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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF BENDAMUSTINE IN RAW MATERIAL AND FINISHED PRODUCT

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ABSTRACT

Bendamustine Hydrochloride Injection is indicated for the treatment of patients with chronic lymphocytic leukemia. Efficacy relative to first line therapies other than chlorambucil has not been established. Bendamustine Hydrochloride Injection is indicated for the treatment of patients with indolent B-cell non-Hodgkin lymphoma that has progressed during or within six months of treatment with rituximab or a rituximab-containing regimen. A reversed-phase HPLC method was developed and validated for the determination of Bendamustine in raw material and to determine impurities and degradants that may developed in the tested samples. The separation was achieved on Thermo Hypersil C18 column (4.6 x 250 mm, 5µm) using mobile phase consisting of 70% Potassium Phosphate Dibasic Buffer (pH 7) and 30% acetonitrile (100% ACN). The flow rate was 1.0 mL/min, injection volume 10µL, and detection was accomplished at 232 nm. The retention time for Bendamustine was 7 minutes. The developed method was validated and met all the acceptance criteria for validation parameters - system suitability, specificity, solution stability, robustness, linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). The LOD was determined to be 0.1 ppm and LOQ was found to be 0.5 ppm.

KEYWORDS: Bendamustine, Degradation, RP-HPLC, Validation and Finished product.

INTRODUCTION

Bendamustine is an alkylating compound, containing a nitrogen mustard moiety, a benzimidazole ring and an alkane carboxylic corrosive side chain. While it was at that point blended in 1963 by Ozegowski also, Krebs,[1-3] and being used for quite a long time in Germany against various malignancies, it was just in 2008 that bendamustine was affirmed by the United States Food and Drug Administration (US FDA) for the treatment of endless lymphocytic leukemia (CLL) also, later for slothful B-cell non-Hodgkin's lymphoma (NHL) that has advanced amid or following treatment with rituximab or a rituximab-containing regimen. To date, no less than 80 clinical preliminaries with bendamustine are dynamic and enrolling patients,[4,5] demonstrating that there is a considerable measure of new enthusiasm for this somewhat old medication. To help clinical preliminaries, we created and approved an LC– MS/MS strategy for the evaluation of bendamustine in plasma and pee.[6-8] As far as anyone is concerned, this article is the first depicting the approval of an examine for this compound. Aside from unaltered bendamustine, the measure moreover permits measurement of - hydroxy-bendamustine (M3) and N-desmethylbendamustine (M4), the two known stage I metabolites of bendamustine, which have cytotoxic action roughly proportionate to and five to ten times not as much as their parent, individually.[9] Moreover, a different examine is depicted to evaluate the item of two-overlap hydrolysis of bendamustine, dihydroxy-bendamustine (HP2), in similar examples. Bendamustine may differ from other alkylators in that it may be more potent in activating p53-dependent stress pathways and inducing apoptosis; it may induce mitotic catastrophe; and it may activate a base excision DNA repair pathway rather than an alkyltransferase DNA repair mechanism. Accordingly, this agent may be more efficacious and less susceptible to drug resistance than other alkylators. Bendamustine IUPAC name is 4-[5-[bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid. Its molecular formula is $C_{16}H_{21}Cl_2N_3O_2$ and its molecular weight is 358.262.

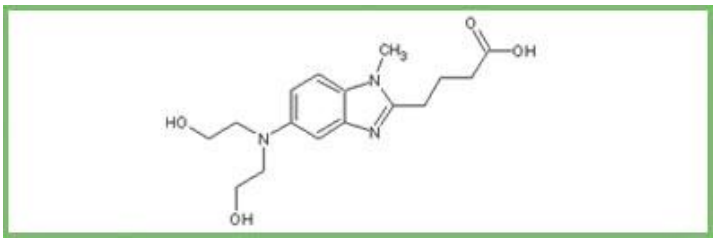

Figure 1: Chemical structure of Bendamustine.

The primary purpose of this research project was to develop and to validate a simple HPLC method for determination of Bendamustine in the raw material and finished product. This is beneficial in any clinical environment where the concentration of Bendamustine is needed to understand any patient issues along with the Pharmaceutical industry to prepare the multiple steps that may be needed to prepare the raw material for production. There are very few analytical methods that have been reported for the determination of Bendamustine in Bulk and Formulations. Studying the stability of a drug and being able to monitor degradation products aids in the clinical treatments/early product development and shelf life for the drug. The present study was aimed at developing simple, specific, accurate and precise HPLC method for the determination of Bendamustine in commercially available pharmaceutical formulations, based on direct UV-detection.

Experimental: Chemicals and reagents: Standard drug Bendamustine was obtained as working standard from Mylan laboratories Hyderabad. HPLC grade acetonitrile, and hydrochloric acid were procured from Merck Ltd., India. Analytical grade sodium hydroxide, hydrogen peroxide and other chemicals used in the study were procured from CDH chemicals Ltd, Mumbai, India.

Instrumentation: Quantitative HPLC was performed on the Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing. Flow rates from50 uL/min to 5 mL/min can be generated for use with 2.1 mm ID columns and larger. The auto-sampler has a maximum capacity of 120 vials (12x32, 2-mL) with programmable temperature control from 4 to 40°C. A heated column compartment provides temperatures from 5 degrees above ambient to 65°C. The detector is a photodiode array (model 2996) with a wavelength range of 190-800 nm and sensitivity settings from 0.0001-2.0000 absorbance units. X-Terra RP-C18 Column (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with Empower-software solution software.

Preparation of standard solutions: Bendamustine standard solution (4000ppm): Weight accurately 400 mg of Bendamustine and transfer in to 40 mL volumetric flask. Add solvent (75% ACN) and shake gently; sonicate the solution till completely dissolved. Make up the volume with diluting solvent (75% ACN) which produces 4000ppm stock solution.

Selection of stationary phase: According to ICH, for a particular compound column must provide the tailing between 0.9-2.0 and theoretical plates must be above 2000. For the Bendamustine research workfour different columns were used.

1. **Column 1:** ThermoHypersil C18 (4.6 x 250 mm, 5µm) Part # 30105254630, Serial # 01535710.
2. **Column 2:** Supelco Discovery C18 (4.6 x 250 mm, 5µm)Part # 39777702 Serial # 1022040N.
3. **Column 3:** Thermo Electron C18 (4.6 x 250, 5µm) Lot # 6854, Part # 30105-254630, Serial # 0541587.
4. **Column 4:** Supelco C18 (4.6 x 250 mm, 5µm) Col: 28105-254630, CN # 6279.

Samples of 1000ppm bendamustine were injected under mobile phase conditions of 30% ACN were injected into the HPLC system. Considering ICH criteria of the column, above 4 columns were tested. Out of four columns used for the method, Column 1 has selected for further studies as the peak symmetry and number of theoretical plates were in accordance with guidelines. Column 2 gave lower Retention time, and as compared to column 1, it's tailing factor also greater. In the case of column 3 peaks appeared broken and other peaks were present so it was rejected. Similarly, for column 4 lot of impurity peaks appeared and were deemed unsuitable for use in analysis. So that Column 1 was selected for the method development and validation of Bendamustine.

Selection of mobile phase: Because the analyte of interest is predominantly polar and of low molecular weight; several binary mixtures containing potassium phosphate dibasic and acetonitrile (20-55% v/v) were evaluated with a Hypersil C-18 (250x4.6mm) column with 5 µm particle size. The retention time of bendamustine was decreased with a high concentration of acetonitrile. Also, the precipitation of potassium phosphate dibasic buffer was observed at the high concentration of organic solvent in the mobile phase in a composition which, was indicated by pressure fluctuations resulting in a rough, noisy base line. Hence acetonitrile strength in mobile phase composition was limited to less than 40% (v/v) which also resulted in a very low 'k'value of bendamustine. Further use of buffer, acetonitrile 70: 30 (v/v) resulted in quality separation of bendamustine in terms of peak symmetry, adequate resolution, acceptable run time and retention time with agreeable 'k' values. Increasing the mobile phase flow rate from 0.8 ml/ min to 1.0 ml/minute decreased the chromatographic run time. To optimize the pH of the mobile phase, three different pH

conditions were used: 2.90, 5.0 & 7.0. At pH 5.0 elution was at 2.629 which interferes with void peaks so, it was not selected for analysis. In case of pH 2.90 Retention time at 1.683 was very early elution, this condition was not selected. But in case of pH 7.0, an excellent peak at 7.609 with tailing factor 1.166 and theoretical plate count 4400 was obtained. So, pH 7.0 was selected for further analysis.

Selection of Detection wavelength: The choice of specific wavelength for the analyte of interest, i.e., Bendamustine was based on the necessity of good sensitivity and a high degree of selectivity. The aliquot portion of the standard stock solution of bendamustine was diluted with 50% ACN was examined in the range of 200-400 nm. The representative UV-Spectrum of bendamustine was shown in Figure-1. The UV-spectrum depicts the maximum absorption at 232 nm for bendamustine, was selected due to less noise followed by no solvent interference was observed at maximum absorption peak. It was also observed that there was no mobile phase interference or baseline disturbances due to buffer or diluting liquids.

Preparation of Linearity dilutions: To select a nominal concentration, serial dilution of analyte that were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 250, 500, 750, 1000, 1250 and 1500ppm. The prepared solutions were filtered through filter paper. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. A calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis) (Table 1).

Acid stress study: Take a measured quantity of 2.5mL of HCl and mix with 2.5mL of 4000 ppm drug in a dry test tube. After this heat this mixture in 75 ^{0}C for 24 hrs. Then cool down to room temperature. Once the mixture comes down to room temperature, neutralize the solution by adding 2.5mL of an equivalent concentration of NaOH. Transfer this to a dry 10mL volumetricflask and make up to the mark with 50% ACN as a diluent solvent, further to dilute this solution to 1000 ppm concentration with diluent. Sonicate the solution followed by filtration before injecting into HPLC system. To optimize the degradation conditions two different concentrations of HCl and different times has been followed.

Base Stress studies: Take a measured quantity of 2.5 ml of NaOH and mix with 2.5mL of 4000 ppm drug in a dry test tube. After this heat this mixture in 75 ^{0}C for 24 hrs. Then cool down to room temperature. Once the mixture comes down to room temperature neutralize the

solution by adding 2.5mL of corresponding HCl. Transfer this to a dry 10mL volumetric flask and make up to the mark with 50% ACN as a diluent solvent. further to dilute this solution to 1000 ppm concentration with diluent. Sonicate the solution followed by filtration before injecting into HPLC system. To optimize the degradation conditions two different concentrations of NaOH and different times has been followed. The chromatograms and results are as follows. From the degradation results it is clearly indicated that the drug is not stable under basic conditions. There are two potential degradant products intensely observed in almost in all concentrations of NaOH. Different degree of degradation was observed by different acid concentrations and for different exposure time.

Oxidation Stress studies: Take ameasured quantity of 2.5 ml of corresponding hydrogen peroxide and mix with 2.5 ml of 4000 ppm drug in a dry test tube. Heat this mixture in 75 ^{0}C for 24 hrs, then cool down to room temperature. Transfer this to a dry 10mL volumetric flask and make up to the mark with 50% ACN as a diluent solvent. further to dilute this solution to 1000 ppm concentration with diluent. Sonicate the solution followed by filtration before injecting into HPLC system. To optimize the degradation conditions, two different concentrations of hydrogen peroxide & different times has been followed.

Heat Stress studies: The stability of bendamustine was studied under thermal stress conditions. The primary stock solution of bendamustine at 4000 ppm was used for the thermal degradations study. 2.5mL of Bendamustine was taken in a test tube and subjected to extreme heat conditions at 75^{o}C for different time intervals. The resulting solution was further diluted with diluents solvent up to the mark in 10 mL volumetric flask and mixed thoroughly, further to dilute this solution to 1000 ppm concentration with diluent. The resulting stress sample of bendamustine were injected into HPLC instrument and analyzed under optimized chromatographic conditions for the stability behavior of bendmustine.

UV sensitivity Study: Stability behavior of bendamustine under light irradiation was studied by using UV light. The bendamustine raw material was transferred into a petri dish and kept under UV-irradiation for 24hrs and 72 hrs. 10 mg of irradiated bendamustine raw material was dissolved in 10mL of 50% ACN in a 10mL volumetric flask, which gives a concentration of 1000 ppm of bendamustine. The solution was mix well and filter before injecting into HPLC system.To optimize the degradation conditions different times has been followed.

RESULTS AND DISCUSSION

Optimized Chromatographic Conditions: A reversed phase isocratic HPLC stability-indicating method was developed for the determination of Bendamustine and a reversed phase gradient elution technique is developed for the separation of degradants using reversed-phase liquid chromatography.

(a) The optimum conditions used for the developed method for the determination of Bendamustine are as follows:

- Elution Technique: Isocratic (reversed-phase separation)
- HPLC: 1100 Series HPLC System with MWD (UV/VIS Detector), Agilent Technologies.
- Column: Hypersil- C 18 5µ 4.6mm x 250mm column
- Mobile Phase: Mobile Phase A.25mM Potassium Dibasic Phosphate Buffer pH 7; B.100% ACN.
- Solvent Strength: 70% Buffer (phosphate pH 7) and 30% ACN.
- Absorbance: 330 nm
- Flow rate: 1.0 mL/min.
- Injection Volume: 10 µL
- Run time: 15 minutes.

(b) The optimum conditions used for the developed method for the separation of Bendamustine degradants are as follows:

- **Elution Technique:** Isocratic (reversed-phase separation)
- **HPLC:** 1100 Series HPLC System with MWD (UV/VIS Detector), Agilent Technologies.
- **Column:** Hypersil C_{18}, (250x 4.6 mm 5µm particle size)
- **Mobile Phase:** 30:70 Acetonitrile /potassium dibasic phosphate buffer pH 7.0.
- **Flow Rate:** 1 mL/min
- **Wavelength:** 330 nm
- **Temperature:** Ambient
- **Injection Volume:** 10 µL
- **Run time:** 15 minutes

Validation Parameters: System suitability: The prepared standard solutions of 1000 µg/ml Bendamustine were injected into the HPLC system under the established optimum separation conditions. Standard solution 1 was injected six times, and standard solution 2 was injected twice. The % RSD values for retention times and peak areas of six replicate injections for Bendamustine standard and two replicate injections for Bendamustine standard 2 were 0.222% and 0.037% respectively. The % Drift, which was calculated using the following equation, is less than 2%. A_S is average peak area of six replicate standard injection and A_C is the peak area of check standard. The tailing factor for Bendamustine peak is less than 2.0 and more than 0.9 and the number of theoretical plates are more than 2000. These results fulfilled the required system suitability acceptance criteria. Table 1 shows the results.

Table 1: System suitability parameters for Bendamustine.

Bendamusitne Hydrochloride								
Standard 1	**Retention Time**	**Retention Time % RSD**		**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	**Peak Area % Drift**
Injection 1	7.410	0.11%		1.335	4326	9028.57	0.11%	0.12%
Injection 2	7.396			1.312	4201	9020.64		
Injection 3	7.395			1.305	4218	9015.28		
Injection 4	7.385			1.334	4252	9004.87		
Injection 5	7.391			1.308	4224	9003.25		
Injection 6	7.394			1.318	4235	9010.29		
Standard 2	**Retention Time**	**Retention Time % RSD**		**Tailing Factor**	**Theoretical Plates**	**Peak Area**	**Peak Area %RSD**	
Injection 1	7.396	0.06%		1.331	4291	9003.67	0.01%	
Injection 2	7.402			1.327	4264	9002.16		

Solution Stability of Bendamustine: Bendamustine solution was injected immediately and then after 24, 48 and 72 hours of sample preparation, simultaneously peak areas were recorded for all the samples injected. All the 3 peak areas were compared with the immediately injected sample peak area and percentage change was calculated. The acceptance criteria for percentage change should be less than 2%. Table 2 shows the results for the solution stability.

Table 2: Solution stability of Bendamustine over a period of 72 hours.

Time	Peak Area	% Change
0	11007.52	N/A
24	10697.32	2.81

48	10309.07	6.34
72	9692.23	11.94

Specificity: Determination of Bendamustine Peak Purity

Once confirmed that the potential degradants are completely resolved from the Bendamustine peak, the next attempt was to ensure that the Bendamustine chromatographic peak in the force degraded sample is pure. For that, the degradation samples are mixed and used to test specificity to show that Bendamustine is pure and separated from their degradants. This sample was injected in an HPLC instrument equipped with a Diode Array Detector (DAD). Peak purity tests were performed on Agilent 1200 HPLC system equipped with Diode Array Detector (DAD). Under the optimum separation conditions, the resulted purity factor is within the threshold limits. Validity of these results was further supported by the three-dimension plot of Bendamustine chromatographic peak (Figures 1 and 2).

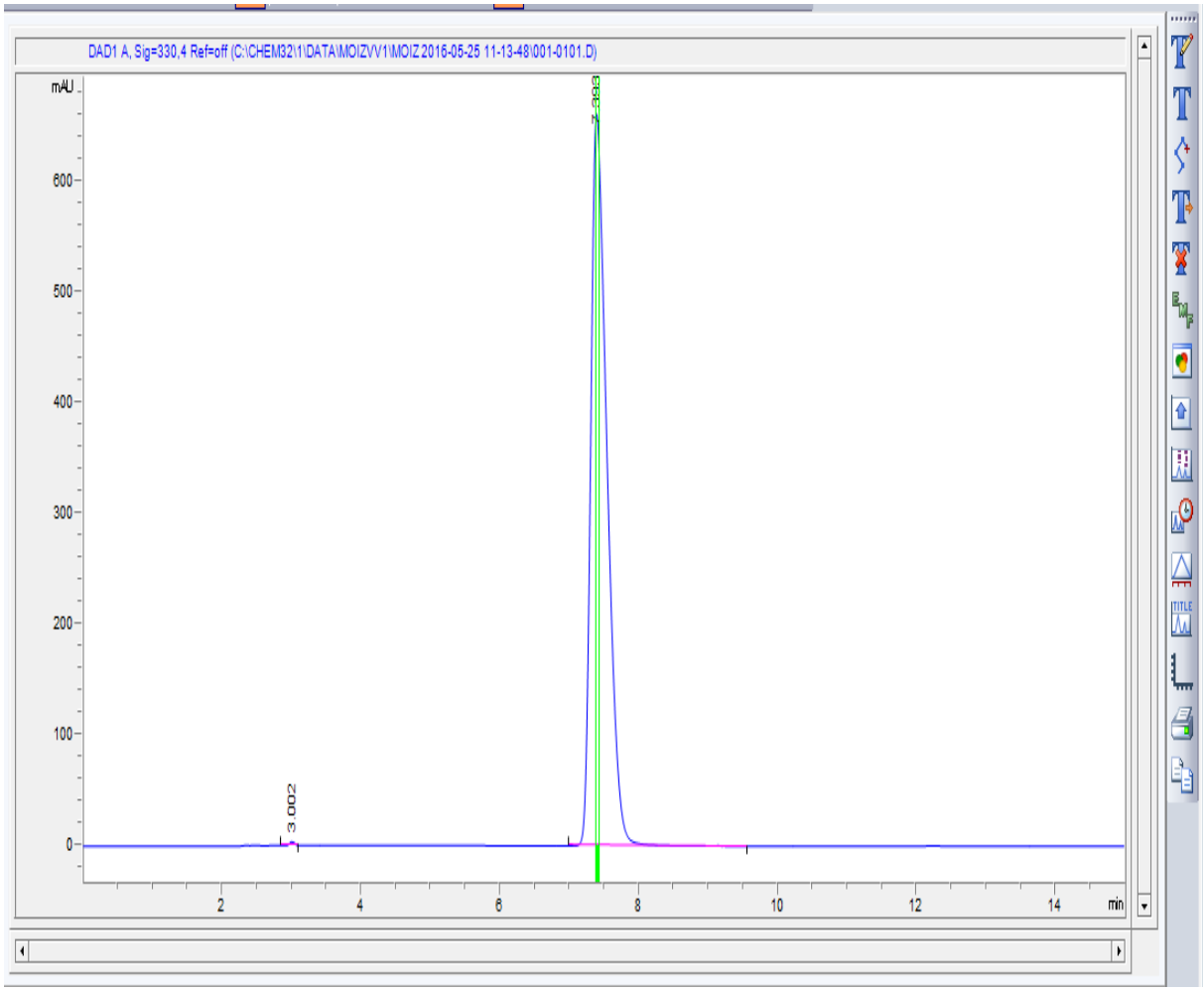

Figure 1: Peak purity of Bendamustine on Agilent 1200 HPLC-DAD.

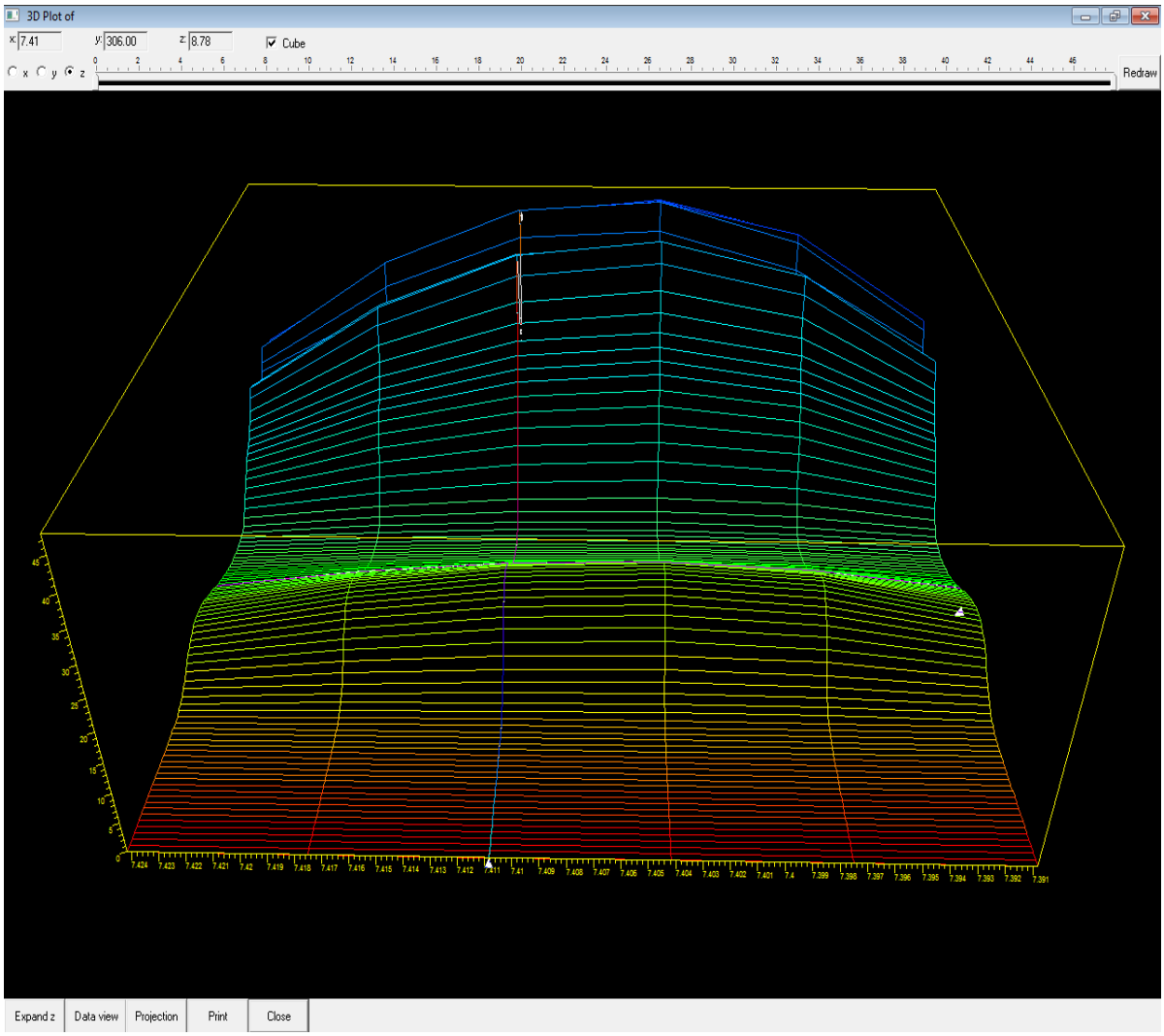

Figure 2: 3-D image of Bendamustine.

Robustness Studies on Bendamustine Raw Material: The impact of deliberately the optimized chromatographic conditions on the retention and resolution of Bendamustine peak was investigated. For this research, the following parameters were studied: solvent strength, pH of buffer, Flow rate and wavelength. The optimized chromatographic conditions were studied by changing the (a) ± 0.1% pH (b) ± 2 nm Wavelength (c) ± 1% B Composition (solvent strength) and (d) ± 0.1% Flow rate. The results of robustness studies are summarized in Table –3.

Linearity: The linearity range tested for Bendamustine raw material was between 600 ppm and 1200 ppm. Solutions with different concentrations of Bendamustine were prepared as shown in Table 4 and injected into the HPLC system. The obtained peak areas for the corresponding concentrations of Bendamustine are summarized in Table-4.

Table 3: Robustness results of bendamustine.

Experimental Parameters	Various Conditions	Retention Time	Tailing Factor
Wavelength	328	7.422	1.552
	330(optimum)	7.362	1.549
	332	7.428	1.559
Flow rate	0.9	7.440	1.508
	1.0 (optimum)	7.362	1.549
	1.1	6.793	1.536
pH of buffer	6.9	7.438	1.521
	7.0(optimum)	7.362	1.549
	7.1	7.440	1.566
% B Composition	29	6.543	1.639
	30(optimum)	7.362	1.549
	31	8.544	1.639

Table 4: Linearity Studies of Bendamustine in raw material.

Concentration	Peak Area
600	7053.88
700	8253.58
900	10330.38
1000	11555.57
1100	12478.58
1200	13583.73

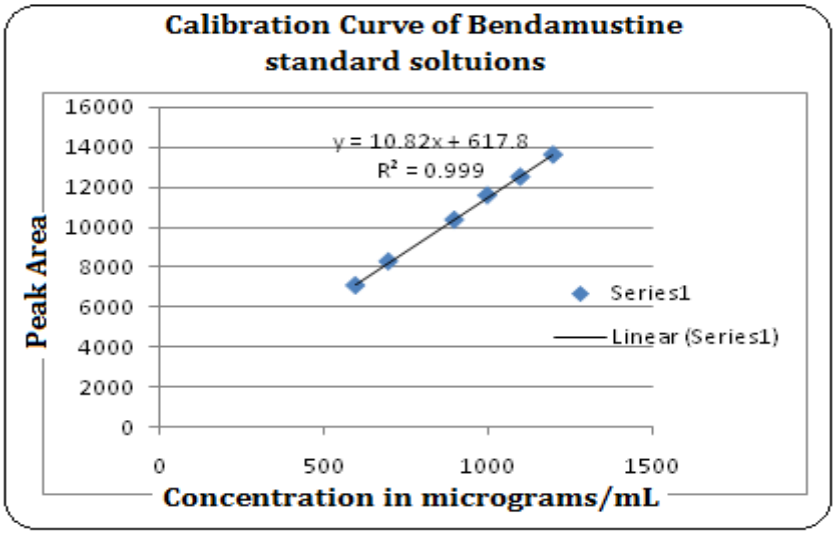

Figure 3: Calibration Curve of Bendamustine standard solutions in linearity study.

Accuracy: The accuracy of the developed method was tested to determine closeness of the measured value to the true value. Accuracy of the developed method was studied by evaluating the recovery of Bendamustine from spike solutions. The different concentrations of Bendamustine were prepared of the nominal concentration. The 4000ppm the of stock

solution was used as standard and serial dilutions of 700ppm, 1000ppm and 1200ppm were prepared. Each sample was prepared in triplicate and injected, giving a total of 9 injections.

Table 5: Summary of Accuracy studies results (Raw Material)

Concentration (ppm)	Peak Area	Average Peak Area	Recovery
700	7841.42	7828.68	99.51%
	7826.30		
	7818.32		
1000	11023.04	11021.31	100.8%
	11016.36		
	11024.53		
1200	12933.48	12929.64	99.57%
	12924.76		
	12930.70		

Precision: The ICH guidelines recommend that repeatability should be assessed using a minimum nine determinations covering the specified range for the procedure (i.e., 3 concentrations and 3 replicates of each concentration (or) using minimum of six determinations at 100% of test concentration.

Instrument Precision (Injection Repeatability): Repeatability of injection of Bendamustine was determined by preparing one solution at 1000ppm of nominal concentration and carrying out six repeated injections. The precision of peak areas of six injections was calculated as relative standard deviation (%RSD). This analysis precision met the defined acceptance criteria of maximum allowable %RSD of NMT 1 %. Then calculate precision and %RSD. The results can be seen in the Table-6. Method precision was demonstrated by calculating % RSD of six independent preparations of 100% target concentration of 1000ppm of Bendamustine. It was also calculated for the impurities of six independent preparations of 1000 ppm and all these were injected into hplc under developed method conditions. The results can be seen in the Table-7.

Table 6: Peak area results of six injections of Bendamustine for injection precision.

Sample	Peak Area	Average Value	Standard Deviation	%RSD
1	10973.35	10965.54	7.621	0.07%
2	10966.95			
3	10961.91			
4	10962.52			
5	10974.31			
6	10954.20			

Table 7: Summary of Method Precision (Raw Material).

Sample	Peak Area	Average Values	Standard Deviation	%RSD
1	10772.86	10758.875	7.65%	0.07%
2	10758.56			
3	10760.20			
4	10755.19			
5	10756.23			
6	10750.21			

Limit of detection (LOD): The LOD is generally expressed as the concentration of the analyte sample (i.e., in ppm or in percentage). The detection limit of the developed method is the lowest analyte concentration that can produce a response detectable above the noise level of a system, typically, three times the noise level (3:1).

The Limit of Detection (LOD) was evaluated over the course of 10 independent preparations of low concentrations of Bendamustine Reference Standard. All these 10 solutions are prepared from the stock solution of 100 ppm, Table 30shows the preparations volume of stock solution. The cut off criteria was when signal to noise ratio exhibits a 3:1 ratio, which was found at 0.25ppm. Table 8 displays the summarized results.

Table 8: Signal-to-Noise ratios of various concentrations of Bendamustine for LOD and LOQ.

Bendamustine hydrochloride	
Concentration (ppm)	**Signal to Noise ratio**
3ppm	80
2.5ppm	40.57
2ppm	53
1.5ppm	50
1.0ppm	28.60.
0.5ppm	**12.0**
0.25ppm	2.6

Acid stress study: The following table 9 shows the different concentrations of HCl and their degradation time and peak areas.

Table 9: Acid degradation study of different concentrations of Bendamustine under optimized chromatographic conditions.

Concentration of HCl	Exposed time	Peak Area	Degradation
Standard	0	534.8	0
0.01 M HCl	2hour	86.8	83.7
0.01 M HCl	1hour	235.4	55.9

0.01 M HCl	30mins	228.8	57.2
0.01 M HCl	20mins	426.6	20.23
0.01 M HCl	15mins	475.3	11.1
0.01 M HCl	14mins	490.4	8.3

Base stress study: The following table 10 shows the different concentrations of NaoH and their degradation time and peak areas.

Table 10: Base degradation study of different concentrations of Bendamustine under optimized chromatographic conditions.

Concentration of NaOH	Exposed time	Peak	Degradation
Standard		534.8	
0.01MNaOH	1hour	116.9	78.1%
0.01MNaOH	10 mins	264.1	50.6%
0.01MNaOH	1hour at room Temperature	442.7	17.2%
0.01MNaOH	40mins at room Temperature	489.9	8.3%
0.01MNaOH	30 mins at room Temperature	514.0	3.8%

Oxidative stress study: The resulted chromatograms are evaluated and the degradation results are given in Table 11.

Table 11: Oxidation study of different concentrations of Bendamustine under optimized chromatographic conditions.

Parameter	Exposed time	Peak Area	Degradation
Standard		534.8	
3% H_2O_2	1 hour	224.6	58%
3% H_2O_2	30 mins	360.1	32%
3% H_2O_2	20 mins	322.9	39%
3% H_2O_2	15 mins	469.6	12.1%
3% H_2O_2	13 mins	499.2	6.6%
3% H_2O_2	10 mins	521.8	2.4%
2% H_2O_2	1 hour	311.9	41.6%
2% H_2O_2	30 mins	252.9	52.7
2% H_2O_2	20 mins	523.1	2.1
2% H_2O_2	10 mins	515.3	3.6
1% H_2O_2	1 hour	263.5	50.7
1% H_2O_2	13 mins	500.1	6.4%

Thermal degradation study: The resulted chromatograms are evalulated, and the degradation results are given in Table 12.

Table 12: Heat stress results of Bendamustine under optimized chromatographic conditions.

Time	Peak Area	Degradation
Standard	534.8	N/A
24hour heat	23.4	N/A
1 hour heat	408.4	23.6%
40mins heat	462.1	13%
35mins heat	453.9	15.1%
37mins heat	444.9	16.8%
20mins heat	483.8	9.5%

UV-Senstivity study: Bendamustine raw drug is stable against UV light. The resulted chromatograms were shown in the Figure 15.

Table 13: UV results of Bendamustine under optimized chromatographic conditions.

Time	Peak Area	Degradation
Standard	534.8	N/A
UV 30 mins	593.4	N/A
UV 1hour	581.4	N/A
UV 24 hours	651.7	N/A
UV 48 hours	578.2	N/A
UV 72 hours	632.8	N/A

CONCLUSION

The developed method was sensitive, accurate and precise Reverse Phase HPLC (isocratic) for the estimation of Bendamustine HCL in bulk drug and in pharmaceutical formulations. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. Thus, the developed method can be easily used for the routine quality control of bulk and parenteral dosage form (sterile powder for injection) of Bendamustine HCL within a short analysis time. The currently developed method was also studied for forced degradation and data was depicted in respective tables. The Bendamustine was stable and can estimate in various stress conditions. The developed method was accurate reverse phase HPLC for the estimation of Bendamustine Hydrochloride in a bulk drug and pharmaceutical formulation. The method was validated for System Suitability, Specificity, Solution's stability, Robustness, Precision, Accuracy, Linearity and LOD and LOQ.

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