Research Article

Formulation and Evaluation of Stable Lyophilized Bendamustine Hydrochloride Injection

Mikkilineni Ravikanth [*1], P Srinivasa Babu[2], Mandava Venkata Basaveswara Rao[3], Yaswanth Allamneni
[1]Department of Regulatory Affairs, Natco Pharma Limited, Kothur, Mahaboobnagar, Andhra Pradesh, India.
[2]Principal, Vignan college of Pharmacy, Guntur, Andhra Pradesh, India.
[3]Dean, Departmemnt of Pharmacy and Chemistry, Krishna University, Nuzividu, Krishna, Andhra Pradesh, India.
***Corresponding author's E-mail:** hiravikanth_mikkilineni@yahoo.co.in

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ABSTRACT

The objective of this study was to develop and manufacture a stable lyophilized formulation for Bendamustine Hydrochloride without using preservatives and antioxidants. The drug substance exhibits polymorphism and is a highly soluble, low permeable Biopharmaceutics Classification System (BCS) Class III compound. As the finished product is received by lyophilization from a solution, physical characteristics of the drug substance e.g. particle size and polymorphism were not considered relevant for the drug product performance. Bendamustine HCl is soluble in methanol and sparingly soluble in water. From the literature it is well known that the active substance is not stable in aqueous solutions due to hydrolysis of the bis (2-chloroethyl) amino group. Tertiary butanol has low toxicity and has several advantages for freeze drying. To improve the product quality after lyophilization low quantity of ethanol was added as further co- solvent. To find out the critical temperature for freezing and primary drying the collapse temperature of the bulk solution was determined by freeze-drying microscopy.

Keywords: Bendamustine hydrochloride, Collapse temperature, Freezing and primary drying, Lyophilized formulation.

INTRODUCTION

Bendamustine hydrochloride is an alkylating antitumour agent of the nitrogen mustard type. The antineoplastic and cytocidal effect is based on cross-linking of DNA single and double strands by alkylation.[1, 2] It is used for the treatment of patients with chronic lymphocytic leukemia. Chemically it is 1H-Benzimidazole-2-butanoic acid, 5-[bis (2-chloroethyl) amino]-1 methyl-, monohydrochloride. Bendamustine hydrochloride is off white to cream colored crystalline powder. The drug substance is a weak base; hence solubility is pH dependent and is more soluble in acidic media. The drug substance exhibits polymorphism. As the finished product is received by lyophilization from a solution, physical characteristics of the drug substance e.g. particle size and polymorphism were not considered relevant for the drug product performance.

In lyophilized preparations, Mannitol is used as a carrier to produce a stiff, homogeneous cake that improves the appearance of the lyophilized plug in a vial.[6, 7] A pyrogen-free form is specifically used for this purpose. Lyophilization is a stabilizing process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (referred to as the primary drying process) and then desorption (known as the secondary drying process) to values that will no longer support biological activity or chemical reactions. Lyophilization is carried out using a simple principle of physics called sublimation. Sublimation is the transition of a substance from the solid to the vapour state, without first passing through an intermediate liquid phase. The lyophilization recipe has to be based on the structural characteristics of the product and on its thermal behaviour when subjected to freezing and drying processes.

Bendamustine Hydrochloride in liquid injection[8] was found to be unstable. The major objective of this experiment is to formulate the Bendamustine Hydrochloride injection by lyophillization technique for better stability and for long term storage.

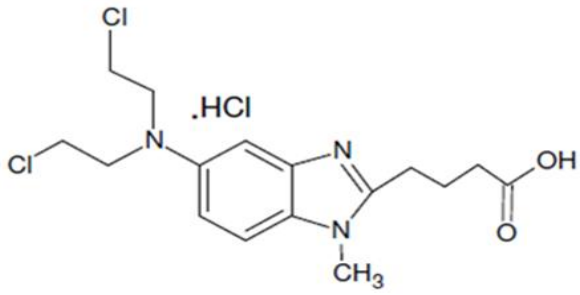

Figure 1: Chemical Structure of Bendamustine Hydrochloride

MATERIALS AND METHODS

Materials

Bendamustine Hydrochloride was provided by Natco Organics, Chennai. Mannitol was supplied by Roquette, Germany, Ethanol and TBA were supplied by Merck limited, India and other reagents were of analytical grade.

Methods

Melting point Determination

Consequently, the melting point of a compound is a criterion for purity as well as for identification. The melting point of an organic solid can be determined by

introducing a tiny amount into a small capillary tube, attaching this to the stem of a thermometer centered in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete.

Loss on Drying

Prepare the weighing bottle and cover by heating 30 minutes in the 105°C oven. Tare balance and accurately weigh both bottle and cover and record weight. Remove the cover and add 2-3 (±0.1) g of sample to the bottle. Replace cover and reweigh immediately. Record weight to the nearest 0.1 mg. Obtain the weight of the sample by difference. Heat the sample at 105°C for 1 hour. At the end of the drying time, remove the bottle and its cover from the oven (remembering to place cover over bottle but in an angled position so that bottle is not sealed) and place them in the desiccators. Allow the material to reach room temperature and then replace cover and weigh. Calculate the Loss on drying value using the following formula and it should be not more than 1.0%.

Loss on Drying =

$$\frac{[\text{Sample + Bottle (Initial Wt.)}]-[\text{Sample + Bottle (Final Wt.)}]}{\text{Sample weight}} \times 100$$

Solubility Studies

The solubility of Bendamustine hydrochloride was determined by mixing an excess quantity of drug with approximately 2 mL of the solvent which was taken in a screw-capped bottle. Bulk solution preparation was carried out at 2-8°C to prevent evaporation of solvents during process which may affect the assay of drug substance. The bottles were rotated on a laboratory rotator at 30 rpm for 24 hours at room temperature. Preliminary studies indicated that this time period was adequate to obtain equilibrium solubility. After the particles had settled, the supernatant was carefully withdrawn and filtered through a 0.22-µm filter and analyzed by UV.

Formulation of Bulk solution

Suitable combinations with organic solvents were tested under various conditions in order to receive a stable bulk solution of bendamustine HCl, allowing further processing within reasonable process times. Organic solvents were chosen based on toxicity and their suitability for freeze drying. The prepared bulk solution is tested for description, assay and related substances. The processing of bulk solution is carried out at 2-8°C to prevent the evaporation of organic solvents.

Method of Preparation

Collect approximately 70% of the required quantity of WFI into SS vessel. TBA & ethanol are added to the collected WFI and the mixture is stirred until ac clear solution is obtained. To the above co-solvent mixture, Mannitol is added under continuous stirring and cool down the Mannitol solution to 2-8°C. After achieving the desired temperature, bendamustine HCl was added and the mixture was kept for stirring to get a clear solution. The bulk solution is filtered and filled into amber colored glass vials. The filled vials are loaded in the lyophilizer and the lyophilization process is carried out as per the cycle which is given in below table. After completion of lyophilization process, vials are unloaded from lyophilizer and capped with aluminium seals.

Lyophilization cycle development

Lyophilization cycle development started with the evaluation of critical temperatures of the formulation in order to define the setting parameters of the freeze drying process. The temperature at which the drying structure began to collapse was higher with the addition of an annealing step. The total collapse was detected at -28.8°C. Hence the recommended freezing/ primary drying temperature are between -30.8 and -35.8°C. Annealing is furthermore advantageous to encourage the majority of the mannitol to adopt a crystalline conformation and therefore obtain a more suitable pore structure for sublimation.

Evaluation of Lyophilized vials

pH of the Reconstituted Solution

Take three vials and reconstitute each vial with 10 mL of water for Injection, dissolve and transfer the reconstituted solution into a suitable glass beaker and measure the pH of the solution using a pH meter at 25°C.[9]

Water Content

Transfer about 100 mL of Hydranal (Coulomat AG-Oven) reagent into the titration vessel and conditioning the cell, for the determination of drift. Set the temperature about 100°C and flow in the range of 60 mL/min depending upon vial capacity. After desired temperature has been reached and the titration cell conditioned (i.e. drift should be less than 20µg), the determination is started at KF colometer.

Reconstitution Time

Reconstitute the vial with water for injection and it should be not more than 5 minutes to reconstitute.[9]

Assay

Take 2 vials and reconstitute each vial with 40 mL of diluent, dissolve and transfer the reconstituted solution into 500 mL amber colored volumetric flask without any loss of solution. Wash the vial with 10 mL of diluent for 2 - 3 times and dilute to volume with diluent, shake and mix well. Transfer 5 mL of the above solution into a 100 mL amber coloured volumetric flask, dilute to volume with diluent (0.02mg/mL). Separately inject (about 10 QL) of diluent as blank, standard preparation and sample preparations into the chromatograph and record the chromatograms and measure the peak area responses for the analyte peaks. Calculate the % content of Bendamustine Hydrochloride in the portion of Bendamustine Hydrochloride for Injection.

Stability Studies

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. The ICH Guidelines have established that long term stability testing should be done at 25^0C/60% RH; stress testing should be done at 40^0C/75%RH for 6 months.[13] If significant change occurs at these stress condition, then the formulation should be tested at an intermediate condition i.e. 30^0C/75%RH.

Table 1: Solubility Studies of Bendamustine HCl in different pH

Buffer pH	Solubility
2.0	Slightly soluble
4.0	In soluble
6.0	Sparingly Soluble
8.0	Sparingly Soluble
10.0	Sparingly Soluble

Table 2: Stability of bendamustine bulk solution at 25°C, variation of TBA content from 30 to 40 % in aqueous solution

Solvent used→	TBA 30%					TBA 35%					TBA 40%				
Parameters	**Initial**	**6 hr**	**12 hr**	**18 hr**	**24 hr**	**Initial**	**6 hr**	**12 hr**	**18 hr**	**24 hr**	**Initial**	**6 hr**	**12 hr**	**18 hr**	**24 hr**
Description	Clear					Clear					Clear				
Assay [%]	100.5	100.2	100.6	101.0	100.0	100.9	100.0	101.2	102.3	100.3	99.3	97.9	101.6	100.9	99.0
Related substances															
Single Max [%]	0.11	0.07	0.21	0.27	0.69	0.11	0.08	0.12	0.15	0.18	0.06	0.07	0.08	0.10	0.12
Total Imp [%]	0.24	0.24	0.51	0.46	1.52	0.17	0.21	0.25	0.29	0.33	0.22	0.20	0.22	0.25	0.27

Table 3: Stability of bendamustine bulk solution at 2-8°C, variation of TBA content and Ethanol content in aqueous solution

Solvent used→	TBA (30%) + Ethanol (5%)				TBA (30%) + Ethanol (10%)				TBA (20%) + Ethanol (5%)				TBA (20%) + Ethanol (10%)			
Parameters	**Initial**	**6hrs**	**12hrs**	**18hrs**	**Initial**	**6hrs**	**12hrs**	**18hrs**	**Initial**	**6hrs**	**12hrs**	**18hrs**	**Initial**	**6hrs**	**12hrs**	**18hrs**
Description	Clear colorless				Clear colorless				Clear colorless				Clear colorless			
pH	3.05	3.07	3.10	3.13	3.06	3.07	3.09	3.104	3.06	3.07	3.11	3.12	3.07	3.06	3.08	3.11
Assay [%]	100.2	99.7	99.1	99.4	99.6	99.8	99.3	99.3	100.2	99.5	99.5	99.4	99.9	99.4	99.3	99.3
Related substances [%]																
Single maximum	0.06	0.08	0.11	0.15	0.06	0.07	0.08	0.10	0.08	0.22	0.35	0.49	0.06	0.13	0.21	0.28
Total impurities	0.24	0.28	0.31	0.35	0.24	0.24	0.29	0.30	0.28	0.42	0.57	0.72	0.25	0.32	0.41	0.49

Table 4: Final Lyophilization Cycle Process for Bendamustine HCl for Injection, 100 mg

Step No	Temperature (°C)	Time (Minutes)		Vacuum (mTorr)
		Ramp	Hold	
Freezing	0	10	20	-
	-30	90	60	–
	-45	90	120	–
	-25	90	360	–
	-45	90	180	–
Primary Drying	-35	300	300	150
	-30	240	1000	150
	-25	180	240	150
	-20	180	360	150
	0	180	120	150
	15	120	180	100
	30	180	600	50
Secondary Drying	40	120	1030	50

Table 5: Analytical Results of Bendamustine HCl for Injection

Test	Result				
	F01	F02	F03	F04	F05
Water content (%)	1.8	1.3	1.5	1.1	0.25
Reconstitution time (Minutes)	2 min 30 sec	2 min 10 sec	1 min 45 sec	1 min 12 sec	55 sec
pH	3.02	3.15	2.92	2.96	2.78
Assay (%)	102.3	100.7	99.6	101.4	100.1
Related substances (%)					
Maximum unknown impurity	0.231	0.210	0.217	0.201	0.156
Total impurities	0.89	0.85	0.90	0.88	0.593

Table 6: Stability study results of Bendamustine HCl for injection (F05)

Test	Result			
	40°C/75%			25°/60%
	1st Month	2nd Month	3rd Month	3rd Month
Water content (%)	0.21	0.25	0.19	0.22
Reconstitution time (Minutes)	51 sec	53 sec	55 sec	52 sec
pH	2.76	2.84	2.75	2.76
Assay (%)	99.9	100.0	100.2	100.3
Related Substances (%)				
Maximum unknown impurity	0.159	0.167	0.170	0.165
Total impurities	0.598	0.601	0.615	0.604

RESULTS AND DISCUSSION

Melting point of Bendamustine Hydrochloride was found to be 167°C. Loss on drying of Bendamustine Hydrochloride was found to be 0.23%. The drug substance is a weak base, hence solubility is pH dependent and is more soluble in acidic media. For formulation development two important criteria were taken into consideration: the stability of bendamustine bulk solution and the product quality after lyophilization. The stability of bendamustine bulk solution is dependent on the content of organic co-solvents and on the temperature. The stability increases with increasing content of the organic component in the mixture and with decreasing temperature. Solutions with higher contents of TBA were not suitable for freeze drying but acceptable results were achieved with aqueous solutions containing TBA and EtOH. Based on the results, the maximum holding time for Bendamustine bulk solution should not exceed 18 hours when stored at a temperature of 2°C-8°C. As a precaution it was decided to reduce the maximum holding time to 12 hours for routine. Formulation trial F05 was finalized based on the physical appearance and analytical results which were tabulated below.

Stability study results of Bendamustine HCl for injection reveals that there is no significant change in the formulation at both accelerated and long term stability conditions and which were tabulated below.

CONCLUSION

Bendamustine hydrochloride in liquid Injection was found to be unstable. Therefore, it was developed as lyophilized formulation for better stability. Bendamustine HCl for injection was compatible with 50 mL amber coloured glass USP Type I vial, bromobutyl rubber closure, nitrogen sparging. The developed formulation was able to withstand three freeze thaw cycles without getting affected product quality. The formulation was stable for 3 months on accelerated stability. However, further research in this field of study is highly required in order to effectively make this drug available for use in the market.

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