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Oil-Based Formulation and Evaluation of Anti-Cancer Drug

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ABSTRACT

B-cell chronic lymphocytic leukemia (B-CLL), also known as chronic lymphoid leukemia (CLL), is the most common type of leukemia. Leukemias are cancers of white blood cells (leukocytes). Bendamustine (INN, trade names Ribomustin, Treanda, Bendeka, and Belrapzo) is nitrogen mustard used in the treatment of chronic lymphocytic leukemias and lymphomas. It belongs to the family of drugs called alkylating agents. Bendamustine Hydrochloride is commercially available in the market as a lyophilized dosage form across the globe whereas, in the US, nonaqueous solution form is also commercially available. Package inserts evaluation of all available 3 products and also evaluating various articles; it is learned that there is a scope to evaluate novel strength requirement for the effective use and hence attempt is made to present novel intermediate strength-based formulation. Also, enough literature is available that Bendamustine Hydrochloride is very unstable in the liquid dosage form. Aqueous-based formulations attempt was not successful and there was a need to revisit the composition. Data from the revisited composition showed an improvement in the control of impurities.

INTRODUCTION

Cancer is a disease of uncontrolled cell division, invasion, and metastasis[1]. It is generally considered to be due to the clonal expansion of a single neoplastic cell. Cancers are classified in two ways[2]: by the type of tissue in which cancer originates (histological type) and by primary site or the location in the body where cancer first developed. It is also being studied for the treatment of sarcoma[3]. Bendamustine was first synthesized in 1963 by Ozegowski and Krebs in East Germany (the former German Democratic Republic). It undergoes hydrolytic degradation in the presence of water[4]. It is a white, water soluble microcrystalline powder with amphoteric properties[5]. Until 1990 it was available only in East Germany. East German investigators found that it was useful for treating chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and lung cancer[6]. The IUPAC name of Bendamustine Hydrochloride is lHbenzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-l methyl-, monohydrochloride. Its empirical molecular formula is $C_{16}H_{21}Cl_2N_3O_2$.HCl, and the molecular weight is 394.7. Bendamustine hydrochloride contains a mechlorethamine group and a benzimidazole heterocyclic ring with a butyric acid substituent and has the following structural formula[7].

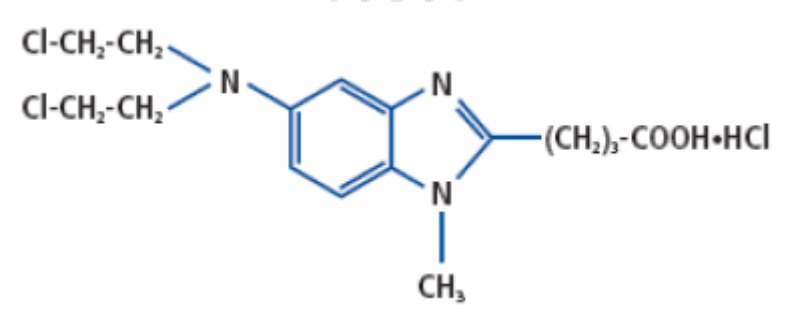

Figure No. 1: Structure of Bendamustine Hydrochloride

In India, the drug Bendamustine Hydrochloride was approved as the Lyophilized powder Injection of 100 mg/vial for the treatment of patients with chronic lymphocytic leukemia. As per the literature available, the lengthy exposure of Bendamustine to water during the reconstitution process increases the potential loss of potency and impurity formation due to the hydrolysis of the product by water. Lyophilization is time consuming, tedious, and involves cumbersome procedures. Further, it involves expensive technology to develop a lyophilized product. One of the main disadvantages of lyophilization is the expensive and the lyophilization cycle development and criticality of the freeze drying process, which requires very low temperatures, can be quite costly. Further, the product needs to be handled with the

precautions while dispensing, manufacturing and lyophilization. The lyophilization cycle recipe needs to be set carefully based on the load of the vials that go into the lyophilizer. Hence, an attempt to develop a non-lyophilized drug product such as liquid formulation would offer convenience for practitioners by avoiding the reconstitution step when preparing the drug for administration.

MATERIALS AND METHODS:

Bendamustine Hydrochloride was procured from Keman Chemicals, Gujarat, DMSO and was sourced from Sigma-Aldrich, Edetate Di-Sodium was received as a gift sample from Merck. Polysorbate 80, Polyox castor oil 35, and Soyabean oil were purchased from commercial sources. All required chemicals used were of standard grade.

Preparation of Bendamustine Hydrochloride Formulations.

A total of 3 formulations were prepared. The concentration chosen of Bendamustine Hydrochloride was 45 mg/mL based on the solubility. The mixture of Polysorbate 80 and Soyabean oil was taken, the drug was added and dissolved. Rest ingredients were added as per the below composition table and 100 % volume make up was made as per the below composition table.

Table No. 1: Formulation of Bendamustine Hydrochloride Injection.

Sr. No.	Ingredients	NBF1	NBF2	NBF3
1.	Bendamustine Hydrochloride	45 mg/mL	45 mg/mL	45 mg/mL
2.	Soya bean Oil	200 mg/mL	250 mg/mL	300 mg/mL
3.	Polysorbate 80	75 mg/mL	75 mg/mL	75 mg/mL
4.	Demethyl Sulfoxide	--	--	300 mg/mL
5.	Edetate Disodium	0.1 mg/mL	0.1 mg/mL	0.1 mg/mL
6.	Polyoxyl castor oil 35	QS to 1 mL	QS to 1 mL	QS to 1 mL

Evaluation of Bendamustine Hydrochloride Formulations:

Physical evaluation

***Description*:** This is a physical observation made by an individual.

pH: pH was measured using a pH meter at about 25°C temperature by diluting the one part of formulations with 10 mL of water.

Water Content: water content of all the formulations was measured using Karl-Fischer USP 921 <Method 1a>.

Light Transmission: All formulations were tested for light transmission at 650 nm using a UV spectrophotometer. The formulations were diluted in a 1:10 ratio with water and then measured.

Chemical Evaluation:

Assay: HPLC method was adopted to measure the active drug content from the 3 formulations. The active obtained is expressed as a percent of the labeled amount of Bendamustine Hydrochloride content. The obtained value of drug content is expected to be within limits of 90.0% to 110.0% (General compendia like USP & BP requirement).

Related Substances: % content of known and unknown impurities were determined by the HPLC method.

RESULTS AND DISCUSSION:

The results are compiled in Table No. 2. A clear colourless to a light yellow colour solution was observed in all three formulations. The pH of all 3 formulations was observed in the range of 2.5 to 3.0 wherein the formulations don't contain pH adjusting agents. This indicates that the pH of the formulations is independent of drug substances though there are qualitative and quantitative changes among the formulation. It is also noted from the pH trend that all the three formulations indicated that formulation stability is towards the acidic nature as the drug substance is salt of weak acid which has butyric acid moiety. Light transmission measured for the three formulations found between 95 to 100% indicating the clear transmission of the liquid formulation when each of the formulations was transmitted through a UV spectrophotometer at 650 nm. The water content results of all three formulations were found satisfactory. Concerning the chemical analysis of all the three formulations, it was observed that all the three formulations have shown assay value of about 98.0 % indicating the correct input of % content of Bendamustine Hydrochloride vs label claim. It also indicates that the analytical method employed for estimating the % content of Bendamustine Hydrochloride is correct. From the analysis of the related substances, it was observed that monohydroxy

Bendamustine (impurity A) was observed in all three formulations in a significant amount and other two known impurities such as dihydroxy Bendamustine (Impurity B) and Dimer Impurity (Impurity C) content are satisfactory. However, the content of the single highest unknown impurity is found high in all three formulations.

Table No. 2: Physical and Chemical Evaluation of Bendamustine Hydrochloride Formulations.

Sr. No.	Formulation Codes	Description	pH	LT (in %)	Water Content	Assay (in %)	Related Substances
1	NBF1	@	2.94	98.5	0.31%	98.7%	Imp A:1.18% Imp B:0.11% Imp C:0.11% Single Highest UNK Imp: 0.12% Total Imp: 1.65%
2	NBF2	@	2.92	99.1	0.28%	98.2%	Imp A:0.89% Imp B:0.12% Imp C:0.09% Single Highest UNK Imp: 0.11% Total Imp: 1.41%
3	NBF3	@	2.85	98.4	0.33%	99.6%	Imp A:0.52% Imp B:0.15% Imp C:0.11% Single Highest UNK Imp: 0.09% Total Imp: 0.85%

NBF stands for Non-Aqueous Bendamustine Formulations.

@: Description: A clear yellow color solution. LT is Light Transmission.

Imp A is Impurity A: Monohydroxy Impurity, Imp B is Impurity B: Dihydroxy Impurity and

Imp C is Impurity C: Dimer Impurity.

CONCLUSION:

The overall characterization of all three formulations concluded that no physical description complications were observed. Analytical results of pH and light transmission test parameters were found satisfactory. Results of the water content estimation of the studied formulations were found satisfactory. Chemical evaluation such as assay test parameter result was observed satisfactory. However, concerning impurity formation such as monohydroxy Bendamustine was observed higher levels in the first two formulations [NBF1 and NBF2]. Soya bean oil quantity in each of the formulation was altered to understand the impact of its quantity on the impurity formation. However, other two known impurities such as dihydroxy and dimer impurities results are found satisfactory. It is also to be noted that the % content of unknown impurities is found to a satisfactory level in all three formulations. From the above experiment, it can be concluded that Bendamustine hydrochloride formulation requires further fine tuning to arrest the further levels of degradation impurity such as Impurity A.

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