

Derivative Spectrophotometric Methods for the Determination of Bendamustine Hydrochloride

*M. Mathrusri Annapurna, S. Pavani, S. Anusha, and Harika Mahanti

Department of Pharmaceutical Analysis and Quality Assurance, GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, India

ARTICLE INFO

Article history:

Received on: 15/10/2012

Revised on: 02/11/2012

Accepted on: 11/11/2012

Available online: 29/11/2012

Key words:

Bendamustine hydrochloride,

Derivative spectroscopy,

Difference spectroscopy,

Validation.

ABSTRACT

Bendamustine hydrochloride is used to treat chronic lymphocytic leukemia. It kills the existing cancer cells and limits the growth of new cancer cells. Three simple, rapid and sensitive spectrophotometric methods were developed for the determination of Bendamustine hydrochloride in phosphate buffer (pH 8.0) (Method A) and boric buffer (pH 9.0) (Method B). Method C is a difference spectroscopy technique in which the amplitude was chosen for the analytical calculations. Bendamustine hydrochloride obeys Beer-Lambert's law over the concentration range 1-40 $\mu\text{g/ml}$, 0.1-40 $\mu\text{g/ml}$ and 5-40 $\mu\text{g/ml}$ with regression equations $y = 0.003x + 0.001$ ($r^2 = 0.998$), $y = 0.0027x + 0.0005$ ($r^2 = 0.999$), and $y = 0.0034x + 0.006$ ($r^2 = 0.994$) for Method A, B and C respectively. The methods were validated as per ICH guidelines and can be applied for the determination of Bendamustine hydrochloride in pharmaceutical formulations.

INTRODUCTION

Bendamustine (4-{5-[bis-(2-chloroethyl) amino]-1-methyl-1H 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 (Budavari 2006).

It has molecular formula $\text{C}_{16}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_2 \cdot \text{HCl}$ with molecular weight 394.7. Bendamustine hydrochloride is indicated for the treatment of patients with chronic lymphocytic leukemia (Friedberg *et al.*, 2008). Besides biotransformation bendamustine, similar to other nitrogen mustards, undergoes degradation by hydrolysis (Teichert *et al.*, 2005, Teichert *et al.*, 2007, Rasschaert *et al.*, 2007). Two hydrolysis products of bendamustine have been detected, namely monohydroxy and dihydroxy derivatives (4-{5-[(2-chloroethyl) - (2-hydroxyethyl) amino] - 1 - methyl - 1H benzimidazol- 2-yl} butanoic acid and 4 {5-[bis-(2-hydroxyethyl) amino]-1-methyl-1H benzimidazol- 2-yl} butanoic acid) (Preiss *et al.*, 1985). 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 inter-strand DNA cross links. The bi-functional covalent linkage can lead to cell death via several pathways (Lissitchkov *et al.*, 2006).

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 (Ivanka *et al.*, 2008) and there is only one stability indicating liquid chromatographic method (Mathrusri Annapurna *et al.*, 2012) and one spectrophotometric method (Mathrusri Annapurna *et al.*, 2012) for the determination of Bendamustine hydrochloride in pharmaceutical dosage forms. In the present study two derivative spectrophotometric methods and one difference spectrophotometric methods have been developed for the determination of Bendamustine hydrochloride in pharmaceutical dosage form (Injections) and validated as per the ICH guidelines (ICH 1996, ICH 2005).

* Corresponding Author

E-mail: mathrusri2000@yahoo.com

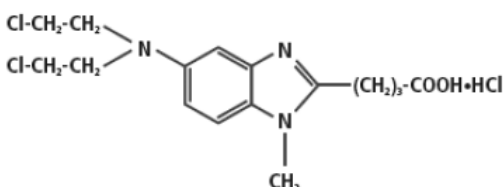


Fig. 1: Chemical Structure of Bendamustine Hydrochloride.

MATERIALS AND METHODS

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

Bendamustine hydrochloride standard (purity 99.0%) was obtained from White Pharmaceuticals (India). It is available as injection in a single dose vial containing 100 mg of BMH as white to off-white lyophilized powder. It is available in the local market with brand names BENDIT® and PURPLZ® (Natco Pharma Limited, India) containing 100 mg of Bendamustine HCl in a single dose vial. Analytical grade potassium di hydrogen phosphate (Merck), sodium hydroxide (Qualigens) and analytical grade boric acid (Qualigens) were purchased.

Preparation of phosphate buffer (pH 8.0)

50.0 ml of 0.2 M Potassium di hydrogen phosphate and 46.1 ml of 0.2M sodium hydroxide were mixed in a 200 ml volumetric flask and made up to volume with distilled water.

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 25 mg of Bendamustine hydrochloride in methanol in a 25 ml volumetric flask. The stock solution was further diluted with phosphate buffer (pH 8.0) and boric buffer (pH 9.0) separately for method A and B respectively as per the requirement.

Method A

The drug solution was scanned (200-400 nm) against reagent blank i.e. phosphate buffer (pH 8.0) and the absorption spectrum was recorded. The absorption spectrum was then converted in to first derivative spectrum by the inbuilt software. A series of solutions (1-40 $\mu\text{g/ml}$) were prepared in phosphate buffer (pH 8.0) and scanned. The resultant absorption spectra were converted in to first order derivative spectra by the inbuilt software

in the instrument and the derivative absorbance ($dA/d\lambda$) was recorded at 224.78-240.24 nm (amplitude).

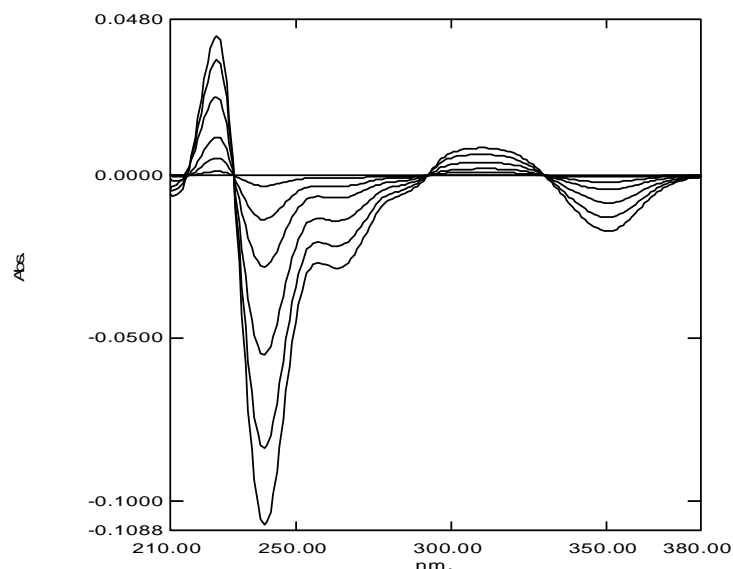


Fig. 2: Overlay first derivative spectrum of Bendamustine hydrochloride in phosphate buffer (pH 8.0) (Method A).

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. The absorption spectrum was then converted in to first derivative spectrum by the inbuilt software. A series of solutions (0.1-40 $\mu\text{g/ml}$) were prepared in boric buffer (pH 9.0) and scanned. The resultant absorption spectra were converted in to first order derivative spectra by the inbuilt software of the instrument and the derivative absorbance ($dA/d\lambda$) was recorded at 239.9 nm (minima).

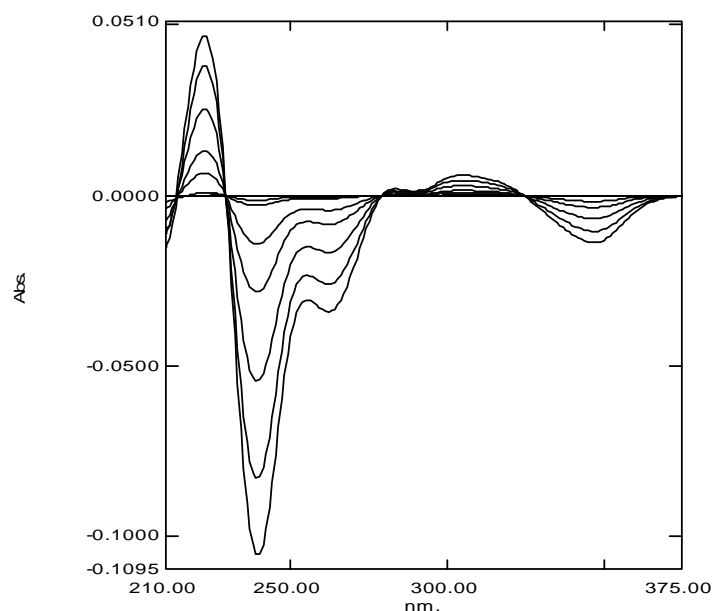


Fig. 3: Overlay first derivative spectrum of Bendamustine hydrochloride in boric buffer (pH 9.0) (Method B).

Method C

A series of Bendamustine hydrochloride drug solutions (5-40 $\mu\text{g/ml}$) were prepared in phosphate buffer (pH 8.0) and scanned against the drug solutions prepared in boric buffer (pH 9.0) (blank) and the resultant difference absorption spectra was recorded (Fig 4). The difference absorption spectrum shows maxima at 268.77 nm and the absorbance at maxima was chosen for all the analytical calculations. A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding difference in absorbance on the y-axis.

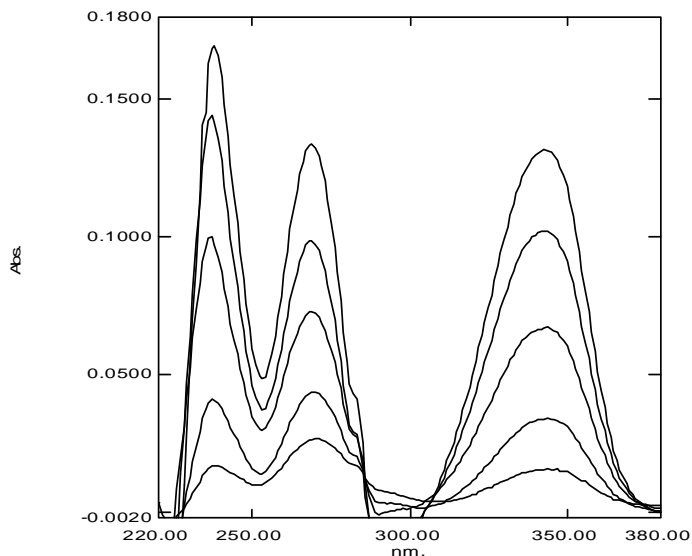


Fig. 4: Overlay Difference Absorption Spectrum of Bendamustine hydrochloride (Method C).

Assay procedure for the commercial formulations (Injections)

Injection volume equivalent to 25 mg of Bendamustine hydrochloride 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 and scanned. The data obtained was substituted in the regression equations and the percentage of purity was determined.

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 $\mu\text{g/ml}$) 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 $\mu\text{g/ml}$) and the % RSD was calculated.

RESULTS AND DISCUSSION

Beer Lambert's law was obeyed in the concentration range of 1-40, 0.1-40 and 5-40 $\mu\text{g/ml}$ for method A, B and C respectively. The linear regression equations were found to be $y = 0.003x + 0.001$ ($r^2 = 0.998$), $y = 0.0027x + 0.0005$ ($r^2 = 0.999$) and

$y = 0.0034x + 0.006$ ($r^2 = 0.994$) for method A, B and C respectively (Fig 5-7). The RSD values in precision studies were found to be 0.63, 0.73 and 0.62 (< 2.0 %) for method A, B and C respectively indicating that the methods are precise. The % RSD values in accuracy studies were also found to be 0.36, 0.42 and 0.89 (< 2.0 %) for method A, B and C respectively indicating that the method is accurate. The percentage recovery values in the accuracy studies were found to be 98.84-98.92, 98.72-98.83 and 98.62-98.76 for method A, B and C respectively. The optical characteristics were shown in Table 1. The percentage recovery was found to be 99.78-99.86, 99.62-99.66 and 99.58-99.74 for method A, B and C respectively and the assay results of the marketed formulations were given in Table 2. The proposed methods are simple and can be applied for the determination of Bendamustine hydrochloride in pharmaceutical formulations.

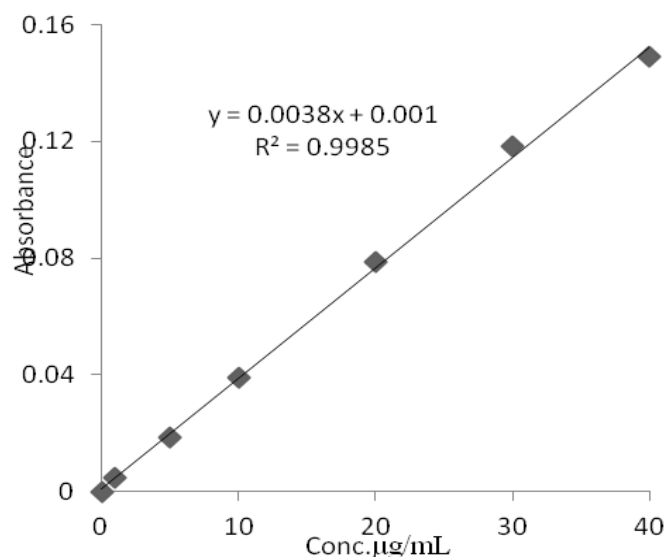


Fig. 5: Calibration curve of Bendamustine hydrochloride in phosphate buffer (pH 8.0)

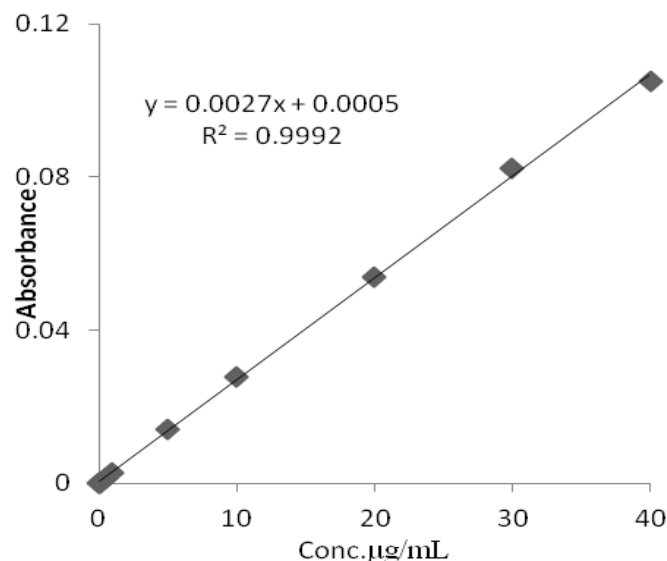


Fig. 6: Calibration curve of Bendamustine hydrochloride in boric buffer (pH 9.0).

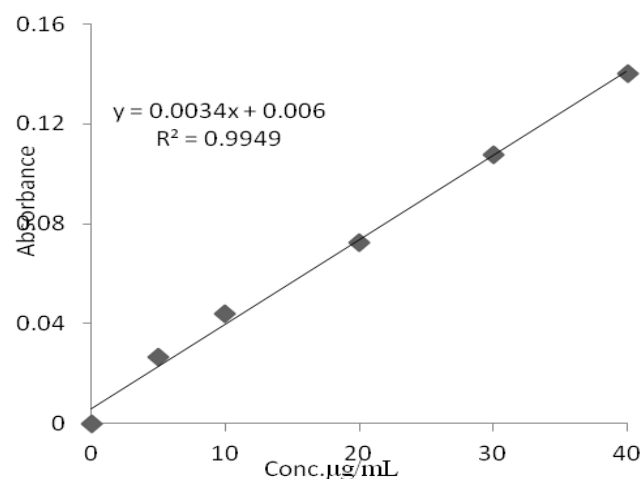
Table. 1: Optical characteristics of Bendamustine hydrochloride

Parameters	Method A	Method B	Method C
λ (nm) Amplitude/Minima/Maxima	224.78-240.24	239.9	268.77
Beer-Lambert's range ($\mu\text{g/mL}$)	1-40	0.1-40	5-40
Sandell's sensitivity ($\mu\text{g cm}^{-2}/0.001$ absorbance unit)	25.445×10^{-1}	35.714×10^{-1}	26.666×10^{-1}
Molar extinction coefficient ($\text{Litre mole}^{-1} \text{cm}^{-1}$)	15.514×10^{-2}	11.0532×10^{-2}	14.803×10^{-2}
Slope	0.003	0.0027	0.0034
Intercept	0.001	0.0005	0.006
Correlation coefficient	0.998	0.999	0.994
Precision (% RSD)	0.63	0.73	0.62
Accuracy (% recovery) (% RSD)	98.84-98.92 (0.36)	98.72-98.83 (0.42)	98.62-98.76(0.89)

Table. 2: Assay of commercial formulations

Sample No	Formulation	Labeled Claim (mg/5ml)	*Amount Found (mg/5ml)			% Recovery		
			A	B	C	A	B	C
1.	Brand I	5	4.989	4.983	4.979	99.78	99.66	99.58
2.	Brand II	5	4.973	4.981	4.987	99.86	99.62	99.74

*Mean of three determinations

**Fig. 7:** Calibration curve of Bendamustine hydrochloride (Method C).

CONCLUSION

The present proposed methods are simple, precise and accurate and therefore can be successfully applied for the determination of Bendamustine hydrochloride in pharmaceutical formulations.

ACKNOWLEDGEMENT

The authors are grateful to M/s GITAM University for providing necessary research facilities to carry out the research work and to White Pharmaceuticals, India for providing the gift sample of the drug.

REFERENCES

- Budavari S. The Merck Index. An Encyclopedia of Chemicals, Drugs and Biologicals, Merck & Co., Inc., 14th ed. Whitehouse Station, NJ (2006).
- Friedberg JW., Cohen P., Chen L., Robinson KS., Forero-Torres A., La Casce AS., Fayad LE., Bessudo A., Camacho ES., Williams ME., Van der Jagt RH., Oliver JW., Cheson BD. Bendamustine in Patients with Rituximab-Refractory Indolent and Transformed Non-Hodgkin's Lymphoma: Results from a Phase II Multicenter, Single-Agent Study. *J. Clin. Oncol.* 2008; 26: 204-210.

Ivanka Pencheva., Anita Bogomilova., Neli Koseva., Danka Obreshkova., Kolio Troev., HPLC Study on the Stability of Bendamustine hydrochloride Immobilized onto Polyphosphoesters. *J. Pharm. Biomed. Analysis.* 2008; 48: 1143-1150.

Lissitchkov T., Arnaudov G., Peytchev D., Merkle Kh., Phase-I/II Study to Evaluate Dose Limiting Toxicity, Maximum Tolerated Dose, and Tolerability of Bendamustine HCl in Pre-Treated Patients with B-Chronic Lymphocytic Leukaemia (Binet Stages B and C) Requiring Therapy. *J. Cancer Research and Clinical Oncology.* 2006; 132: 99-104.

Mathrusri Annapurna M., Pavani S., Anusha S., Harika Mahanti., Venkatesh B. New analytical methods for the determination of Bendamustine hydrochloride - An anti-neoplastic drug. *J. Chem. Pharm. Res.* 2012; 4: 1696-1701.

Mathrusri Annapurna M., Venkatesh B., Anusha S., Neelima B. Stability-Indicating Liquid Chromatographic Method for the Determination of Bendamustine Hydrochloride in Parenterals. *Res. J. Chem. Sci.* 2012; 2: 72-78.

Preiss R., Sohr R., Matthias M., Brockmann B., Huller H. The Pharmacokinetics of Bendamustine (Cytostasane) in Humans. *Pharmazie.* 1985; 40: 782-784.

Rasschaert M., Schrijvers D., Van den Brande J., Dyck J., Bosmans J., Merkle J Vermorken K. A Phase I Study of Bendamustine hydrochloride Administered Once Every Three Weeks in Patients with Solid Tumors. *Brit. J. Cancer.* 2007; 96: 1692-1698.

Teichert J., Baumann F., Chao Q., Franklin C., Bailey B., Hennig L., Caca K., Schoppmeyer K., Patzak U., Preiss R. Characterization of two phase I metabolites of Bendamustine in human liver microsomes and in cancer patients treated with Bendamustine hydrochloride. *Cancer Chemother. Pharmacol.* 2007; 59: 759-770.

Teichert J., Sohr R., Baumann F., Hennig L., Merkle K., Caca K., Preiss R. Synthesis and Characterization of Some New Phase II Metabolites of the Alkylator Bendamustine and their Identification in Human Bile, Urine, and Plasma from Patients with Cholangiocarcinoma. *Drug Metab. Dispos.* 2005; 33: 984-992.

Validation of Analytical Procedures: Methodology (Q2B), ICH Harmonized Tripartite Guidelines, Geneva (1996).

International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use: Validation of Analytical procedures, Text and methodology, Q2 (R1) (2005).

How to cite this article:

M. Mathrusri Annapurna, S. Pavani, S. Anusha, and Harika Mahanti, Derivative Spectrophotometric Methods for the Determination of Bendamustine Hydrochloride. *J App Pharm Sci.* 2012; 2 (11): 139-142