Derivative Spectrophotometric Methods for the Determination of Bendamustine Hydrochloride

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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 C_{10}H_{13}ClN_{3}O_{2}•HC1 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).

**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 µg/ml, 0.1-40 µg/ml and 5-40 µg/ml with regression equations y = 0.003x + 0.001 (r² = 0.998), y = 0.0027x + 0.0005 (r² = 0.999), and y = 0.0034x + 0.006 (r² = 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.

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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 (0.1-40 µg/ml) were prepared in phosphate 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/dλ) 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 µg/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/dλ) 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 μg/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 μg/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 μg/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 μg/ml for method A, B and C respectively. The linear regression equations were found to be $y = 0.0034x + 0.006$ ($r^2 = 0.994$) 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ (nm) Amplitude/Minima/Maxima</td>
<td>224.78-240.24</td>
<td>239.9</td>
<td>268.77</td>
</tr>
<tr>
<td>Beer-Lambert’s range (μg/mL)</td>
<td>1-40</td>
<td>0.1-40</td>
<td>5-40</td>
</tr>
<tr>
<td>Sandell’s sensitivity (μg cm⁻²/0.001 absorbance unit)</td>
<td>25.445×10⁻¹</td>
<td>35.714×10⁻¹</td>
<td>26.66×10⁻¹</td>
</tr>
<tr>
<td>Molar extinction coefficient (Litre mole⁻¹ cm⁻¹)</td>
<td>15.514×10⁻²</td>
<td>11.053×10⁻¹</td>
<td>14.803×10⁻²</td>
</tr>
<tr>
<td>Slope</td>
<td>0.003</td>
<td>0.0027</td>
<td>0.0034</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.001</td>
<td>0.0005</td>
<td>0.006</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
<td>0.999</td>
<td>0.994</td>
</tr>
<tr>
<td>Precision (% RSD)</td>
<td>0.63</td>
<td>0.73</td>
<td>0.62</td>
</tr>
<tr>
<td>Accuracy (% recovery) (% RSD)</td>
<td>98.84-98.92 (0.36)</td>
<td>98.72-98.83 (0.42)</td>
<td>98.62-98.76 (0.89)</td>
</tr>
</tbody>
</table>

**Table 2:** Assay of commercial formulations

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Formulation</th>
<th>Labeled Claim (mg/5ml)</th>
<th>*Amount Found (mg/5ml)</th>
<th>% Recovery</th>
</tr>
</thead>
</table>

*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.

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**REFERENCES**


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