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A STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF BENDAMUSTINE HCL IN PARENTERALS

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Abstract: A simple, precise, accurate, economical and reproducible HPLC method for estimation of Bendamustine HCl in Parenteral dosage form has been developed. Quantitative HPLC was performed with AGILENT 1120 COMPACT LC HPLC with software EZ CHROME ELITE with UV-Visible detector). Agilent Zorbax poroshell 120EC –C_{18} RP column, 100 x 4.6 mm, 2.7µm was used in the study. The mobile phase of water and Acetonitrile (with 0.01% TFA) were mixed in the ratio of 50:50 of pH 6.9-7.2 were used in this study. The conditions optimized were: flow rate (0.5 mL/minute), wavelength (254 nm) and run time was 10 min. Retention time was found to be 2.720 min. The linearity was found to be in the concentration range of 80-120% of target concentration. The developed method was evaluated in the assay of commercially available vial Bendit/Purplz label to contain Bendamustine HCl 100mg/vial strength. Results of analysis were validated statistically and by recovery studies. The recovery studies 99.67% was indicative of the accuracy of proposed method. The precision was calculated as repeatability, inter and intraday variation (%RSD) for the drug.

Keywords: Bendamustine HCl, Accuracy, Precision, Specificity, Linearity.

INTRODUCTION

Bendamustine HCl [15,16,17,18,19,20,21,21,22] chemical Name 4-[5-[Bis(2-chloroethyl) amino]-1-methyl benzimidazol-2-yl] butanoicacid hydrochloride, as per BCS the drug belongs to Class II i.e. high solubility and low permeability. Chemical formula of Bendamustine HCl is $C_{16}H_{21}Cl_2N_3O_2$·HCl and its molecular Weight is 394.72 g/mol.

Bendamustine HCl is a bifunctional mechlorethamine derivative containing a purine-like benzimidazole ring. 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 activation of DNA damage stress response and apoptosis, inhibition of mitotic checkpoints, and induction of mitotic catastrophe. It was approved by the US Food and Drug Administration (FDA) for Chronic Lymphocytic Leukaemia in March 2008 [21, 22]; Bendamustine HCl was developed by Cephalon in the United States as Treanda.

Literature survey reveals few analytical methods for the determination of Bendamustine HCl in pharmaceutical preparations, including: spectrophotometry[1] and HPLC [2, 3, 4, 5]. However the reported methods suffer from the low sensitivity, for this reason, our target was to develop a simple sensitive HPLC method for the determination of Bendamustine HCl in solid dosage forms. Because HPLC [2, 3] methods have been widely used for routine quality-control assessment of drugs, because of their sensitivity, repeatability and specificity [6, 7, 8]; we have developed a simple and specific RP-HPLC method for determination of Bendamustine HCl in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC-UV method was validated in accordance with International Conference on Harmonization (ICH) guidelines, by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantitation [8].

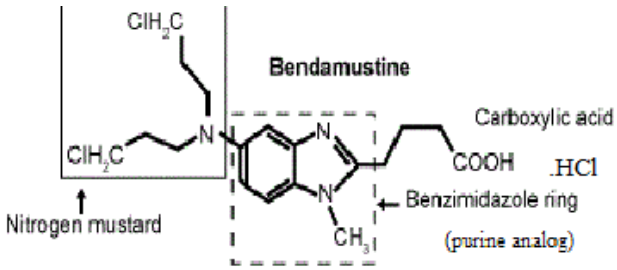

Figure 1: Structural formula of Bendamustine HCl

MATERIALS AND METHODS

Instruments, Chromatographic System and Conditions: The HPLC System is the AGILENT 1120

COMPACT LC HPLC with software EZ CHROME ELITE, UV-Visible detector, pump, which ensures consistent system-to-system performance and high reproducibility, consisted of UV detector and auto-injector.

HPLC Programme: Isocratic

Column: Zorbax poroshell 120EC –C_{18} RP column, 100 x 4.6 mm, 2.7µm. All materials and reagents used were of Analytical Reagent grade.

- Reference standards
- Bendit/Purplz; labelled to contain Bendamustine HCl 100 mg strength/vial, were purchased from commercial sources in the Indian market.
- Acetonitrile and HPLC Grade water
- Trifluoroacetic acid.

Preparation of Mobile Phase and Stock Solutions:

Mobile phase: consists of a mixture water and Acetonitrile (with 0.01% TFA) were mixed in the ratio of 50:50 of pH 6.9-7.2.The run time was 10 min at a flow rate was 0.5 ml min^{-1}., detector wavelength 254 nm. The injection volume was 10µl.

Standard solutions: 10 mg of Bendamustine HCl standard was taken into a 25 ml clean dry volumetric flask, about 2 ml of methanol was added, sonicated for 5 minutes, and diluted to volume with blank. Filtered through 0.2µ membrane filter.

Diluent preparation: Pipette out 0.25ml from the standard stock solution, into a 25 ml clean dry volumetric flask, and dilute to the mark with 10ml of blank.

Calibration curve for Bendamustine HCl: Transfer an accurately weighed quantity of about 10 mg of Bendamustine HCl into a 10ml volumetric flask. Add about 2ml of the methanol was added and sonicate to dissolve. Make the volume up to the mark with the diluent. From the stock serial dilutions were made by taking 0.2, 0.22, 0.25, 0.27, and 0.3ml into 10ml volumetric flask and diluted with the diluent up to the mark. Inject these solutions into the HPLC system and record the area of analyte peaks. Plot a graph of concentration (in x-axis) vs analyte peak area (in y-axis).evaluate the correlation coefficient between concentration and peak area on y-intercept of the correlation plot.

Analysis of Marketed Formulations: Weighed and transferred accurately equivalent amount of about 10 mg of the lyophilised powder from vial into 10ml clean dry volumetric flask, about 2 ml of methanol was added, sonicated for 5 minutes, and diluted to volume with blank. Filtered the solution through the membrane filter paper of 0.2µm, from the filtrate pipette out 0.25ml of sample solution into a 10ml volumetric flask, make upto the volume with diluent (mobile phase). Typical chromatogram of a standard and sample can be seen in Figure 3 and 4 respectively.

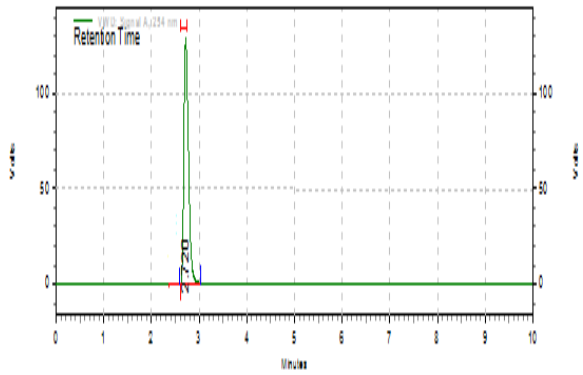

Figure 2: Typical chromatogram of Bendamustine HCl (Standard)

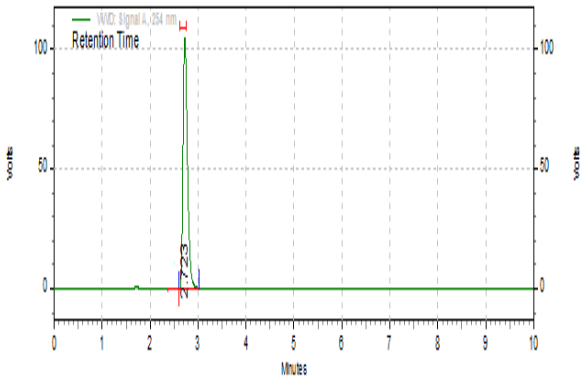

Figure 3: Typical chromatogram of Bendamustine HCl (Sample)

Method Validation:

Accuracy: The accuracy of analytical method is defined as the similarity of the results obtained by this method to the true value and the precision as the degree of that similarity. To prove the accuracy of the proposed method, the results were compared with those obtained using the reference method.

Acceptance criteria: The mean percent recovery of at each spike level should be not less than 98% and not more than 102%.

Method precision: The precision of test method was determined by preparing six test preparations using the product blend as per manufacturing formula. And the relative standard deviation of assay results was calculated. To evaluate the precision for assay method, six samples were prepared and analyzed as per test method. Calculated % assay and RSD of six samples and found to be within the acceptance criteria. Data can be seen in Table.2.

Intermediate precision:

a) **Intermediate precision 1:** Prepare replicate injections of the standard solution of the same concentration and inject six times one after the other on the day 1 by analyst 1.

b) **Intermediate precision 2:** Prepare replicate injections of the standard solution of the same concentration and inject six times one after the other on the day 2 by analyst 1.

c) **Intermediate precision 3:** Prepare replicate injections of the standard solution of the same concentration and inject six times one after the other on the day 2 by analyst 2.

To evaluate intermediate precisions for assay method, six samples were prepared and analyzed by using different HPLC system, different column, by different analyst on different day. Calculated % assay, % RSD for % assay results of method precision and intermediate precision and found to be within the acceptable limits

Acceptance criteria: Each individual preparation should meet the acceptance criteria of 98.0% to102.0%. The RSD for six sample assay results should not be more than 2.0%. Overall RSD for assay results (twelve assay results) in method precision and intermediate precision should not be more than 2.0%.

Linearity: Under the above described experimental conditions, a linear relationship was established by plotting the peak area ratio against the drug concentration. The results are abridged in Table 6. Linear regression analysis of the data can be seen in Figure 4:

Conclusion: Square of Correlation coefficient value of Bendamustine HCl observed as, r^2 = 0.9999 and there is no placebo interference observed with the analyte peak.

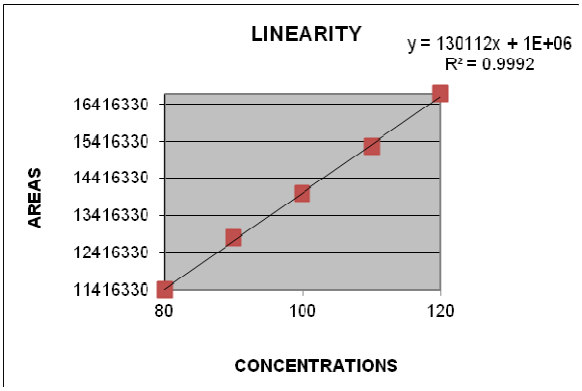

Figure 4: Linearity plot of Bendamustine HCl

Robustness: In order to measure the extent of the method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged, and in parallel the chromatographic profile was observed and recorded. The studied parameters were: the composition of the mobile phase, pH, flow rate, and column temperature.

Acceptance criteria: They shouldn't be any alteration in Retention time and other Parameters.

Stability for Standard and Test solutions: Solution Stability: Standard solution is injected 5 times and sample solution of same concentration is injected twice at different intervals at 0 hr, 1 hr, 3 hrs, 5 hrs, 10 hrs, 24 hrs.

Acceptance criteria: The % assay result should not differ from initial value by more than ± 2.0 for test solution Similarity factor for standard should be in the range of 0.98 to 1.02.

System suitability: Inject 10 µl of the diluted standard solution in five replicate injections, into the chromatograph and record the chromatograms. Parameters can be seen Table 10.

Specificity:

Evaluated the interference of blank, placebo with the analyte peak as per the methodology and the results are given below.

Blank interference: Blank was prepared and injected as per test method. It was observed that no blank peaks were interfering with analytical peaks.

Placebo interference: Placebo solutions were prepared in duplicate and injected as per test method. It was observed that no placebo peaks were interfering with analytical peaks.

Impurity interference: All known impurities solution was prepared at about 1% of the test concentration and analyzed as per test method. It was observed that no co elution of the all known impurities peaks with analytical peaks.

Stress degradation study:

To determine whether the analytical method was stability indicating, Bendamustine HCl was stressed under various conditions includes photolytic degradation (Exposed to 1, 00,000 Lux.), acid hydrolysis (0.5N Hydrochloric acid), base hydrolysis (0.5N sodium hydroxide), Oxidative degradation (5% Hydrogen peroxide) and Thermal degradation (at 125°C).

Photolytic degradation:

The sample solution was exposed to Exposed to 1,00,000 Lux hours of white light to determine the

effects of light irradiation on the stability of Bendamustine HCl.

Acid, alkaline, oxidative and thermal degradation:

Forced degradation in acidic media, alkaline media, H_2O_2 was performed with 1mg mL-1 concentration to determine the effects of chemical irradiation on the stability of Bendamustine HCl.

RESULTS AND DISCUSSION

After extensive various preliminary experimental trials using different combinations of buffers, organic modifiers (acetonitrile, methanol, ethanol), and after trials using different C_8 and C_{18} reverse phased-phase (RP) columns, best chromatographic conditions to obtain good separation, resolution and symmetrical peaks for determination of Bendamustine HCl was achieved when using a Agilent Zorbax poroshell 120EC –C_{18} RP column, 100 x 4.6 mm, 2.7µm (µm particle size), The column hold up value was the first deviation of the base line obtained. Mobile phase: consists of a mixture of mixture water and Acetonitrile (with 0.01% TFA) were mixed in the ratio of 50:50 of pH 6.9-7.2.The run time was 10 min at a flow rate was 0.5 ml min^{-1}, detector wavelength 254nm. The injection volume was 10µl. Moreover the proposed method allowed the determination of Bendamustine HCl in their formulated parenteral vials. Figure. 2 show a typical chromatogram of Bendamustine HCl. Validation studies were performed according to ICH Guidelines and the proposed method is specific, rapid, reliable and reproducible. The proposed HPLC method is applicable for the determination of Bendamustine HCl in the formulated dosage forms. It has distinct advantages over other existing method regarding sensitivity, time saving and minimum detection limits.

Table 1: Accuracy Data

Concentration of Bendamustine HCl	Peak Area	Amount Found	%recovery		
		standard 95%			
Pre1	13653280	81.47	101.83	**MEAN**	100.0
Pre2	13640443	78.587	98.234	**SD**	0.05773
Pre3	13613666	79.94	99.92	**%RSD**	0.05771
		standard 100%			
Pre1	14491849	101.116	101.12	**MEAN**	99.933
Pre2	14526011	101.68	101.68	**SD**	0.25166
Pre3	14542713	99.57	99.57	**%RSD**	0.010825
		standard 105%			
Pre1	15596551	118.797	98.99	**MEAN**	100.2
Pre2	15593220	119.89	99.91	**SD**	0.1
Pre3	15415886	119.92	99.93	**%RSD**	0.31005

Table 2: Method Precision Data:

Sample	Rt	Peak Area	Assay
1	2.740	14235545	99
2	2.737	14327158	100
3	2.733	14351536	100
4	2.737	14340786	100
5	2.733	14275739	99
6	2.733	14289570	100
Average	2.736	14303389	99.6666699
%RSD	0.109649123	0.310150193	0.518124862

Table 3: Intermediate precision 1 data:

Sample	Rt	Peak Area
1	2.723	14319948
2	2.720	14332145
3	2.737	14327158
4	2.727	14297690
5	2.733	14340786
6	2.727	14237733
Mean	2.727833333	14309576.67
SD	0.006274286	37200.67368
% RSD	0.2300098735	0.25997047

Table 4: Intermediate precision 2 data:

Sample	Rt	Peak Area
1	2.737	14340786
2	2.707	14295545
3	2.723	14311026
4	2.717	14333395
5	2.720	14319146
6	2.720	14275938
Mean	2.7206	14312639.33
SD	0.00973	24112.96829
% RSD	0.003568	0.168473248

Table 5: Intermediate precision 3 data:

Sample	Rt	Peak Area
1	2.727	14339985
2	2.713	14312995
3	2.720	14319798
4	2.720	14323598
5	2.720	14299698
6	2.703	14325852
Mean	2.7171	14320321
SD	0.008232051	13470.37876
% RSD	0.003029645	0.094064782

Table 6: Linearity data

S. No.	Concentration %	Peak Area
1	80	11416333
2	90	12810992
3	100	13998756
4	110	15278964
5	120	16687925

Table 7: Summary of Robustness:

Parameters	Optimum range	Remarks
Wavelength (nm)	± 2 nm	At lower wavelength tailing factor was increased and plate count was decreased.
Flowrate ml/min	0.4-0.6	At lower flow rates the asymmetry factor was increased and at higher flow rates the relative retentions was slightly decreased
Temperature	38-42°C	Beyond the optimum range peak shape and symmetry was lost

Table 8: Solution stability data

Sample (hrs)	Rt	Peak Area	% difference in assay
0	2.733	14351536	nil
	2.723	14311026	nil
1	2.717	14350987	nil
	2.723	14313928	nil
3	2.740	14349878	nil
	2.733	14312795	nil
5	2.723	14350208	nil
	2.723	14320920	nil
10	2.737	14350495	nil
	2.723	14319632	nil
24	2.703	14335839	nil
	2.707	14316978	nil
Mean	2.723	14329703.18	nil
SD	0.011161012	17862.21543	nil
% RSD	0.004097664	0.124651678	nil

Table 9: System suitability parameters

System suitability parameters	Bendamustine HCl
% RSD for five replicate injections of standard	0.1
Tailing factor	1.3
Theoretical plates	4058
% Check standard recovery	100.0

Table 10: Impurity interference

Peak Name	RT (min)		Acceptance criteria
	Standard Individual impurity sample	Spiked sample	
IMPURITY A	5.01	5.03	Chromatogram of known impurities solution should not show any peak at the retention time of the peak.
IMPURITY B	6.90	6.85	
IMPURITY C	8.98	8.963	
IMPURITY D	10.8	11.9	
IMPURITY E	12.9	13.5	

Table 11: Difference between % assay of spiked and un-spiked sample

Parameters	Bendamustine HCl
Un spiked sample (Method precision)% Assay	99.9
Spiked sample (Specificity) % assay	99.7
% assay difference	0.2
Acceptance criteria	Difference in the mean of % assay results of spiked and unspiked samples should not be more than ± 2.0

Table.12: Results for Degradation behavior of Bendamustine HCl

Degradation mechanism / condition	% Assay	% Degradation	Remarks
Un-degraded sample	99.8	-	Passed
Thermal at	97.0	2.6	Passed
Photolytic	98.8	1.0	Passed
Acid /0.5 N HCl	93.0	7.0	Passed
Base /0.5 N NaOH	93.0	4.8	Passed
Heat on water bath for 2.5 hrs	96.7	3.1	Passed
Peroxide /5.0% H_2O_2	99.6	0.2	Passed

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