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Research Article

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New analytical methods for the determination of Bendamustine hydrochloride: An anti-neoplastic drug

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ABSTRACT

Bendamustine hydrochloride is an active nitrogen mustard fragment and a residue of butanoic acid used for the treatment of chronic lymphocytic leukemia. Three simple, rapid and sensitive spectrophotometric methods are developed for the determination of Bendamustine hydrochloride in pharmaceutical dosage forms. The absorption maxima was found to be at 232.41 nm in phosphate buffer (pH 6.8) (Method A) and shows linearity over the concentration range of 0.1-50 µg ml^{-1} with regression equation 0.064x + 0.038 (r^2 = 0.998). For Method B boric buffer (pH 9.0) was chosen in which the absorption maxima was observed at 229.25 nm and linearity was followed over the concentration range 0.5-50 µg ml^{-1} with regression equation is found to be 0.062x + 0.022 (r^2 = 0.999). In Method C difference spectroscopy technique was used in which the maxima (343.32 nm) was chosen for the spectral analysis and linearity was followed over the concentration range 5-40 µg ml^{-1} with regression equation 0.006x + 0.001 (r^2 = 0.998).The proposed methods can be successfully applied for the determination of Bendamustine hydrochloride in pharmaceutical formulations and validated according to ICH guidelines.

Keywords: Bendamustine hydrochloride, Difference spectroscopy, Validation, ICH.

INTRODUCTION

Bendamustine hydrochloride (BDM) is indicated for the treatment of patients with chronic lymphocytic leukemia [1]. Bendamustine (4-*{*5-[bis-(2-chloroethyl) amino]-1-methyl-1*H*benzimidazol-2-yl*}* butanoic acid) is a bifunctional alkylating agent with an atypical structure that includes a benzimidazole ring, an active nitrogen mustard fragment and a residue of butanoic acid. Its empirical molecular formula is $C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$, and the molecular weight is 394.7. Besides biotransformation [2-5], bendamustine, similar to other nitrogen mustards, undergoes degradation by hydrolysis. Two hydrolysis products of bendamustine have been detected, namely monohydroxy and dihydroxy derivatives (4-*{*5-[(2-chloroethyl)-(2-hydroxyethyl) amino]-1-methyl-1*H*-benzimidazol-2-yl*}*butanoic acid and 4-*{*5-[bis-(2-hydroxyethyl)amino]-1-methyl-1*H*benzimidazol- 2-yl*}*butanoic acid) [6]. Because of the hydrolytic degradation in aqueous solutions, nitrogen mustards are often supplied for administration in a lyophilized form that requires reconstitution, usually in water. Bendamustine hydrochloride (Fig 1) contains a mechlorethamine group and a benzimidazole heterocyclic ring with a butyric acid substituent. Mechlorethamine and its derivatives form electrophilic alkyl groups. These groups form covalent bonds with electron-rich nucleophilic moieties, resulting in interstrand DNA crosslinks. The bifunctional covalent linkage can lead to cell death via several pathways [7]. Bendamustine is active against both quiescent and dividing cells.

Literature review revealed that there is only one HPLC method for the determination of stability of Bendamustine hydrochloride immobilized onto polyphosphoesters [8] and there is not even a single spectrophotometric method for the determination of Bendamustine hydrochloride in pharmaceutical dosage forms. In the present study three simple, rapid, precise, and accurate spectrophotometric methods have been developed for the determination of

Bendamustine hydrochloride in pharmaceutical dosage form (Injections) and validated as per the ICH guidelines [9-10].

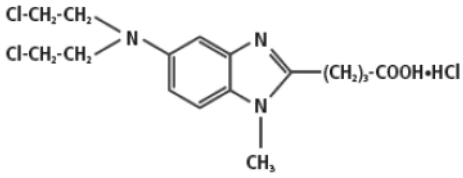

Fig 1: Chemical Structure of Bendamustine hydrochloride (BDM)

EXPERIMENTAL SECTION

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Denver, Germany).

Chemicals and reagents

Analytical grade boric acid (Qualigens), sodium hydroxide (Merck), Potassium di hydrogen phosphate (Merck) and disodium hydrogen phosphate (Merck) were purchased. Bendamustine hydrochloride (white to off-white lyophilized powder) was obtained as gift sample from White pharmaceuticals (India) was used as such without further purification.

Preparation of Phosphate buffer (pH 6.8)

28.8 grams of disodium hydrogen phosphate and 11.45 grams of potassium dihydrogen phosphate were dissolved in sufficient water in a 1000 ml volumetric flask.

Preparation of Boric buffer (pH 9.0)

6.2 grams of boric acid was dissolved in about 500 ml of water and then pH was adjusted to 9.0 with 1M sodium hydroxide and diluted with water in a 1000 ml volumetric flask.

Preparation of Stock and sample Solution

The standard solution of Bendamustine hydrochloride was prepared by dissolving accurately about 25 mg of the Bendamustine hydrochloride with Methanol in a 25 ml volumetric flask.

The stock solution was further diluted with phosphate buffer (pH 6.8) and boric buffer (pH 9.0) separately for method A and B respectively as per the requirement.

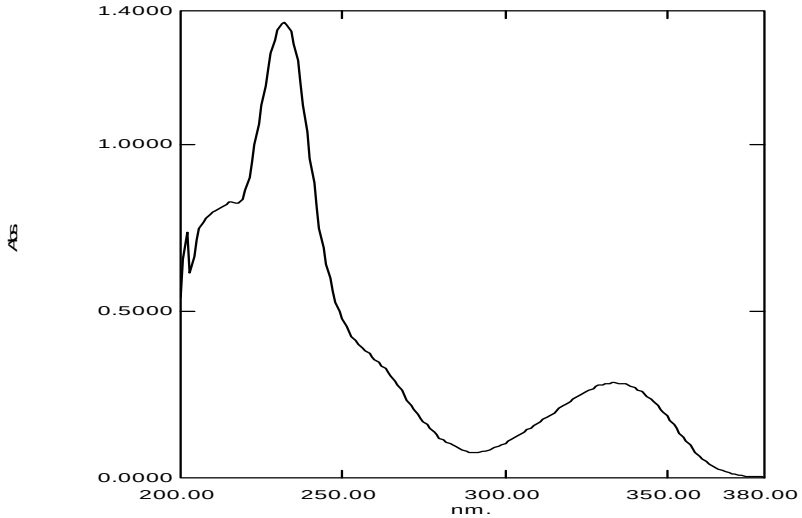

Fig 2: Absorption Spectrum of Bendamustine Hydrochloride (20 µg ml^{-1}) in phosphate buffer (pH 6.8) (Method A)

Procedure:

Method A

The drug solution was scanned (200-400 nm) against reagent blank i.e. phosphate buffer (pH 6.8) and the absorption spectrum (Fig 2) was recorded. The absorption maximum (λ_{max}) was observed at 232.41 nm and the absorbance of all the sample solutions (0.1-50 µg ml^{-1}) was recorded at that λ_{max}. A graph was plotted by taking the concentration of the solutions on the x-axis and the corresponding absorbance values on the y-axis.

Method B

The drug solution was scanned (200-400 nm) against reagent blank i.e. boric buffer (pH 9.0) and the absorption spectrum was recorded (Fig 3) The absorption maximum (λ_{max}) was observed at 229.25 nm and the absorbance of all the sample solutions (0.5-50 µg ml^{-1}) was recorded at that λ_{max}. A graph was plotted by taking the concentration of the solutions on the x-axis and the corresponding absorbance values on the y-axis.

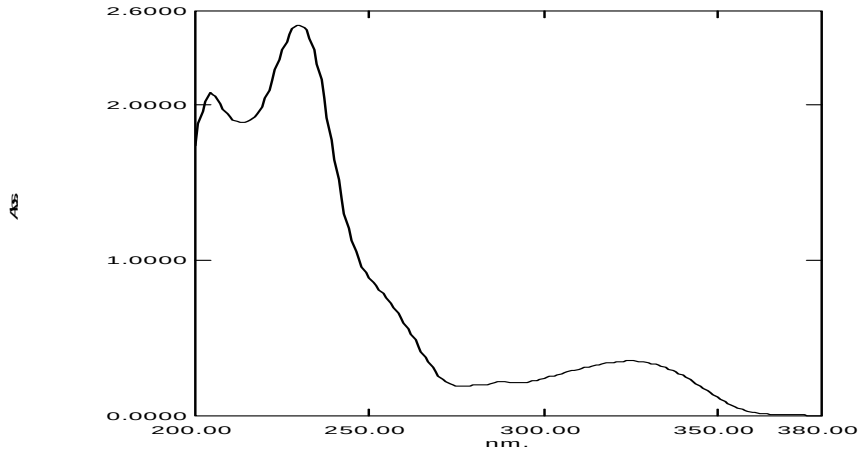

Fig 3: Absorption Spectrum of Bendamustine Hydrochloride (40 µg ml^{-1}) in boric buffer (pH 9.0) (Method B)

Method C

A series of Bendamustine Hydrochloride drug solutions (5.0-40 µg ml^{-1}) were prepared in boric buffer (pH 9.0) and scanned (200-400 nm) against the same solutions prepared in phosphate buffer (pH 6.8) as blank and the difference absorption spectra was recorded (Fig 4) in which the maxima at 343.32 nm was chosen for the analytical calculations. A graph was plotted by taking the concentration of the sample solutions on the x-axis and the corresponding maxima values on y-axis.

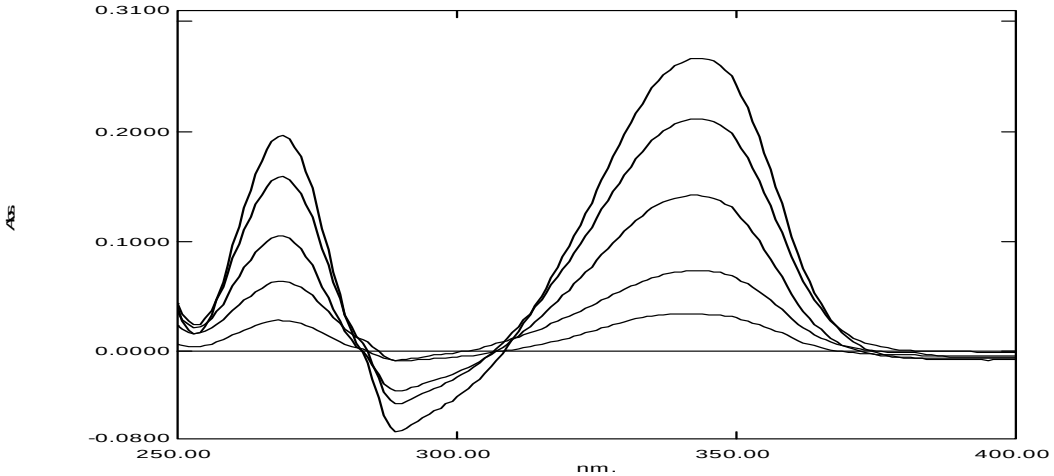

Fig 4: **Overlay Difference Absorption Spectrum of Bendamustine Hydrochloride (Method C)**

Assay procedure for the commercial formulations (Injections)

Bendamustine Hydrochloride is available as injection in a single dose vial containing 100 mg of bendamustine HCl

as white to off-white lyophilized powder. Bendamustine Hydrochloride is available in the local market with brand names BENDIT® (White Pharmaceuticals, India) and PURPLZ® (India) containing 100 mg of Bendamustine HCl in a single dose vial. Injection volume equivalent to 25 mg of Bendamustine HCl was drawn from the vial and extracted with phosphate buffer (Method A) and boric buffer (Method B) separately and appropriate dilutions were made as per the requirement.

A series of solutions were prepared for Method A, B and C, scanned and the corresponding values were recorded as per the procedure given and the calculations were made from the calibration curves drawn.

Precision and Accuracy

The precision study was done as per the ICH guidelines by recording the absorbance of three replicates at three different levels for Method A, B and C (10, 20 and 30 μg ml^{-1}) and the % RSD was calculated.

Accuracy was evaluated as per the ICH guidelines by the percent recovery studies by the addition of 80%, 100%, and 120% of pure sample solution to the pre-analysed formulation solution (10 μg ml^{-1}) and the % RSD was calculated.

RESULTS AND DISCUSSION

Beer Lambert's law was obeyed in the concentration range of 0.1–50.0, 0.5–50.0 and 5.0–40.0 μg ml^{-1} for method A, B and C respectively. The linear regression equations were found to be y = 0.064 x + 0.038 (r^2 = 0.998), y = 0.062 x + 0.022 (r^2 = 0.999) and y = 0.006 x + 0.001 (r^2 = 0.998) for method A, B and C (Fig 5-7) respectively.

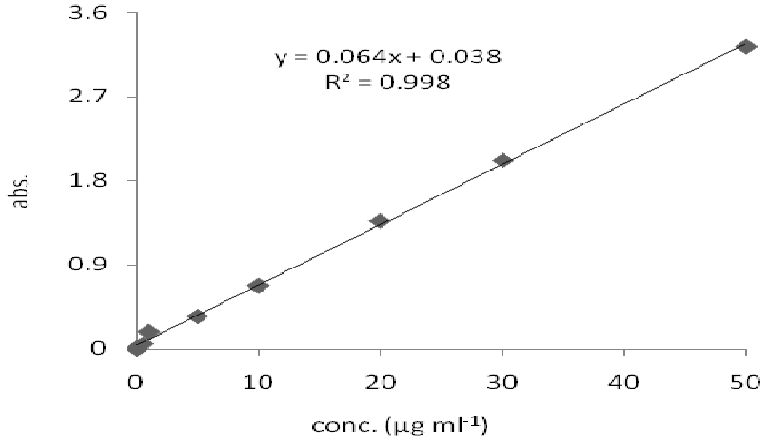

Fig 5: Linearity in Phosphate Buffer (pH 6.8)
(Method A)

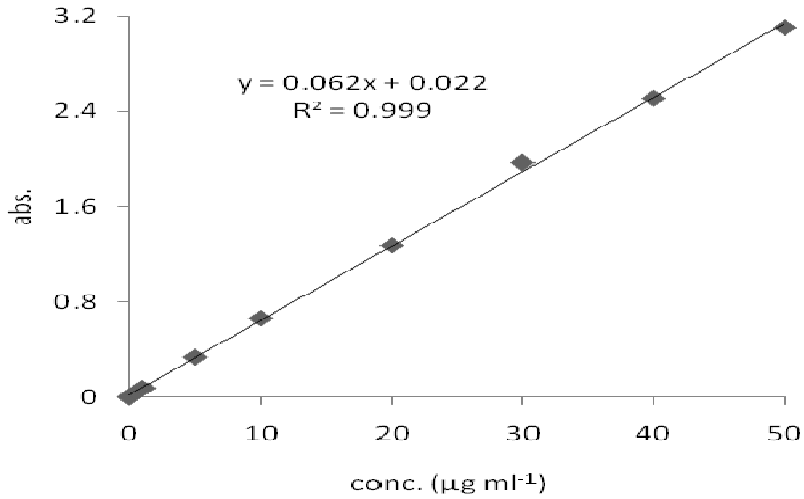

Fig 6: Linearity in Boric Buffer (pH 9.0)
(Method B)

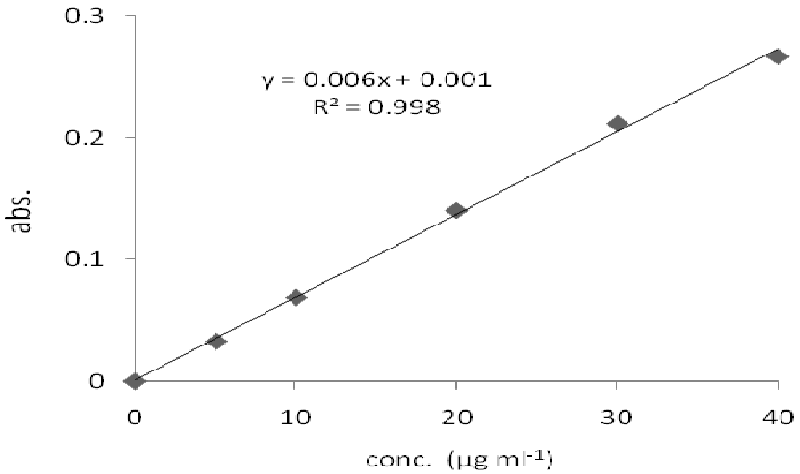

Fig 7: Linearity in Difference Spectroscopy (Method C)

The RSD values in precision studies were found to be 0.43-0.54, 0.33-0.42 and 0.53-0.74 for method A, B and C respectively which are less than 2.0 % indicating that the method is more precise. The % RSD values in accuracy studies were also found to be less than 2.0 % (0.81-0.87) for method A, B and C indicating that the method is more accurate. The optical characteristics are shown in Table 1.

Table 1: Optical characteristics of Bendamustine Hydrochloride

Parameters	Method A	Method B	Method C
λ_{max} (nm)	232.41	229.25	343.32 (Maxima)
Beer-Lambert's range (µg ml^{-1})	0.1-50	0.5-50	5-40
Sandell's sensitivity (µg cm^{-2}/0.001 absorbance unit)	0.01467	0.01595	-
Molar extinction coefficient (Litre $mole^{-1}$ cm^{-1})	2.691 x 10^4	2.475 x 10^4	-
Slope	0.064	0.062	0.006
Intercept	0.038	0.022	0.001
Correlation coefficient	0.998	0.999	0.998
Precision (RSD, %)	0.43-0.54	0.33-0.42	0.53-0.74
Accuracy (% recovery)	99.91-99.96	99.71-99.83	99.81-99.92

The percentage of purity in the marketed formulations was found to be 99.73-99.91 with RSD less than 2.0% (0.86-1.03). The percentage recovery values are given in Table 2.

Table 2: Assay of commercial formulations

S. No.	Labelled Amount (mg)	*Amount obtained (mg)			% Recovery			% RSD*		
		Method A			Method B			Method C		
		A	B	C	A	B	C	A	B	C
Brand I	100	99.91	99.89	99.83	99.91	99.89	99.83	0.86	0.95	0.96
Brand II	100	99.73	99.86	99.78	99.73	99.86	99.78	0.98	1.02	1.03

**Each value is average of three determinations*

CONCLUSION

The present proposed methods can be successfully employed for the determination of Bendamustine Hydrochloride in pharmaceutical formulations and the methods are validated as per the ICH guide lines.

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