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Analytical Method Validation of Assay of Bendamustine in Bendamustine HCL for Injection

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ABSTRACT

Analytical Method Development & Validation:

An in-house analytical method development for assay, related substances (for drug substance and drug product) and tertiary butyl content (for drug product) was developed and further the developed method was found to stability indicating. As a part of the analytical method validation of assay, related substances and tertiary butyl content of the finished product, forced degradation study was carried and it was found that the drug product is found sensitive to alkali, neutral, heat and peroxide conditions. The analytical method validation was carried out satisfactorily with the parameters like precision, accuracy, robustness and linearity.

INTRODUCTION:

Analytical method validation of Assay method Parameters considered for Bendamustine HCl for injection[1-9]. Are as follows:

- System suitability
- Specificity
- Forced degradation
- Precision
- System precision
- Method precision
- Intermediate precision
- Stability in analytical solution
- Linearity
- Accuracy
- Range

DETAILS OF DRUG PRODUCT:

Chemical name: 1H-benzimidazole-2-butanoic aicd,5-[bis(2-chloroethyl)amino]-1-methyl-monohydrochloride.

Molecular Formulae: $C_{16}H_{21}Cl_2N_3O_2$

Molecular weight: 394.7

Product Details:

Bendamustine Hydrochloride for Injection [100 mg/vial]

Table No. 1: Label claim details of Bendamustine HCl for injection 100 mg/vial

Sl. No.	Ingredients	Qty/mL	Qty/vial
1.	Bendamustine HCl IH	12.5 mg	100 mg
2.	Mannitol Pyrogen free USP/BP/PhEur	21.25 mg	170 mg
3.	Tertiary butyl alcohol IH *	0.5 mL	q.s
4.	Water for injection USP/BP/Ph.Eur *	q.s to 1 mL	q.s to 8 mL

Note: * Will be removed during Lyophilization process

Bendamustine Hydrochloride for Injection 25 mg/vial

Table No. 2: Label Claim details of Bendamustine Hydrochloride 25 mg

Sl. No.	Ingredients	Qty/mL	Qty/vial
1.	Bendamustine HCl IH	12.5 mg	25 mg
2.	Mannitol Pyrogen free USP/BP/Ph Eur	21.25 mg	42.5 mg
3.	Tertiary butyl alcohol IH *	0.5 mL	q.s
4.	Water for injection USP/BP/Ph.Eur *	q.s to 1 mL	q.s to 2 mL

Note: * Will be removed during Lyophilization process

METHOD DESCRIPTION:

Principle: Reverse phase liquid chromatography with Isocratic elution and UVdetector.

Table No. 3: Chromatographic conditions:

Column	:	Inertsil BDS C-18 (4.6 x 250 mm)5µm
Wavelength	:	236 nm
Flow rate	:	1.0 mL/min
Injection volume	:	20.0 µl
Run time	:	10.0 minutes
Column oven temperature	:	25°C
Sample cooler temperature	:	4°C
Diluent	:	Mobile phase

Preparation of Buffer:

Weigh and transfer 3.85 g of Ammonium acetate into 1L of water, add 2.0 mL of Triethylamine and adjust the pH to 4.7 with dilute acetic acid.

Preparation of Mobile phase:

Mix 580 mL of Buffer and 420 mL of Acetonitrile, filter and degas.

Standard Preparation:

Weigh and transfer 20.0 mg of Bendamustine Hydrochloride into 20 mL volumetric flask previously kept in the chilled water tray, dissolve and dilute to volume with diluent (Previously chilled). Further dilute 1.0 mL of this solution to 10 mL with diluent (Previously chilled).

Sample preparation:

For 25 mg/vial:

Reconstitute 5 sample vials, each with 5 mL of diluent (previously chilled), pool together into 100 mL volumetric flask previously placed in the chilled water tray, dilute to volume with diluent. Further dilute 2.0 mL of this solution to 25 mL with diluent (Previously chilled).

For 100 mg/vial:

Reconstitute 2 sample vials, each with 10 mL of diluent (previously chilled), pool together into 100 mL volumetric flask previously placed in the chilled water tray, diluteto volume with diluent (previously chilled). Further dilute 1.0 mL of this solution to 20 mL with diluent (previously chilled).

System suitability:

The RSD from five replicate injections of standard preparation should be NMT 2.0%. The tailing factor for the Bendamustine HCl peak should be NMT 2.0.

Theoretical Plates for Bendamustine HCl peak should be NLT 2000.

Procedure:

Inject blank (diluent) (one injection), standard preparation (5 injections), and check the system suitability parameters.

If the system suitability parameter passes, then inject sample preparation (2 injections) and record the chromatograms.

Calculate the Assay of Bendamustine Hydrochloride by using the followingformula.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{DT}{LC} \times \frac{P}{DS} \times \frac{100}{N}$$

Where,

AT	:	The average area of response of Bendamustine HCl peak from the sample chromatogram
AS	:	The average area of response of Bendamustine HCl peak from the standard chromatogram
WS	:	Weight of standard taken in mg
DS	:	Dilution of Standard preparation in mL
DT	:	Dilution of sample preparation in mL

N	:	Number of vials taken
P	:	The potency of standard in % on as-is basis
LC	:	Label claim of Bendamustine HCl in mg per vial

Specification Limit:

Bendamustine HCl	:90.0-110.0 % of the label claim 25mg/vial or 100 mg/vial

SYSTEM SUITABILITY:

To verify that the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set.

Injected Blank (one injection) and Standard preparation (5 injections), recorded chromatograms and checked the system suitability parameters.

Table No.: 4: RSD Details

The RSD from five replicate injections of standard preparation should be NMT 2.0%.	1.4 %
The tailing factor for Bendamustine HCl peak should be NMT 2.0.	1.1
Theoretical Plates for Bendamustine HCl peak should be NLT 2000.	6092

SPECIFICITY:

Specificity is the ability of the analytical method to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

Performed the specificity parameter of the method by injecting Diluent, Standard preparation, Sample preparation, and Placebo spiked with 100% Standard solution into the chromatographic system.

Recorded the retention times of Diluent, Standard preparation, Sample preparation and Placebo preparation.

Table No.: 5: Solutions & RT details

Solutions	Retention time (in min.)
Blank(Diluent)	-
Standard preparation	3.653
Placebo	-
Sample preparation	3.667

SPECIFICITY BY DEGRADATION STUDIES:

Specificity by forced degradation:

Forced degradation of Bendamustine HCl for injection has been carried out, to confirm that during stability study or throughout the shelf life, any degradation if found should not interfere with the Bendamustine HCl peak. In addition, the forced degradation study will help to identify the type of degradation pathway (whether oxidative, alkali hydrolysis, acid hydrolysis, water hydrolysis, photolytic and dry heat) for each of the degradants.

Preparation of sample solutions:

Note: Performed Specificity by forced degradation for Higher Strength (Final concentration is 1000ppm).

Sample as such:

For 100mg/vial:

Reconstituted 5 sample vials with mobile phase, pooled together into 100 mL volumetric flask, and diluted to volume with diluent. Further diluted 1.0 mL of thissolution to 50 mL with diluent.

Placebo as such:

Reconstituted one placebo vial with mobile phase, pooled together into 20 mL volumetric flask, diluted to volume with diluent. Further diluted 1.0 mL of thissolution to 50 mL with diluent.

UV light exposed sample:

Exposed one vial of the sample was to UV light (264 nm & 365 nm) for 8 hours, dissolved in 20 mL volumetric flask and dilute to volume with diluent. Further diluted 1mL of this solution to 50mL with diluent.

Sunlight-exposed sample:

Exposed one vial of sample to Sun light for 8 hours. Allowed to attain room temperature, dissolved and diluted to 20 mL volumetric flask with diluent. Further diluted 1mL of this solution to 50mL with diluent.

Thermal Stressed (Dry heat) sample:

Exposed one vial of sample to 80°C in a hot air oven for 4 hours. Allowed to attain room temperature, dissolved in 20 mL volumetric flask and diluted to volume with diluent. Further diluted 1mL of this solution to 50mL with diluent.

Acid Stressed sample:

Added 1.0mL of 0.1N HCl to one vial of sample and kept in water bath at 60°C for 30 minutes. Allowed to attain room temperature and neutralized with 1mL of 0.1N NaOH and diluted to volume with diluent. Further diluted 1mL of this solution to 50 mL with diluent.

Alkali Stressed sample:

Added 2.0mL of 0.1N NaOH to one vial of sample and kept in water bath at 60°C for 30 minutes. Allowed to attain room temperature and neutralized with 2 mL of 0.1N HCl and diluted to volume with diluent. Further diluted 1mL of this solution to 50 mLwith diluent.

Peroxide Stressed sample:

Added 2.0mL of 1% v/v Peroxide solution to one vial of sample and kept in water bath at 60°C for 30 minutes. Allowed to attain room temperature, and diluted to volume with diluent. Further diluted 1mL of this solution to 50 mL with diluent.

Neutral Stressed sample:

Added 2.0mL of water to one vial of sample and kept in water bath at 60°C for 30 minutes.

Allowed to attain room temperature and diluted to volume with diluent. Further diluted 1mL of this solution to 50 mL with diluent.

Table No.: 6: Forced degradation study compilation

Stressed Condition	**Bendamustine HCl (in Assay %)**	**% of Degradation**
As such	98.5	-
0.1N HCL	82.2	15.3
0.1N NaOH	81.5	16.0
3 % Peroxide	87.0	12.5
Neutral	92.2	7.3
Sun light	98.7	0.8
UV-light	98.1	1.4
Thermal	98.4	1.1

PRECISION:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurements.

METHOD PRECISION:

In method precision, a homogeneous sample of a single batch should be analyzed sixtimes. This indicates whether a method is giving consisting results of a single batch.

Analyzed the samples of Bendamustine HCl for injection six times of a same batch as per analytical procedure. Calculated the % Assay of Bendamustine HCl.

Table No.: 7: Percentage Assay results of Bendamustine HCl for injection inassay test parameter validation

% Assay of Bendamustine HCl		
Injection No.	**25 mg/vial**	**100 mg/vial**
1	100.2	98.6
2	99.2	99.1
3	98.6	99.0
4	100.0	98.9
5	98.7	98.7
6	98.9	98.4
Mean	99.3	98.8
% RSD	**0.7**	**0.3**

LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Performed the linearity with Bendamustine HCl standard in the range of 50 to 150% of the specification limit.

Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Tested the intercept for statistical equivalence to zero.

Plotted a graph of Bendamustine HCl concentration (ppm) on X-axis and Area response on the Y-axis.

Table No.: 8: Linearity Details

Level	Concentration in ppm	Area Response
0	0.0000	0
1	49.9083	4379716
2	59.8899	5184876
3	69.8716	5972511
4	79.8532	6678143
5	89.8349	7421270
6	99.8165	8578902
7	109.7982	9542070
8	119.7798	10229690
9	129.7615	11470242
10	149.7248	12692452
Correlation coefficient		**0.999**
Regression coefficient		**0.998**

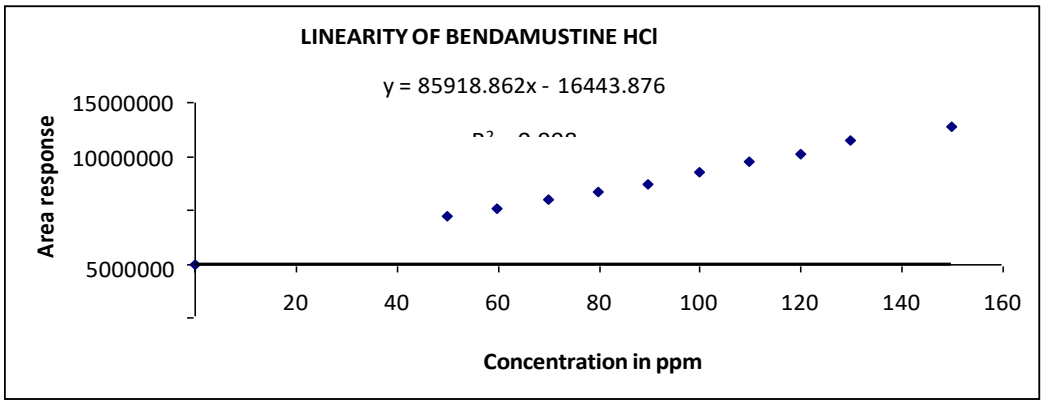

Fig No.: 3: Linearity Graphical Presentation

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by thatmethod to the true value (standard value).

Spiked known quantity of Bendamustine HCl standard at 50%, 100%, and 150% of Assay specification limit into the placebo. Analyzed these samples in triplicate for each level.

Calculated the % recovery from the results of Accuracy.

Table No.9: Recovery Details of Bendamustine HCl:

Level (about)	mg Added	mg Recovered	%Recovery	Mean % Recovery
50%	0.9985	1.0040	**100.5**	**100.9**
	0.9985	1.0074	**100.9**	
	0.9985	1.0103	**101.2**	
100%	1.9970	1.9895	**99.6**	**99.6**
	1.9970	1.9887	**99.6**	
	1.9970	1.9882	**99.6**	
150%	2.9955	2.9992	**100.1**	**99.9**
	2.9955	2.9868	**99.7**	
	2.9955	2.9884	**99.8**	

RANGE:

The range of the analytical method is the interval between the upper and lower levels of analyte that has been demonstrated to be determined with suitable accuracy and linearity.

Derived the specified range from the Linearity and Accuracy studies.

Table No.: 10: Range Details

Level (Concentration in %)	Mean Area response
50	4379716
100	8578902
150	12692452
Correlation coefficient	**1.000**

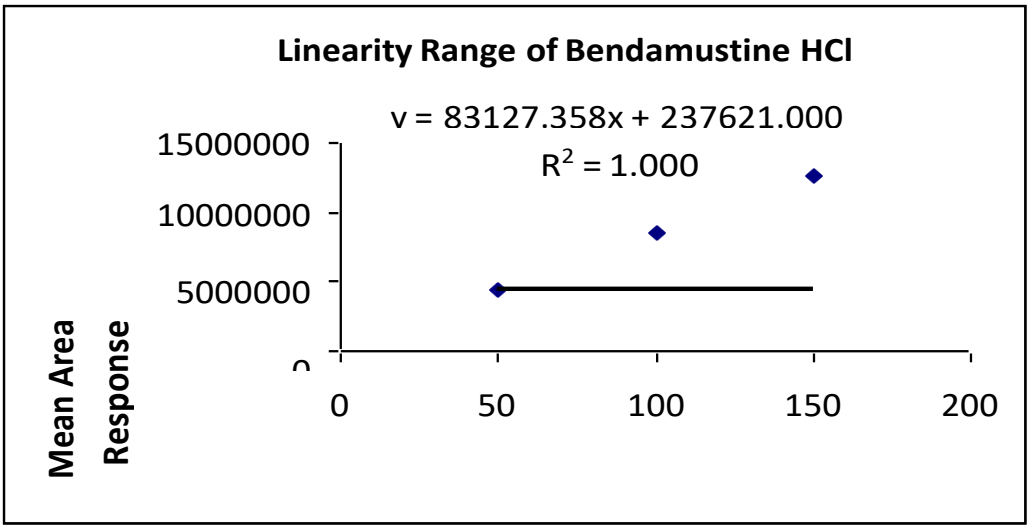

Fig No.:1: Linearity Rage Graphical Presentation

Table No. 11: Accuracy Range of Bendamustine HCl:

Level (Concentration in %)	**Mean Area response**
50	4358171
100	8605009
150	12943627
Correlation coefficient	**1.000**
% RSD	**0.6**

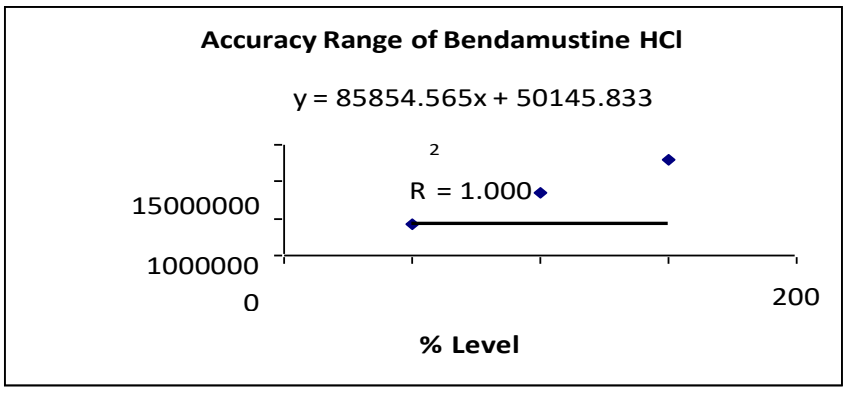

Fig No.: 2: Accuracy Rage Graphical Presentation

Typical Chromatograms of Method Development & Method Validation: Analytical Method Development of Assay Test Parameter.

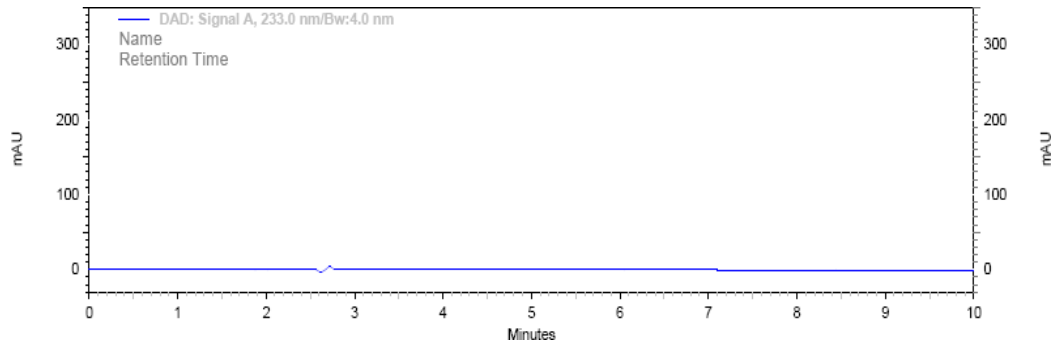

Fig. No.: 3: Chromatogram (Blank)

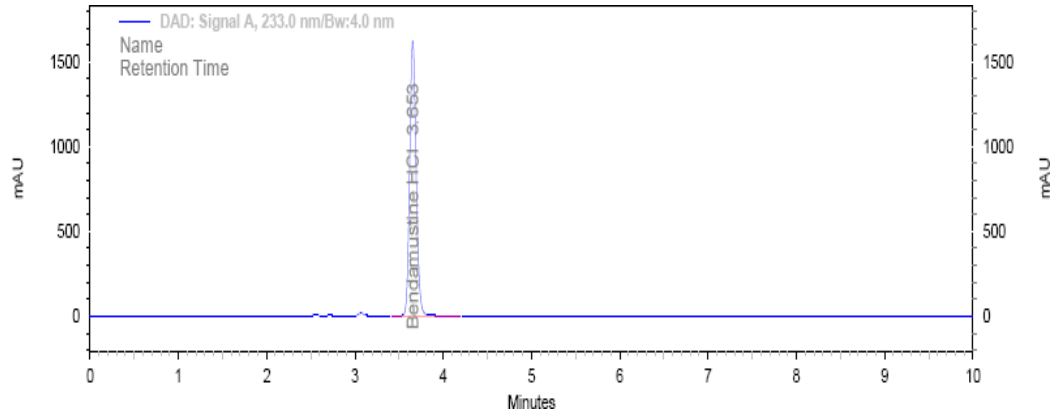

Fig. No.: 4: Chromatogram (Standard)

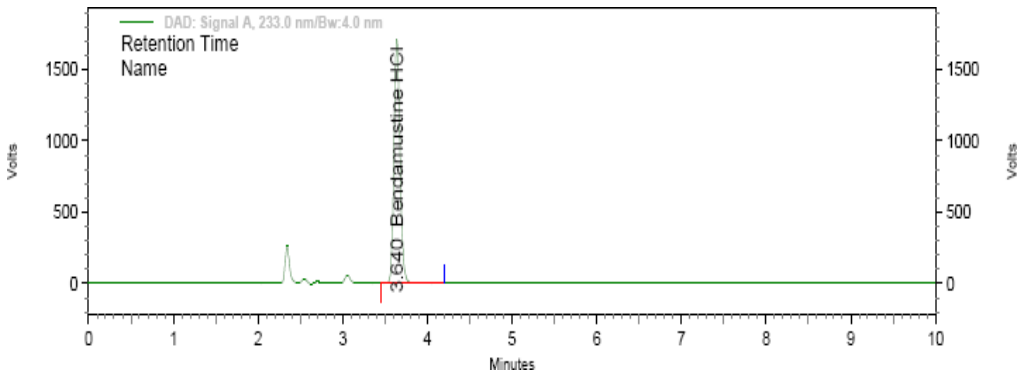

Fig. No.: 5: Chromatogram (Sample)

Typical Chromatograms of Method Development specificity by degradation studies under assay test parameter:

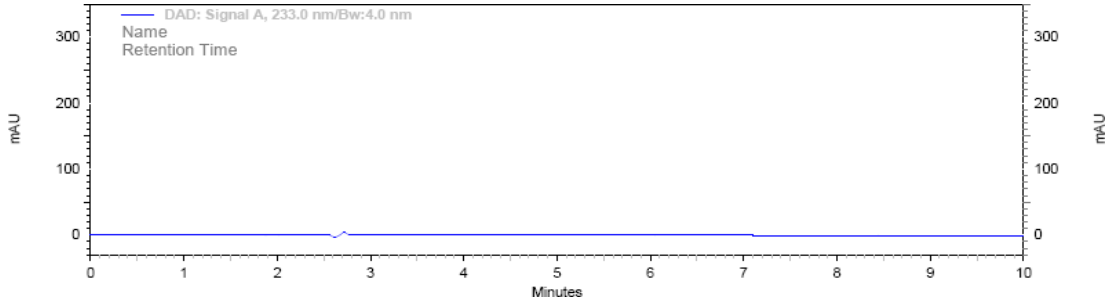

Fig. No.: 6: Chromatogram of Blank:

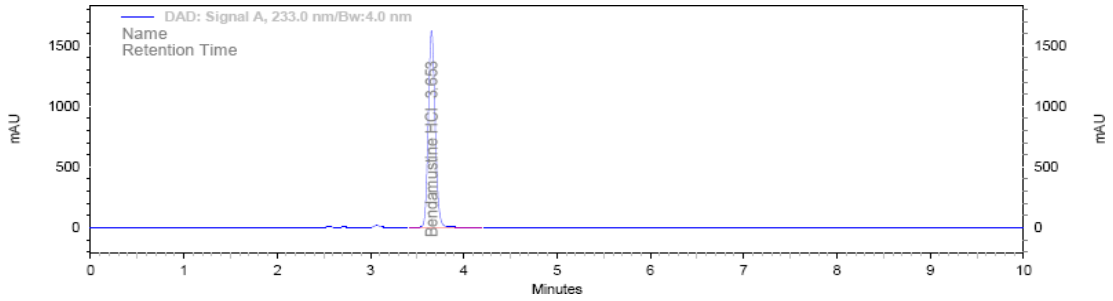

Fig. No.: 7: Chromatogram of Standard preparation:

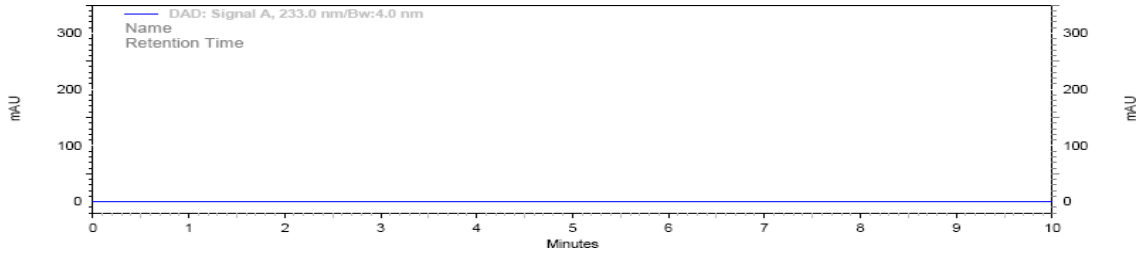

Fig. No.: 8: Chromatogram of Placebo:

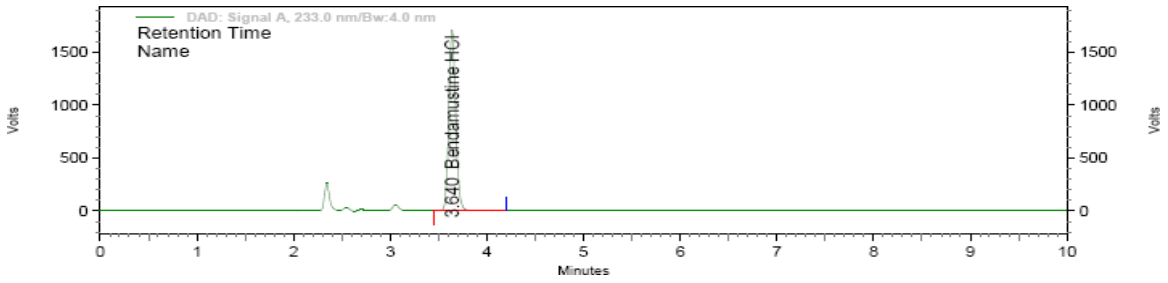

Fig. No.: 9: Chromatogram of Sample preparation:

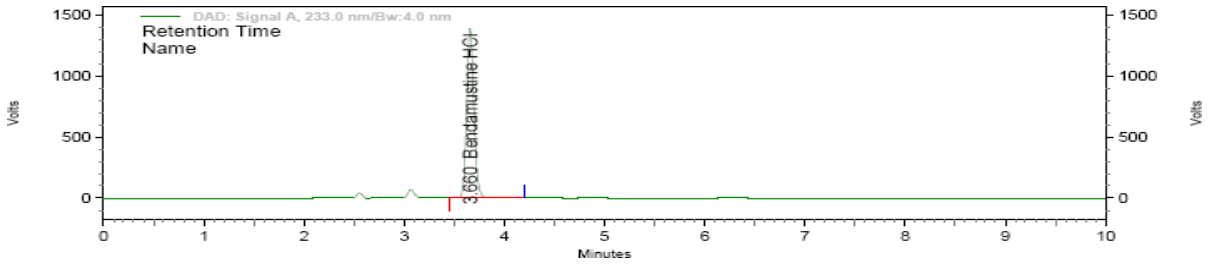

Fig. No.: 10: Chromatogram of Acid stressed Sample:

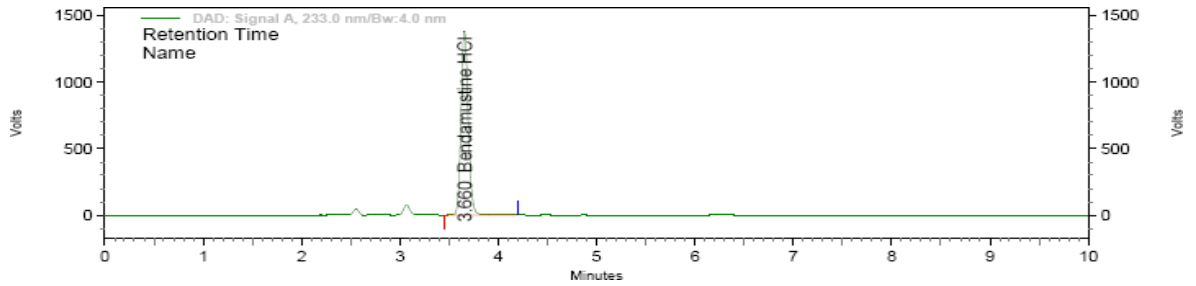

Fig. No.: 11: Chromatogram of Alkali stressed Sample:

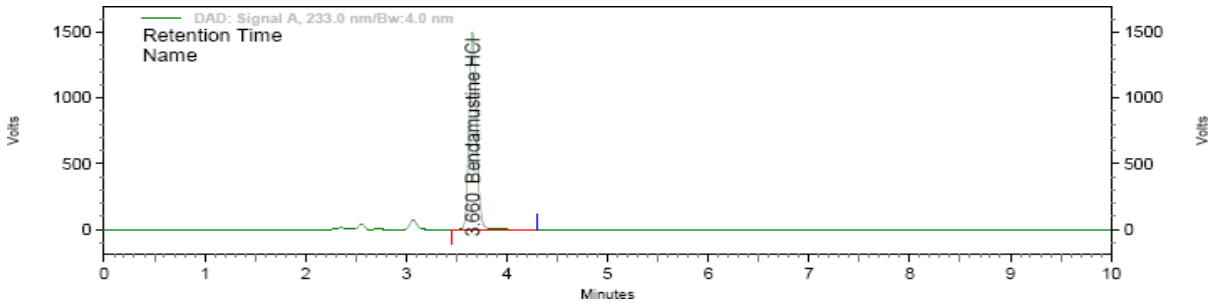

Fig. No.: 12: Chromatogram of Neutral stressed Sample:

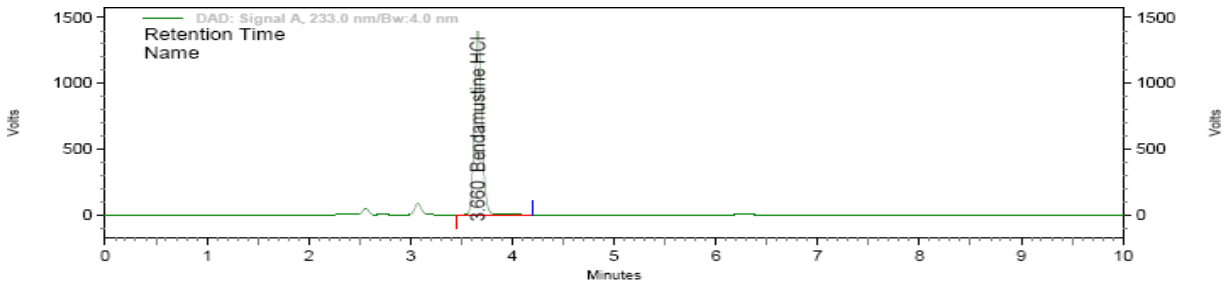

Fig. No.: 13: Chromatogram of Peroxide stressed Sample:

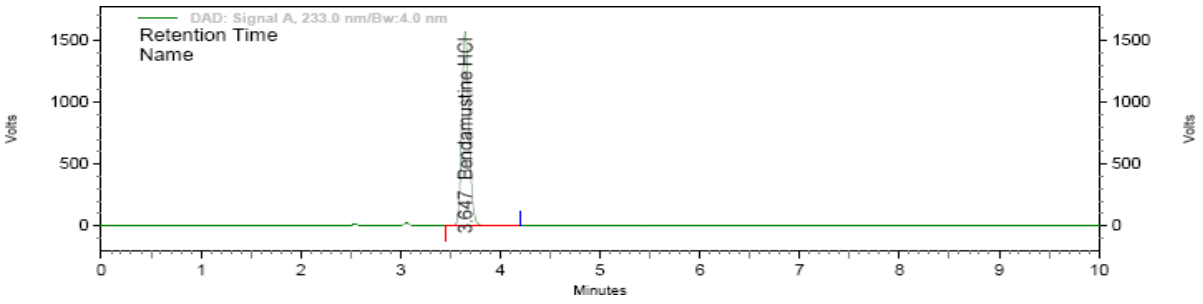

Fig. No.: 14: Chromatogram of Sunlight exposed Sample:

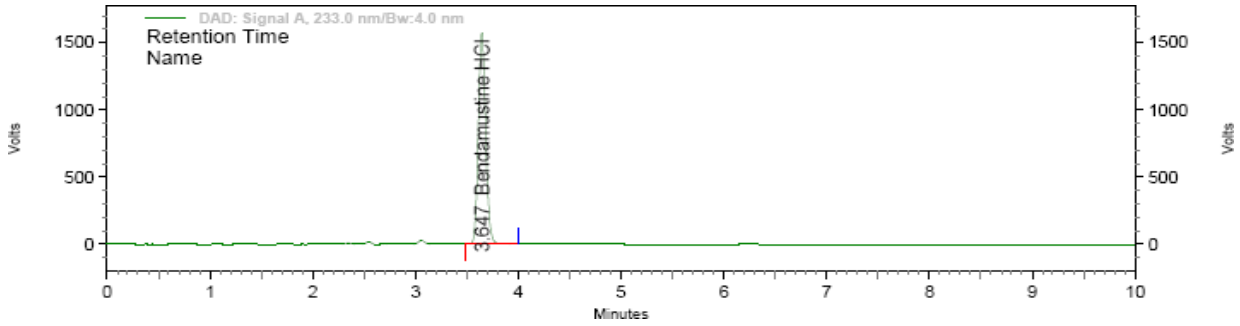

Fig. No.: 15: Chromatogram of UV light Sample:

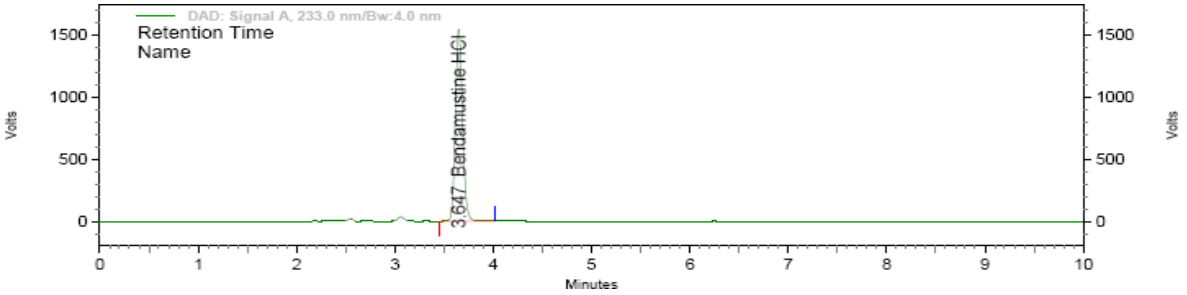

Fig. No.: 16: Chromatogram of Thermal stressed Sample:

Analytical Method Development & Validation:

An in-house analytical method development for assay, related substances (for drug substance and drug product) and tertiary butyl content (for drug product) was developed and further the developed method was found to stability indicating.

As a part of analytical method validation of assay, related substances and tertiary butyl content of the finished product, a forced degradation study was carried and it was found that the drug product is found sensitive to alkali, neutral, heat and peroxide conditions. The analytical method validation was carried out satisfactorily with the parameters like

precision, accuracy, robustness and linearity.

Photostability: The Bendamustine hydrochloride drug product of both strengths is available in amber-colored vials. In order to understand the light/photostability of the drug product, the photostability evaluation was carried as per ICH Q1B conditions. The drug product of 100 mg/vial was taken for the study evaluation as this is 20 mL vial which has more surface area and the worst case when compared to 25 mg/vial strength. The drug product of 100 mg/vial in clear vial and amber vial was exposed simultaneously to 200 watt-hours/m^2 of near UV light and 1.2 million Lux hours of cool fluorescent light in a photostability chamber maintained at 25°C.

When the vials were exposed to the photostability chamber, discoloration of the drug product was observed in the clear vial indicating the drug product's sensitivity to the light. However, the drug product in the amber vial didn’t turn into discoloration indicating the compatibility of amber vial to the drug product.

Reconstitution Solution Stability:

Further the product Bendamustine hydrochloride for injection 100 mg/vial & 25mg/vial was subjected for evaluating the reconstitution solution stability along with one of the Indian marketed available sample of Bendamustine Hydrochloride for injection 25 mg/vial. As per the pack insert, the product needs to be reconstituted with5 mL of sterile water for injection for 25 mg/vial and 20 mL for 100 mg/vial and within the 30 minutes from the time of reconstitution the reconstituted solution needs to be diluted with recommended IV fluids. From the analytical data, it was concluded that evaluated analytical parameters are meeting the requirements and are comparable to the Indian marketed drug product.

Physiological Solution Compatibility: Also, when the drug product after reconstitution, the solution was further diluted with intravenous fluids like 0.9% sodium chloride injection and 0.45% sodium chloride/2.5% Dextrose Injection at 0.2 mg/mL and 0.6 mg/mL concentration of Bendamustine hydrochloride when stored for 24 Hours room temperature and at refrigerated.

Lab Scale Batches:

The analytical results of lab scale batches of Bendamustine hydrochloride for injection 25 mg/vial & 100 mg/vial were found satisfactory. Based on the analytical results confirmation of lab scale batches, the stability study of optimized batches was evaluated.

Market Available Formulation Evaluation:

Before assessing the stability evaluation, the available Indian market samples were characterized and evaluated for critical analytical parameters. From the analytical parameters tested of both the strengths, it is concluded that % impurity A content is found more and rest of other parameters are found satisfactory. Further, the % impurityA content is found similar in all three marketed products.

BIBLIOGRAPHY

1. Cancer Research UK : CancerHelp UK". Retrieved 11 May 2012.
2. Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB "Cancer is a preventable disease that requires major lifestyle changes". Pharm. Res. 25. 2008 ;(9): 2097–116.
3. Kinzler, Kenneth W.; Vogelstein, Bert "Introduction". The genetic basis of human cancer (2nd, illustrated, revised ed.). New York: McGraw-Hill, Medical Pub. Division. p. 5. ISBN 978-0-07-137050-9. (2002).
4. Jemal A, Bray, F, Center, MM, Ferlay, J, Ward, E, Forman, D. GlobalCancer Statistics". CA: A cancer journal for clinicians. 2011. 61 (2): 69–90.
5. Varricchio, Claudette G. A cancer source book for nurses. Boston: Jones and Bartlett Publishers. p. (2004). 229. ISBN 0-7637-3276-1.
6. Harris NL, Jaffe ES, Diebold J, et al. "World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997". J. Clin. Oncol. 17 (12): 3835–49. PMID 10577857.
7. Chiorazzi N, Rai KR, Ferrarini M "Chronic lymphocytic leukemia". N. Engl. J. Med. 2005; 352 (8): 804–15.
8. Janssens et al "Rituximab for Chronic Lymphocytic Leukemia in Treatment- Naïve and Treatment-Experienced Patients". Contemporary Oncology. 2011. 3 (3): 24–36.
9. "Non-Hodgkin lymphomas " at Dorland's Medical Dictionary.
10. Swerdlow, Steven H; Campo, Elias; Harris, Nancy Lee, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Oxford Univ Pr. 2008.ISBN 978-92-832-2431-0.
11. Kramer et al. Current Status of the Epidemiologic Evidence Linking Polychlorinated Biphenyls and Non-Hodgkin Lymphoma and the Role of Immune Dysregulation". Environmental Health Perspectives 120 (8): 1067– 75.
12. Hardell et al. Concentrations of Organohalogen Compounds and Titres of Antibodies to Epstein-Barr Virus Antigens and the Risk for Non-Hodgkin Lymphoma. Oncology Reports. (2009). 21 (6): 1567–76.
13. http://www.elmhurst.edu/~chm/vchembook/655cancer.html
14. Vines T, Faunce T "Assessing the safety and cost-effectiveness of early nanodrugs". J Law Med. 2009;. 16 (5): 822–45. PMID 19554862.
15. Heller R, Gilbert R, Jaroszeski MJ. Clinical Applications of Electrochemotherapy. Adv Drug Deliv Rev. 1999. 35 (1): 119–129.