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RESEARCH ARTICLE

Preparation and Evaluation of Bendamustine Hydrochloride Aqueous Formulations

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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 the white blood cells (leukocytes). CLL affects B cell lymphocytes. B cells originate in the bone marrow, develop in the lymph nodes, and normally fight infection by producing antibodies. Bendamustine (INN, trade names Ribomustin and Treanda; also known as SDX-105) is a nitrogen mustard used in the treatment of chronic lymphocytic leukemias (CLL) and lymphomas. It belongs to the family of drugs called alkylating agents. It is also being studied for the treatment of sarcoma[1]. Bendamustine Hydrochloride is commercially available in the market as lyophilizzed dosage form. Also enough literature is available that Bendamustine Hydrochloride is very unstable in the liquid dosage form. It undergoes hydrolytic degradation in the presence of water[2]. Hence an attempt for developing a simple, aqueous and non aqueous based Bendamustine Hydrochloride formulations have been attempted.

KEYWORDS

Bendamsutine Hydrochloride, Hydroxypropyl beta cyclodextrin, Mannitol.

INTRODUCTION

Bendamustine was first synthesized in 1963 by Ozegowski and Krebs in East Germany (the former German Democratic Republic). It is a white, water soluble microcrystalline powder with amphoteric properties[3]. 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[4]. The IUPAC name of bendamustine Hydrochloride is lH-benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-l methyl-, monohydrochloride. Its empirical molecular formula is $C_{16}H_{21}Cl_2N_3O_2$.HC1, 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[5].

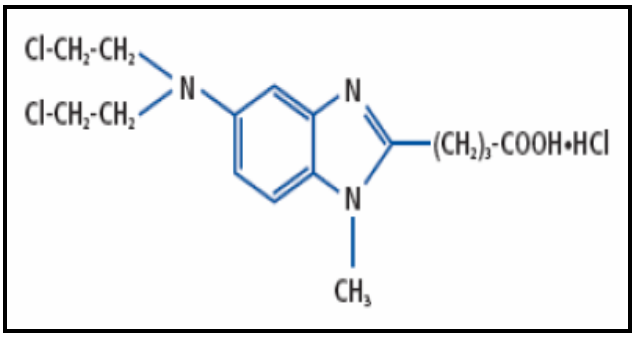

Figure 1: Structure Bendamustine

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EXPERIMENTAL

Chemicals and Reagents

Bendamustine Hydrochloride was procured from Shilpa Medicare Limited, Raichur, Hydroxypropyl betacyclodextrin was received as gift sample from Cydex Inc. Germany. All the other chemicals used were of standard grade.

Preparation of Aqueous based Bendamustine Hydrochloride

A total of aqueous based four formulations were prepared. The concentration chosen of Bendamustine Hydrochloride was 5 mg/mL based on the solubility. Aqueous formulations containing Hydroxypropyl betacyclodextrin were prepared apart from containing Mannitol as an osmolality contributing agent. There are no pH adjusting agent used in the formulation as the injection formulation pH was observed in the range of 2.5 to 3.0 without adjustment.

Evaluation of Aqueous based Bendamustine Hydrochloride Formulations

Physical evaluation

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

pH: pH of the each formulations were measured using Metrohm pH meter at about 25°C temperature.

Light Transmission: The light transmission of the aqueous formulations were transmitted at 650 nm using UV spectrophotomer.

Chemical Evaluation

Assay: HPLC method was used to determine the active drug content from the 4 formulations. The recovered amount of active drug is the expressed as percent of labeled amount of Bendamustine Hydrochloride content. The obtained value of drug content should be within established limits of 90.0% to 110.0% (General compendia like USP & BP requirement)

Related Substances: HPLC method was used to determine % content of known and unknown impurities.

RESULTS AND DISCUSSION

The results are compiled in the table 2. A clear colorless solution from AF1 to AF4 were observed. pH of all 4 formulations were observed in the range of 2.5 to 3.0 indicating the pH of the formulations independent of drug substances though there is a quantitative change in the formulation. Also the pH trend observed from four formulations indicates that formulation stability towards the acidic nature as the drug substance is salt of weak acid as it contains butyric acid moiety. Light transmission measured each of all 4 formulations were in the range of between 98 to 100% indicating the clear transmission of the liquid formulation when transmitted through UV spectrophotometer at 650 nm. If there were a haziness or precipitation in the description, the transmission would have been less and showed variation. With respect to the chemical analysis of the four formulations, it was observed that all the four formulations has shown assay value 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

Formulation of Aqueous Bendamustine Hydrochloride Injection

Sr. No.	Ingredients	AF1	AF2	AF3	AF4
1	Benndamustine Hydrochloride	5 mg/mL	5 mg/mL	5 mg/mL	5 mg/mL
2	Mannitol	20 mg/mL	20 mg/mL	20 mg/mL	20 mg/mL
3	Hydroxypropyl betacyclodextrin	10 mg/mL	15 mg/mL	20 mg/mL	25 mg/mL
4	Water For Injection	Qs to 1mL	Qs to 1mL	Qs to 1mL	Qs to 1mL

Table 2: Physical and Chemical Evaluation of Aqueous Bendamustine Hydrochloride Formulations.

Sl. No.	Formulation Codes	Description	pH	LT	Assay	Related Substances
1	AF1	#	2.62	98.98%	97.95%	Imp A:3.28% Imp B:0.12% Imp C:0.08% Highest UNK Imp: 0.25% Total Imp: 3.92%
2	AF2	#	2.68	99.12%	98.12%	Imp A:3.12% Imp B:0.16% Imp C:0.04% Highest UNK Imp: 0.29% Total Imp: 4.02%
3	AF3	#	2.79	99.98%	97.31%	Imp A: 3.28% Imp B:0.18% Imp C:0.11% Highest UNK Imp: 0.19% Total Imp: 3.89%
4	AF4	#	2.92	99.14%	98.45%	Imp A: 3.28% Imp B:0.11% Imp C:0.09% Highest UNK Imp:0.20% Total Imp:3.82%

#: A clear colorless solution

LT: Light Transmission, Imp: Impurity, UNK: Unknown

of Bendamsutine Hydrochloride is correct. From the related substances analysis, it was observed that monohydroxy Bendamustine (impurity A) was observed in all the four formulations in a significant amount and other two known % impurities content are satisfactory but single maximum unknown impurity is found high.

CONCLUSION

Form the overall characterization of aqueous based formulations of Bendamustine Hydrochloride, it can be concluded that no physical description complication were observed with aqueous based formulations. Also the assay test parameter result was observed satisfactory.

But With respect to the results of related substances, the impurity A monohydroxy bendamustine was observed in the significant levels which is about 3.3% indicating the hydrolytic degradation nature of impurity A. though the Hydroxypropyl betacyclodextrin quantity was increased from AF1 to AF4 but the higher quantities are not able to reduce the % content of Impurity A. However, other two known impurities are well within the control. % content of unknown impurities is not satisfactory. From the above experiment, it can be concluded that bendamustine hydrochloride cant't be formulated in the aqueous based formulations as the impurity A which is a hydrolytic impurity is observed in the significant levels which is also not in line with the requirements of ICH Q3 B R(2). As an alternate, the scope of developing non aqueous Bendamustine Hydrochloride Injection shall be attempted.

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