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Visible Spectrophotometric Determination of Bendamustine By Complex Formation

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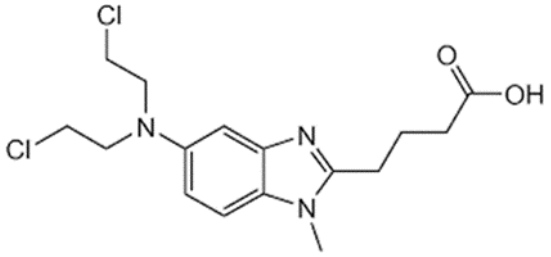
Abstract : *A validate rapid, economical and sensitive visible spectrophotometric method has been developed for quantitative determination of bendamustine hydrochloride in bulk drug and formulation sampleswith no interference from the excipients or solvents of injectable solution. This method is validated for irinotecan with chromogenic reagent 2,2'-bipyridyl.The calibration curve is linear over a concentration range from 2-12µg/ml .The relative standard deviation (R.S.D) is less than 1% and average recovery is above 100.00 %.The proposed method is fast, sensitive, precise, accurate and efficient and can be used for analysis in quality control laboratories.*

Keywords - *Ultraviolet-Visible Spectrophotometry, bendamustine hydrochloride, 2,2'-bipyridyl*

I. INTRODUCTION

Bendamustine hydrochloride is chemically 1H-benzimidazole-2-butanoic acid, 5-[bis (2-chloroethyl) amino]-1 methyl mono hydrochloride. Its molecular formula is $C_{16}H_{21}Cl_2N_3O_2$•HCl and molecular weight 394.72 g/mol.The drug is official present in Martindale, The Extra Pharmacopoeia. It is commercially available as injection formulations as Treanda[1].It is primarily used in the treatment of hormone receptor positive metastatic breast cancer in post-menopausal women with disease progression following anti-estrogen therapy[2]. Estrogen is a female hormone that produces growth stimulatory effects on a significant portion of breast cancer cells. By binding to estrogen receptors, bendamustine hydrochloride inhibits the growth stimulatory effects that estrogen would normally produce. In addition, bendamustine hydrochloride causes the estrogen receptors to degrade, leaving fewer receptors for estrogen to bind [3]. Bendamustine alone or in combination with other drugs is reported to be estimated by HPLC[4-6] and LC-MS[7,8].No visible spectrophotometric method for determination of bendamustine complexing with 2,2'-bipyridyl in bulk drug samples and formulations was reported[9,10].The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods for the estimation of bendamutsine hydrochloride in bulk samples and injection dosage forms.



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II. MATERIALS AND METHOD

Instrument

ELICO-SL-164 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements.Digisun model DI-707 pH meter was used for all the pH measurements.

Materials

Bendamutsine hydrochloride is obtained as gift sample from Mylan Laboratories, Hyderabad. The reagents 1:10 phenanthroline, ferric chloride, o-phosporic acid, methanol, H_3PO_4, NaH_2PO_4 and Na_2HPO_4 used were of analytical grade and were used as they are purchased without any further purification.

Preparation of standard drug solution

A standard drug solution of bendamustine hydrochloride was prepared by dissolving 100mg of drug in 100ml of methanol in a standard volumetric flask to obtain a stock solution of 1mg/ml. This solution was further diluted with methanol to get working standard solutions of 100µg/ml.

Preparation of sample solution

Volume of injection equivalent to 20mg of drug was diluted to 20ml with methanol and analyzed by taking an aliquot and treated as per the procedure for standard.

Methodology for bulk drug sample

Aliquots of standard drug solution (0.5-3.0 ml) were transferred into series of 10ml graduated test tubes, 2ml of ferric chloride and 2ml of 2, 2'-bipyridyl were added to each test tube. The test tubes heated to 75^0C,stirredfor 15 min and then cooled to room temperature.1ml of o-phosphoric acid (0.2 M) was added to each test tube and the total volume was brought to 10ml with methanol. The absorbance of the blood red colored species was measured at 520 nm against reagent blank.

Methodology for formulation sample

About 1ml of sample solution was transferred into 10ml graduated test tube, 2ml of ferric chloride and 2ml of 2, 2'-bipyridyl were added to each test tube. The test tube was then heated to75^0C, stirred for 15 min and cooled to room temperature.1ml of o-phosphoric acid (0.2 M) was added to the test tube and total volume made to 10ml with methanol. The absorbance of the blood red colored species was measured at 520 nm against reagent blank.

Accuracy

Commercially available injection samples of bendamustine hydrochloride is analyzed by the proposed method and as additional check recovery studies were carried out. Percentage recovery was satisfactory. Assay results of the proposed method were compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The results showed that $P > 0.05$ and the means of the proposed method are not significantly different from that of reference method. The assay and accuracy results were presented in Table 1.

Table 1: Result of recovery studies (n=6)

Sample[a]	**Labelled Amount (mg)**	**Amount obtained (mg)[b]**		**Percentage Recovery[b,c]**
		Proposed method	**Reference method**	
T_1	45mg/0.5 ml	82±0.54	100.70±0.98	101.20±1.84
T_2	45mg/0.5 ml	99±0.45	100.70±0.98	99.866±0.84

a - T1 and T2 are the injections from different batches (Faslodex, USA).
b – Mean ± SD of 6 determinations.
c – 10 mg of pure drug was added and recovered

For both the samples T1 and T2 One-way ANOVA with post-test followed by Dunnett multiple comparison test was performed. The results showed that $P > 0.05$ and the means of the proposed methods are not significantly different from that of reference method.

Linearity

By using the method of least squares regression analysis was performed to evaluate the slope (m), intercept (b) and correlation coefficient (r) was computed from various concentrations .The graph showed negligible intercept as described by the regression equation $y = mx + b$ where y is the absorbance and x is the

concentration in µg/ml. Calibration curve for bendamustine hydrochloride is shown in Figures 1. The spectral analysis showed the $\lambda_{max}$of bendamustine hydrochloride is 520nm. The calibration curve was obtained for a series of Beer's concentration range of 2-12µg/ml.

Table 2: Values of Optical characteristics and precision

A. Parameter	*1) Values*
λ_{max} (nm)	520
Beer's law range (µg/ml)	2-12
Molar extinction coefficient(L.mole^{-1} cm^{-1})	12.44 x 10^4
Sandell's sensitivity (µg/cm^2/0.001)	0.0132
Regression equation (y = mx + b) * Slope (m) Intercept (b)	 0.0752 -0.0013
Correlation coefficient (r)	0.9999
Precision (%Relative Standard Deviation)	0.09

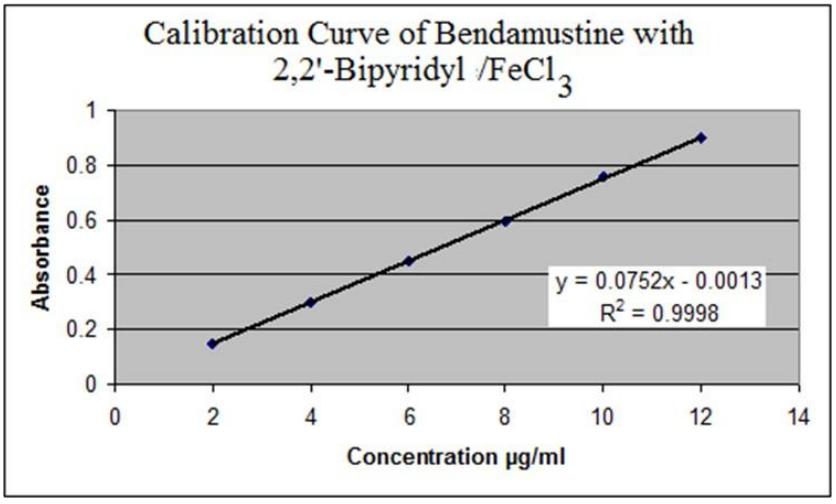

Figure 1: Calibration Curve of Bendamustine hydrochloride

Precision

The reproducibility of this method was evaluated by analysing the bendamustine hydrochloride sample of concentration 12µg/ml in six replicates. The relative standard deviation (RSD) was proved satisfactory. Intraday precession studies were carried out by preparing drug solution of same concentration and analysing it at three different times of same day. The same procedure was followed for 3 different days to determine interday precession.

Table 3:Inter-day precision studies

Concentration	Absorbance	Stastical analysis	
12	0.902		
12	0.902	Mean	0.902
12	0.903	SD	0.0008
12	0.902	%RSD	0.092%
12	0.904		
12	0.902		

Table 4:Intra-day precision studies

Concentration	Day 1	Day 2	Day 3	Average RSD
12	0.903	0.902	0.904	
12	0.904	0.902	0.902	
12	0.902	0.903	0.903	
12	0.903	0.904	0.902	0.09%
12	0.904	0.902	0.903	
12	0.903	0.902	0.905	
% RSD	0.08%	0.09%	0.12%	

III. RESULTS AND DISCUSSION

Optimization of parameters

The optimum conditions were established by changing one parameter while fixing the other parameters and noting the effect on absorbance of chromogen. In the present work a method have been developed for the estimation of bendamustine hydrochloride from injection formulation, based on formation of colored complexes with 2,2'-bipyridyl -$FeCl_3$.The conditions required for the formation of colored complex were optimized.

About 1ml of sample solution was transferred into 10ml graduated test tube, 2ml of ferric chloride(0.3% w/v) and 2ml of 2, 2'-bipyridyl were added to the test tube. Addition of less than 2ml of 2, 2'-bipyridyl results in low absorbance particularly with high concentrations of Beer's law limits,addition of more than 2ml results in high blank value. The test tube was then heated to 72^0C on water bath, maintained for 15 min and then cooled to room temperature. 1ml of o-phosphoric acid (0.2 M) added to the test tube , the total volume made to 10ml with methanol.The absorbance of the blood red colored species was measured at 520 nm against reagent blank.

Chemistry of formation of colored species

Bendamustine has a hydroxyl group in the molecular structure making it possible to form iron complexes of the drug with the reagent 2,2'-bipyridyl in ferric chloride solution to form blood red colored chromogen.

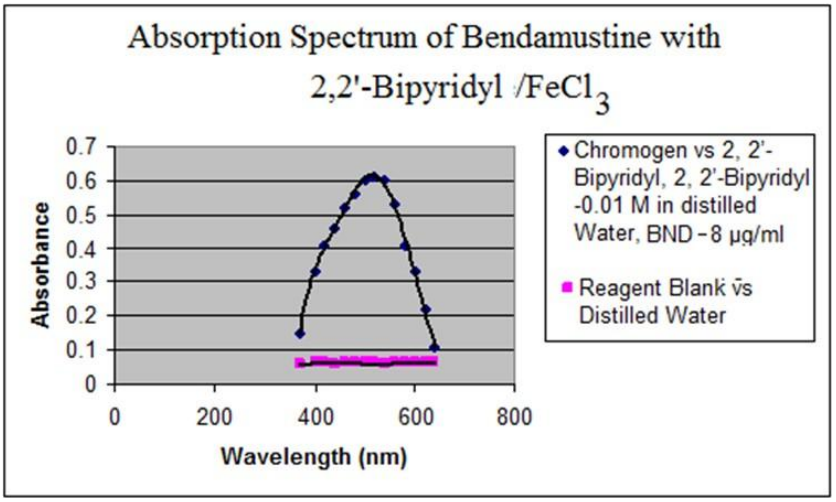

Figure 2: Absorption spectrum of Bendamustine hydrochloride

Absorption Maxima

Absorption spectra of bendamustine hydrochloride by proposed was shown in figures 2.The λ_{max} isat 520nm.

IV. CONCLUSION

The proposed visible spectrophotometric method enable quantitative determination of bendamustinehydrochloride in bulk drug samples and injections. Efficient visible spectrophotometric detection at 520nm absorption maxima enabled determination with no interference from the excipients or solvents of injectable solution. The calibration curve was linear over a concentration range from 2-12µg/ml.The relative standard deviation (RSD) was less than 1% and average recovery was above 100.00 %. Assay results of the proposed method were compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The mean of the proposed method is not significantly different from that of reference method ($p>0.05$). The proposed method is fast, sensitive, precise, accurate, and efficient and can be used in for analysis in quality control laboratories.

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