profile of a pharmaceutical composition, as lyophilization is known to sometimes improve the ability of a composition to dissolve in aqueous solution.

In a typical lyophilization process, a solution of bendamustine hydrochloride, water, alcohol, for example t-butanol or ethanol, and an excipient, for example mannitol, is mechanically sterilized by passing it through a filter. The sterile solution is then aseptically loaded into vials, frozen, and sublimed to remove the water and alcohol, leaving behind a sterile, solid lyophilized cake comprising bendamustine hydrochloride

and the excipient. Both in the United States and abroad, bendamustine is provided to clinicians as a lyophilized powder that is reconstituted with Sterile Water for Injection and 0.9% Sodium Chloride Injection immediately prior to administration. It is critical that the lyophilized solid dissolve quickly because of the instability of bendamustine in aqueous solution. Moreover, the lyophilized solid must dissolve completely prior to administration because of the adverse consequences associated with injecting particulate matter into the bloodstream. TREANDA's instructions for reconstitution, for example, state that the lyophilized powder should completely dissolve in 5 minutes and that reconstituted product having particulate matter should not be used.

While the sterile lyophilized form of bendamustine has been used successfully for nearly 40 years for the treatment of NHL, multiple myeloma, and CLL, there is a long felt need for methods of producing a sterile form of bendamustine having an acceptable dissolution profile that does not require lyophilization and is non-degrading.

SUMMARY

The present invention is directed to methods of sterilizing a solid that comprises bendamustine, or a pharmaceutically acceptable salt form thereof. Preferred methods of sterilization include dry heat sterilization using non-standard conditions, gamma irradiation, and e beam radiation. Sterile, pharmaceutical compositions consisting essentially of bendamustine or a pharmaceutically acceptable salt form thereof, are also described.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention is directed to methods of sterilizing bendamustine, or a pharmaceutically acceptable salt form thereof, comprising providing a solid comprising bendamustine or a pharmaceutically acceptable salt form thereof, and sterilizing the solid. Preferably, the solids consist essentially of, or in the alternative, consist of, bendamustine or a pharmaceutically acceptable salt form thereof. Preferred methods of sterilization include dry heat sterilization using temperatures and times that are outside the scope of the standard dry heat sterilization conditions used in the art, gamma irradiation, and e beam radiation.

As used herein, a material will be considered "sterile" when the probability of a surviving microorganism is less than one in a million, which is expressed as a sterility assurance level ("SAL") of 10^{-6} or better. A SAL of 10^{-6} means that statistically, less than

one in every million samples of material carries a viable organism. SAL can be determined using methods known in the art, for example, U.S. Pharmacopeia Chapter 71.

"Dry heat sterilization," as used herein, refers to sterilization methods that use hot air having little to no water vapor. In a typical dry heat sterilization, a composition will be sterile after exposure to dry heat in a 160 °C chamber for about 2 hours (120 minutes) or a 170 °C chamber for about 1 hour (60 minutes). These conditions, which are accepted by those skilled in the art as standard dry heat sterilization conditions, are not suitable for bendamustine hydrochloride, however, because bendamustine hydrochloride chars at 160 °C and melts at 170 °C.

While standard dry heat sterilization conditions are not suitable for sterilizing a solid comprising bendamustine hydrochloride, it has been surprisingly found that a solid comprising bendamustine hydrochloride can be sterilized by heating the solid in a dry heat sterilization chamber at about 140 °C. It has also been surprisingly found that a solid comprising bendamustine hydrochloride can be sterilized by heating the solid in a dry heat sterilization chamber at about 150 °C. Preferably, the solid is heated in either a 140 °C chamber or a 150 °C chamber for about 180 minutes or less. More preferably, the solid is heated in either a 140 °C chamber or a 150 °C chamber for about 150 minutes to about 180 minutes. In an exemplary embodiment, the solid is heated in a 140 °C chamber for about 180 minutes. In another exemplary embodiment, the solid is heated in a 150 °C for about 150 minutes.

"Gamma irradiation sterilization," as used herein, refers to sterilization methods that use gamma radiation. Gamma rays typically have frequencies above 10^{19} Hz and wavelengths less than 10 pm. Exposure to gamma radiation can result in alteration of molecular bonds of some compositions and it would have been presumed by those skilled in the art that exposure to gamma irradiation sterilization would have resulted in the alteration of the labile bis-chloroethylamine moiety. Surprisingly, however, it has been discovered that a solid comprising bendamustine or a pharmaceutically acceptable salt form can be sterilized using gamma irradiation sterilization. In one embodiment, a solid comprising bendamustine or a pharmaceutically acceptable salt form can be sterilized by irradiating the solid with an absorbed dose of up to about 35 kGy. In certain embodiments, the solid is irradiated with an absorbed dose of about 29 kGy to about 33 kGy. Preferably, the solid is irradiated with an absorbed dose of about 33 kGy.

"Electron beam sterilization," also referred to as "e-beam sterilization," refers to a sterilization method that uses a concentrated, highly charged stream of electrons.

Exposure to e beam radiation can result in alteration of molecular bonds of some compositions and it would have been presumed by those skilled in the art that exposure to e beam radiation would have resulted in the alteration of the labile bis-chloroethylamine moiety. Surprisingly, however, it has been discovered that a solid comprising bendamustine or a pharmaceutically acceptable salt form can be sterilized using e beam radiation. In one embodiment, a solid comprising bendamustine or a pharmaceutically acceptable salt form can be sterilized by irradiating the solid with an absorbed dose of up to about 35 kGy. Preferably, the solid is irradiated with an absorbed dose of about 30 kGy.

As used herein, "absorbed dose" is the measure of the energy deposited into the material being sterilized by gamma or e-beam radiation. It is equal to the energy deposited per unit mass of medium and has the unit J/kg or Gy (Gray).

As used herein, "pharmaceutically acceptable salts" refers to derivatives of bendamustine wherein the bendamustine has been modified by making the acid or base salt thereof. Examples of such salts include those derived from organic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like, as well as the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like.

Surprisingly, sterilization of bendamustine and its pharmaceutically acceptable salt forms, according to the methods described herein, does not detrimentally affect the purity of the composition, as measured using methods standard in the art, for example HPLC. This is unexpected in view of the presence of labile bisethylchloroamine moiety present in bendamustine. For example, when bendamustine hydrochloride is sterilized using the dry heat sterilization methods described herein, the purity of the sterilized material will be at least 95%, preferably at least 99%, as measured using standard methods, for example HPLC. When bendamustine or its pharmaceutically acceptable salt form is sterilized using the gamma irradiation sterilization methods described herein, the purity of the sterilized material will be at least 95%, preferably at least 99%, as measured using standard methods, for example HPLC. When bendamustine or its pharmaceutically acceptable salt form is sterilized using the e-beam irradiation sterilization methods described herein, the purity of the sterilized material will be at least 95%, preferably at least 99%, as measured using standard methods, for example HPLC.

Also within the scope of the invention are pharmaceutical compositions consisting essentially of bendamustine or a pharmaceutically acceptable salt form thereof, wherein said composition is sterile. Preferably, the pharmaceutical compositions are substantially free of any lyophilization excipients. Preferably, these pharmaceutical compositions are solids that have been sterilized using the methods set forth herein. In some embodiments, pharmaceutical compositions of the invention consist of a solid that is bendamustine or a pharmaceutically acceptable salt form that has been sterilized using the methods set forth herein.

Prior to the invention, sterile pharmaceutical compositions of bendamustine were lyophilized compositions that included a pharmaceutically acceptable salt form of bendamustine and a lyophilization excipient such as mannitol. The pharmaceutical compositions within the scope of the invention are not lyophilized compositions and do not include an agent useful in the lyophilization of bendamustine and its pharmaceutically acceptable salt forms. For example, the pharmaceutical compositions of the invention are solids that do not include mannitol. The pharmaceutical compositions of the invention may, however, include other excipients. "Excipients" are substances used to formulate bendamustine or a pharmaceutically acceptable salt form thereof, that does not lower or undesirably interfere with the primary therapeutic effect of the bendamustine. Preferably, the excipient is therapeutically inert and includes solubilizers, stabilizers, and binders that are generally regarded as safe by the U.S. Food and Drug Administration in the Code of Federal Regulations at 21 CFR §§ 182, 184.

EXAMPLES

Preparation of bendamustine hydrochloride

Bendamustine hydrochloride is prepared according to methods described in the art. See, for example, *J. Prakt. Chem.* **20**, 178-186 (1963), *Zentralblatt Fuer Pharmazie, Pharmakotherapie und Laboratoriumsdiagnostic* **110** (10), 1013-1019 (1971), and International Publication No. WO 2010/042568 A1.

Procedures for Dry Heat Sterilization

100 mg each of bendamustine HCl was weighed into a 20 mL tubing vial, a 20 mL amber vial, and a 20 mL clear vial. Rubber stoppers were inserted and aluminum caps crimped on. The vials were placed inside a GC oven set to 140 °C for 3 hours (180

minutes). The vials were then removed from the oven and allowed to cool to ambient temperature prior purity and sterility testing.

100 mg each of bendamustine HCl was weighed into a 20 mL tubing vial, a 20 mL amber vial, and a 20 mL clear vial. Rubber stoppers were inserted and aluminum caps crimped on. The vials were placed inside a GC oven set to 150 °C for 2 1/2 hours (150 minutes). The vials were then removed from the oven and allowed to cool to ambient temperature prior to purity and sterility testing.

Procedure for Gamma Irradiation

100 mg each of bendamustine HCl was weighed into a 20 mL tubing vial, a 20 mL amber vial, and a 20 mL clear vial. Rubber stoppers were inserted and aluminum caps crimped on. The vials were passed through a gamma irradiation line and received doses in the range of 29.3 kGy to about 32.3 kGy. Purity and sterility testing was then performed.

Procedure for E-Beam Irradiation

100 mg each of bendamustine HCl was weighed into a 20 mL tubing vial, a 20 mL amber vial, and a 20 mL clear vial. Rubber stoppers were inserted and aluminum caps crimped on. The vials were passed through an electron beam irradiation line and received a dose of about 30 kGy. Purity and sterility testing was then performed.

Purity Determination

To each 10 mg of bendamustine HCl, sterilized according to the methods described above, was added 10 mL N-methyl-2-pyrrolidone (NMP). A reference standard of bendamustine HCl was prepared in NMP having a concentration of 1 mg/mL. HPLC was performed according to conventional methods. The results are shown below.

Sample Type	**HPLC purity (% area)**
E-beam clear vial	99.70
E-beam amber vial	99.70
Gamma clear vial	99.64
Gamma amber vial	99.70
Untreated	99.71

Sterility Testing

All sterility testing was performed as per U.S. Pharmacopeia Chapter <71> ("USP <71>"). The results of the sterility testing are shown below.

Analysis	Condition	Result
Sterility USP <71>	Dry heat sterilization	No growth observed
Sterility USP <71>	E-beam sterilization	No growth observed
Sterility USP <71>	Gamma Irradiation	No growth observed

What is Claimed:

1. A method of sterilizing bendamustine or a pharmaceutically acceptable salt form thereof, comprising:

providing a solid comprising bendamustine, or a pharmaceutically acceptable salt form thereof;

sterilizing the solid.

2. The method of claim 1, wherein the pharmaceutically acceptable salt form is bendamustine hydrochloride.

3. The method of claim 1, wherein the purity of the bendamustine, or the pharmaceutically acceptable salt form thereof, is at least 95%, as measured by HPLC, after the sterilization step.

4. The method of claim 1, wherein the purity of the bendamustine, or the pharmaceutically acceptable salt form thereof, is at least 99%, as measured by HPLC, after the sterilization step.

5. The method of claim 1, wherein the sterilization step comprises dry heat sterilization and the bendamustine is bendamustine hydrochloride.

6. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber for about 180 minutes or less.

7. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber for about 150 minutes to about 180 minutes.

8. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber that is about 140 °C.

9. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber that is about 140 °C for about 180 minutes.

10. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber that is about 150 °C.

11. The method of claim 5, wherein the dry heat sterilization comprises heating the solid in a chamber that is about 150 °C for about 150 minutes.

12. The method of claim 1, wherein the sterilization step comprises gamma irradiation.

13. The method of claim 12, wherein the sterilization step comprises irradiating the solid with an absorbed dose of about 33 kGy.

14. The method of claim 12, wherein the sterilization step comprises irradiating the solid with an absorbed dose of about 29 kGy to about 33 kGy.

15. The method of claim 1, wherein the sterilization step comprises e-beam irradiation.

16. The method of claim 15, wherein the sterilization step comprises irradiating the solid with an absorbed dose of about 30 kGy.

17. A pharmaceutical composition consisting essentially of bendamustine or a pharmaceutically acceptable salt form thereof, wherein said composition is sterile.

18. The sterile pharmaceutical composition of claim 17, wherein the pharmaceutically acceptable salt form is bendamustine hydrochloride.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/043614

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L2/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2006/159713 A1 (BRITTAIN JASON E [US] ET AL) 20 July 2006 (2006-07-20) paragraphs [0092] - [0098]	1-7,17, 18 8-16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2011	23/09/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bjola, Bogdan

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/043614

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8-11(completely); 1-7, 17, 18(partially)

   method for sterilizing a solid comprising bendamustine or a pharmaceutically acceptable salt form thereof by heating the solid at about 140°C or 150°C

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2. claims: 12-14(completely); 1-7, 17, 18(partially)

   method for sterilizing a solid comprising bendamustine or a pharmaceutically acceptable salt form thereof by gamma irradiation

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3. claims: 15, 16(completely); 1-7, 17, 18(partially)

   method for sterilizing a solid comprising bendamustine or a pharmaceutically acceptable salt form thereof by electron beam irradiation

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/043614

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006159713 A1	20-07-2006	AR 052877 A1	11-04-2007
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