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Analytical Method Development of Related Substances by HPLC for Bendamustine Hydrochloride

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ABSTRACT

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:

Based on the extensive literature survey, there was a lacuna for the stable dosage form of Bendamustine Hydrochloride[1-5]. It is concluded that there is a need to develop a stable dosage form of anti-neoplastic drug candidate called Bendamustine Hydrochloride. As a part of the research work, the Bendamsutine hydrochloride will be evaluated for the suitability of aqueous, non-aqueous liquid injection, lyophilized formulation with only water as solvent and lyophilized formulation with co-solvent system comprising water and solvent in a suitable ratio would be attempted. Based on the results and discussion, the conclusion will be made to identify a suitable dosage form of Bendamustine hydrochloride which gives the stable dosage form of Bendamustine hydrochloride.

Photostability[6-11]: The Bendamustine hydrochloride drug product of both strengths is available in amber-colored vials. In order to understand the light/photo stability 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.

The primary aim of the proposed project is based on the need for the study.

To meet the aim, the following objectives will be studied as a part of research work:

- Analytical method development for assay for drug substance and drug product.
- Analytical method development for related substances for drug substance and drug product.
- Analytical method development of tertiary butyl alcohol content in the finished product.
- Analytical method validation of assay, related substances and residual solvent method for the formulation containing antineoplastic drug candidate.
- Stability Study of the selected formulation following ICH guidelines including photostability.
- Reconstitution solution stability of the final formulation.
- Physiological solution compatibility study of the final formulation.

Analytical Method Development of Related Substances by HPLC

Table No. 1: Chromatographic conditions:

Column	**:**	*4.6 mm x 25 cm containing 5µm packing, Zorbax SB- C18 or equivalent*
Wavelength	**:**	230 nm
Flow rate	**:**	1.0 mL/min
Injection volume	**:**	10.0 µl
Run time	**:**	60.01 minutes
Column oven temperature	**:**	30°C
Sample cooler temperature	**:**	5°C
Diluent	**:**	Methanol

Table No. 2: Gradient program:

Time (Minutes)	**Solution A (%)**	**Solution B (%)**
0	100	0
3	100	0
16	50	50
33	30	70
35	10	90
50	10	90
55	100	0
60	100	0
60.01	STOP	

Reagents Preparation:

Preparation of Mobile phase A: 0.5% Trifluoroacetic acid in Water**:** Acetonitrile (90:10)

Preparation of Mobile phase B: 0.5% Trifluoroacetic acid in Water**:** Acetonitrile (50:50)

Preparation of Diluted Standard:

Weigh and transfer 20.0 mg of Bendamustine Hydrochloride standard into a 20 mL volumetric flask previously kept in a chilled water tray, dissolve and dilute to volume with methanol. Dilute 2.0 mL of this solution to 100.0 mL with methanol. Further, dilute 2.0 mL of this solution to 20.0 mL with methanol. (Or Prepare concentration of 0.002 mg per mL). Note: Standard preparation is stable up to 38 hours at 2-8°C.

Placebo preparation:

For 25 mg/vial:

Reconstitute 1 placebo vial with 1.0 mL of water, swirl gently and immediately transfer to the 25 mL volumetric flask, then rinse sample vial with 2 mL of methanol for three times, transfer the content into the volumetric flask dissolve and dilute to the volume with methanol.

For 100 mg/vial:

Reconstitute 1 placebo vial with 2.0 mL of water, swirl gently and immediately transfer to the 100 mL volumetric flask, then rinse sample vial with 5 mL of methanol for three times, transfer the content into the volumetric flask dissolve and dilute to the volume with methanol.

Sample Preparation:

For 25 mg/vial:

Reconstitute 1 sample vial with 1.0 mL of water, swirl gently and immediately transfer to the 25 mL volumetric flask, then rinse sample vial with 2 mL of methanol for three times, transfer the content into the volumetric flask dissolve and dilute to the volume with methanol.

For 100 mg / vial:

Reconstitute 1 sample vial with 2.0 mL of water, swirl gently and immediately transfer to the 100 mL volumetric flask, then rinse sample vial with 5 mL of methanol for three times, transfer the content into the volumetric flask dissolve and dilute to the volume with methanol.

Note: Sample preparation is stable up to 14 hours at 20-25°C.

Procedure:

Inject Blank (Diluent) one injection, standard preparation (six injections) and check the system suitability parameters.

If the system suitability parameter passes, then inject blank (one injection), Placebo (one injection) and sample preparation (one injection) and record the chromatogram.

The bracketing standard should be injected after 6 injections of sample. Calculate the % RSD of 6 standard injections (Initial 5 + 1 bracketing standard), it should be NMT 5.0.

System suitability:

The % RSD from six replicate injections of diluted standard preparation should be NMT 5.0.

The tailing factor for the Bendamustine HCl peak should be NMT 1.5.

Theoretical Plates for Bendamustine HCl peak should be NLT 3000.

Table No.3: The RT and RRTs of Bendamustine Hydrochloride peak

Name of the peak	RRT (About)	RF
Bendamustine Hydrochloride	1.00	----
Impurity-A	0.64	1.1
Impurity-B	1.24	1.8
Impurity-C	1.53	1.1

Calculation:

Calculate the content of impurities expressed as percentage of Bendamustine Hydrochloride.

$$\% \text{ Impurity} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{V} \times \frac{P}{100} \times \frac{100}{LA} \times RF$$

Where,

AT : Area response of impurity from the sample chromatogram

AS : Average area of peak response of Diluted standard chromatogram

WS : Weight of standard taken in mg

DS : Dilution of Standard preparation in mL

DT : Dilution of sample preparation in mL

V : Number of vials taken for reconstitution

P : Potency of standard in % on as-is basis

LA : Label the Amount of Bendamustine HCl in mg per vial

RF : Response factor

%Total Impurities = Impurity-A+ Impurity-B + Impurity-C+ All Unknown impurities

Analytical method development for Tertiary butyl alcohol in the finished product of Bendamustine Hydrochloride for Injection

Table No.: 4 Gas Chromatographic conditions

Column	: DB-624; 30 m x 0.530 mm x 3µm Capillary column or its equivalent
Oven temperature	: 40°C (10 min) $\xrightarrow{20^{o}C/min}$ 220ºC (0 min)
Carrier gas	: Nitrogen
Carrier gas flow rate	: 4.0 mL/min.
Injector temperature	: 200°C
Split ratio	: 101
Detector	: FID
Detector temperature	: 220°C
Hydrogen flow	: 30 mL/min.
Air flow	: 300 mL/min.
Makeup flow	: 20 mL/min.
Run time	: 19 minutes

Table No.: 5 Head space parameters

Oven temperature	:	80 °C
Loop temperature	:	90 °C
Transfer line temperature	:	110 °C
Vial equilibration time	:	20.0 min.
Vial pressurization time	:	0.02 min.
Loop fill time	:	0.05 min.
Loop equilibration time	:	0.05 min.
Injection time	:	1.0 min
GC cycle time	:	20.0 min
Vial pressure	:	14psi

Diluent Water

Blank Preparation

Transfer 5.0 mL of diluent into a Headspace vial and seal the vial immediately.

Standard stock Preparation

Weigh 400 mg of Tert-butanol standard into a 100 mL volumetric flask containing about 10 mL of diluent, diluted to volume with diluent, and mix well.

Standard Preparation

Transfer 5.0 mL of standard stock preparation into a 100 mL volumetric flask containing about 10 mL of diluent, dilute to volume with diluent and mix well. Transfer 5.0 mL of this standard preparation into a 20 mL Headspace vial and seal the vial immediately.

Sample Preparation

Weigh and transfer 200 mg of sample into a 20 ml headspace vial, add 5.0 mL of diluent and seal the vial immediately.

Procedure

Inject Blank preparation (one injection) and standard preparation (six injections) into the chromatograph and check the system suitability parameters.

If System suitability passes, inject Blank preparation (one injection), then inject Sample preparation (two injections).

System suitability

The RSD for the area response of Tertiary butanol peak from 6 injections of Standard preparation should be NMT 15.0 %.

Table No.: 6 Specification Limits

S.No.	Solvent	Limit (in ppm)
1	Tertiary butanol	NMT-5000 ppm

Calculation and Formulae

Calculate the residual solvent by using the following formula

$$\textbf{Tertiary butanol (ppm)} = \frac{A_T}{A_S} \times \frac{W_S}{D_S} \times \frac{D_T}{W_T} \times \frac{P}{100} \times 10^6$$

Where,

AT = Average area response from sample chromatogram

AS = Average area response from Standard chromatogram

WS = Weight of Standard (in mg)

DS = Dilution of Standard (in mL)

DT = Dilution of Sample (in mL)

WT = Weight of Sample (in mg)

P = Purity of Tertiary butanol

D = Density of Tertiary butanol

Analytical method validation of TBA content in Bendamustine HCl for injection:

Parameters considered for analytical method validation of Tertiary butanol content method for Bendamustine HCl for injection.

- System suitability
- Specificity
- Forced degradation

- Precision
- System precision
- Method precision
- Intermediate precision
- Stability in analytical solution
- Linearity
- Limit of Detection and Limit of Quantitation
- Accuracy
- Range

Details of the drug product:

Chemical Name:1-Methyl-5-bis(2-chloroethyl)amino-2-benzimidazolinebutryric acid hydrochloride; 4-[5-[Bis(2-chloroethyl)amino]-1-methyl benzimidazole-2- yl]

butanoic acid hydrochloride

Molecular formulae: $C_{16}H_{21}Cl_2N_3O_2 \cdot HCl$

Molecular Weight: 394.72 g/mol

CAS No.: 3543-75-7

Table No.: 7 Product Details: TBA Content Validation

Sr.No.	Materials	100 mg/Vial	25 mg/Vial
1	Bendamustine HCl	100 mg	25 mg
2	Mannitol	170 mg	42.5 mg
3	TBA	4.0 mL	1.0 mL
4	Water for Injection	q.s to 8.0 mL	q.s to 2.0 mL

METHOD DESCRIPTION:

Principle: Gas Chromatograph with FID with Headspace.

Table No.:8 GC conditions: TBA Content Validation

Column	:	AT-624; 30 m x 0.530 mm x 3µm Capillary column
		or its equivalent
Oven temperature	:	40°C (10 min) $\xrightarrow{20^{o}C/min}$ 220°C (0 min)
Carrier gas	:	Helium
Carrier gas flow	:	4.0 mL/min.
Injection temperature	:	180°C
Split ratio	:	5:1
Detector	:	FID
Detection temperature	:	200°C
Hydrogen flow	:	35 mL/min.
Air flow	:	300 mL/min.
Makeup flow	:	25 mL/min

Table No.: 9 Head space conditions:

Incubation temperature	:	70 °C
Loop temperature	:	80 °C
Transfer line temperature	:	100 °C
Vial equilibration time	:	25.0 min.
Vial pressurization time	:	0.05 min.
Loop fill time	:	0.05 min.
Loop equilibration time	:	0.05 min.
Injection time	:	1.0 min
GC cycle time	:	20.0 min

Diluent: Water

Blank: 5 mL of Water in 20mL HS vial

Standard solution:

Transfer 400 mg of Tert-butanol into a 100 mL volumetric flask, diluted to volume with diluent.

Transfer 5.0 mL of the above standard stock solution into a 100 mL volumetric flask, dilute to volume with diluent and mix well.

Transfer 5.0 mL of this solution into 20 mL HS vial.

Sample solution:

Transfer 200 mg of the sample into a 20 ml HS vial, to it add 5.0 mL of diluent.

Procedure:

- Inject Blank, Standard solution (6 injections) into the chromatograph and check the system's suitability.
- After ensuring system suitability, inject Blank (one injection), then inject Sample solution (two injections).

System suitability:

- % RSD should be NMT 15.0 %.for the area response of Tertiary butanol peak from 6 injections of Standard solution.

Specification Limit:

Tertiary butanol: NMT 5000 ppm

Calculate the Tertiary butanol (ppm) residual solvent by using the following formula:

$$= \frac{A_T}{A_S} \times \frac{W_S}{D_S} \times \frac{D_T}{W_T} \times \frac{P}{100} \times 10^6$$

Where,

AT = Average area response from sample chromatogram

AS = Average area response from Standard chromatogram

WS = Weight of Standard (in mg)

DS = Dilution of Standard (in mL)

DT = Dilution of Sample (in mL)

WT = Weight of Sample (in mg)

P = Purity of Tertiary butanol

D = Density of Tertiary butanol

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 (1 injection) and Standard preparation (6 injections) and, recorded chromatograms and checked the system suitability parameter.

Table No.: 10: Acceptance Criteria

Acceptance criteria	**Results**
The RSD for the area response of Tertiary butanol peak from 6 injections of Standard preparation should be NMT 15.0 %.	2.6%

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 Blank, Standard preparation, Sample preparation, and Sample spiked with 100% Standard and Tertiary butanol into the chromatographic system and record the retention times.]

Table No.: 11 RT Details

Solutions	**Retention time (in minutes)**
Blank	-
Standard	5.864
Sample	5.868
Smple+100 % Standard(spiked)	5.871
Tertiary butanol	5.853

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 samples. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of series of measurements.

METHOD PRECISION:

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

Analysed the samples of Bendamustine HCl six times of the same batch as per analytical procedure. Calculated the content in ppm of Tertiary butanol.

Table No.: 12 Method Precision Details

	25mg/Vial	**100mg/Vial**
Sample	**t-Butanol Conc.(ppm)**	**t-Butanol Conc.(ppm)**
1	825	819
2	885	691
3	819	771
4	801	850
5	815	850
6	885	753
Mean	**838**	**789**
% RSD	**4.4**	**7.9**

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 Tertiary butanol standard in the range of LOQ to 200% of 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 Tertiary butanol concentration (ppm) on the X-axis and Area response on the Y-axis.

Table No.: 13 Linearity Details of Tertiary Butanol:

Level	Concentration in ppm	Area response
0	0	0
1	0.5002	1787
2	1.0003	3530
3	2.0006	6586
4	4.0013	11820
5	8.0025	25066
6	10.0032	30320
7	20.0063	62502
8	50.0158	164252
9	100.032	297024
10	500.158	1543774
11	1000.315	3018243
12	2500.788	7244822
13	4001.260	11035621
14	5001.575	13859508
15	5501.733	15660811
16	6001.890	16370988
17	7502.363	20563418
18	10003.150	27872875
Correlation coefficient		**1.000**
Regression coefficient		**0.999**

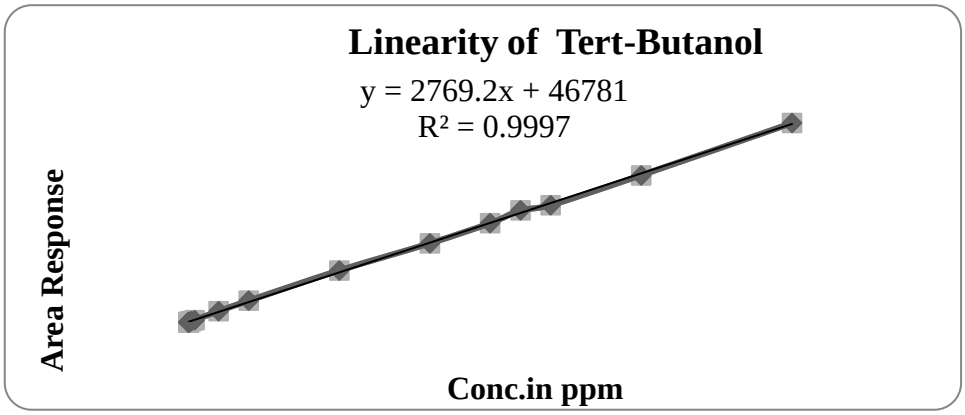

Fig No.:1 Linearity graphical presentation of Tert-Butanol

LIMIT OF DETECTION & LIMIT OF QUANTITATION:

The limit of detection is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

The limit of quantitation is the lowest amount of analyte in a sample that can be quantitated with acceptable accuracy and precision, under the stated experimental conditions.

Calculated Slope and the Residual standard deviation from the linearity curve.

From the Slope and Residual standard deviation, calculated LOD and LOQ.

LIMIT OF DETECTION (LOD):

Calculated limit of detection from linearity curve as per formulae given below.

$$LOD = \frac{3.3 \times \text{Residual Standard deviation}}{\text{Slope}}$$

LIMIT OF QUANTITATION (LOQ):

Calculated limit of quantitation from linearity curve as per formulae given below.

$$LOQ = \frac{10 \times \text{Residual Standard deviation}}{\text{Slope}}$$

Calculated the percentage of LOD and LOQ concerning sample concentration.

Table No.: 14 LOD and LOQ:

Sr. No.	**Tertiary butanol**
LOD (ppm)	0.51
LOQ (ppm)	1.52

ACCURACY:

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

Spiked known quantity of Tertiary butanol standard at 50%, 100%, 150%, and 200 % of specification limit into the Sample. Analyzed these samples in triplicate for each level. Calculated the % recovery from the results of Accuracy.

Table No.: 15 Recovery of Tertiary butanol:

Level (about)	**mg Added**	**mg Recovered**	**%Recovery**	**Mean % Recovery**
50%	0.5002	0.4943	98.8	**96.4**
	0.5002	0.4832	96.6	
	0.5002	0.4699	93.9	
100%	1.0003	0.9547	95.4	**94.1**
	1.0003	0.9359	93.6	
	1.0003	0.9334	93.3	
150%	1.5005	1.4136	94.2	**94.2**
	1.5005	1.3964	93.1	
	1.5005	1.4315	95.4	
200 %	2.0006	1.8093	90.4	**90.0**
	2.0006	1.8138	90.7	
	2.0006	1.7776	88.9	

Table No.: 16 Recovery of Tertiary butanol at LOQ level:

Level (about)	**mg Added**	**mg Recovered**	**%Recovery**	**Mean % Recovery**
LOQ	0.00032	0.00033	103.1	**104.2**
	0.00032	0.00036	112.5	
	0.00032	0.00031	96.9	

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 a suitable accuracy and linearity.

Derived the specified range from the Linearity and Accuracy studies.

Table No.: 17 Linearity Range of Tertiary butanol:

Level (Concentration in %)	Area response
50	7244822
100	13859508
150	20563418
200	27872875
Correlation coefficient	**1.000**

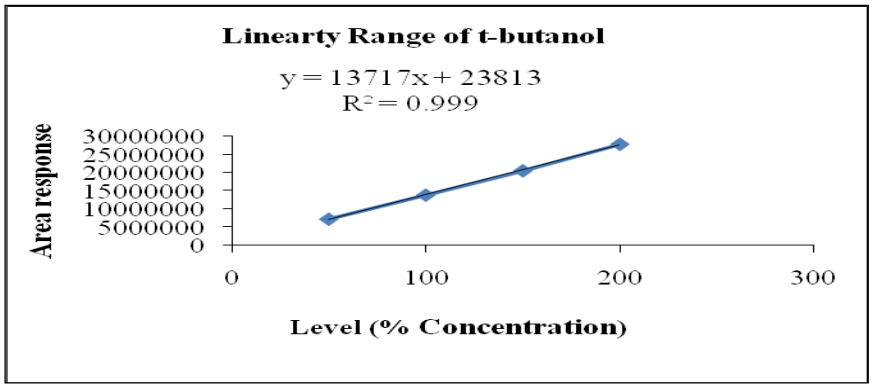

Fig No.:2 Linearity range graphical presentation of Tert-Butanol

Table No. 18: Accuracy Range of Tertiary butanol:

Level (Concentration in %)	Mean Area response
50	7028125
100	13711582
150	20593779
200	26222145
Correlation coefficient	**0.999**
%RSD	**2.9**

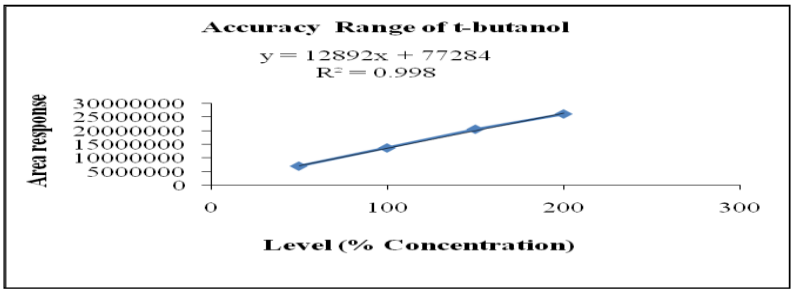

Fig No.:3 Accuracy range graphical preentation ofTert-Butanol

Related substances by HPLC:

Related substances test was carried out using validated HPLC method. Results were discussed in subsequent chapters.

Tertiary butyl alcohol content:

T-butanol content was carried using validated HPLC method. Results were discussed in subsequent chapters.

Reconstitution Solution Study Plan

Bendamustine Hydrochloride for Injection 25mg/ vial & 100 mg/vial has labeled storage condition of Bendamustine Hydrochloride for injection is Store at 20°C to 25°C (68° to 77° F). The objective of the study is to evaluate the reconstituted solution stability of Bendamustine Hydrochloride for Injection, 25 mg/vial & 100 mg/vial with the parallel comparison of one brand of marketed product, Bendamustine Hydrochloride for Injection 100 mg/vial.

As per the pack insert, the Bendamustine hydrochloride for injection 25 mg/vial is to be reconstituted with 5 mL of sterile water for injection and 100 mg/vial is with 20 mL of sterile water for injection to get 5 mg/mL of Bendamustine Hydrochloride.

Reconstitution stability*(Description, Assay & Related substances)	Initial
	30 minutes after reconstitution at Room Temperature

Reconstitution to be performed with 5 mL of sterile water for Injection USP for 25 mg/vial strength & 20 mL of sterile water for Injection USP for 100 mg/vial strength. Solutions shall be used for initial and after 30 minutes after reconstitution at room temperature.

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 the 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.

Photostability: The Bendamustine hydrochloride drug product of both strengths is available in amber colored vials. In order to understand the light/photo stability 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 is the worst case when compared to 25 mg/vial strength. The drug product of 100 mg/vial in a clear vial and amber vial were 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 & 25 mg/vial was subjected for evaluating the reconstitution solution stability along with one of the Indian marketed available samples of Bendmaustine Hydrochloride for injection 25 mg/vial. As per the pack insert, the product needs to be reconstituted with 5 mL of sterile water for injection for 25 mg/vial and 20 mL for 100 mg/vial and within 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 meet the requirements and 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 Bendasmustine hydrochloride when stored for 2 Hours room temperature and at refrigerated.

Conclusion and summary

The ultimate objective of this research work was to increase stability of Bendamustine hydrochloride lyophilized formulation which is used for treating the cancer. Since, the Bendamustine hydrochloride is soluble in water and exhibits severe degradation if formulated as liquid injectable dosage form and also nonaqueous dosage form. Lyophilization is one of the techniques employed to increase the stability of the drug and also by employing the in the present research work. Bendamustine hydrochloride was formulated by lyophilization technique for parenteral administration using co solvent system comprising tertiary butyl alcohol and water.

Hence there was a need to focus on developing stable formulations of Bendamustine hydrochloride which will be having enough shelf life and a better stability profile.

The analytical method developed and validated was found stability indicating.

The method adopted is lyophilization and formulations were prepared using co solvent system of tertiary butyl alcohol and water for injection. All the formulated lyophilized formulations were analyzed initially and subjected to short-term accelerated stability studies and further evaluation parameters of lyophilized formulations were studied till the time interval of 6^{th} month of accelerated and real time studies.

The stability results at the end of 6^{th} month of both conditions concluded that formulation containing bulk co-solvent system of 1:1 tertiary butyl alcohol and water and lyophilization cycle found to be the best formulation passing all the evaluation criteria like description, pH, assay, related substances, percentage water content, reconstitution time and tertiary butyl alcohol content were within the limits. Also reconstitution solution stability and physiological solution compatibility studies have proven that the drug product stability behavior is comparable to the reference product. The TBA content in the finished product from the initial time point to over the stability window period was well in the controlled levels. Though, TBA is not a listed ICH solvent, but based on permissible daily dose levels, maximum of

5000 parts per million limits shall be proposed. Finally, it is concluded that the cycle lyophilization developed was robust for process for the formulated Bendamustine hydrochloride.

9. 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. Global Cancer 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"