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Lyophilized Based Formulations of Bendamustine HCL

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ABSTRACT

The ultimate objective of this research work was to increase the stability of Bendamustine hydrochloride lyophilized formulation which is used for treating cancer. Since, the Bendamustine hydrochloride is soluble in water and exhibits severe degradation if formulated in liquid injectable dosage form and also 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 better stability profile. The present research work is to formulate novel injectable formulation of Bendamustine hydrochloride by lyophilization using co-solvent system. As the marketed injectable formulation of Bendamustine hydrochloride has stability and short shelf life problem. The present work is envisaged to overcome the above limitations by formulating it into a stable lyophilized form.

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INTRODUCTION:

Lyophilization[1-8] or Freeze drying fills an important need in pharmaceutical manufacturing technology by allowing drying of heat-sensitive drugs and biologicals at low temperatures under conditions that allow removal of water by sublimation, or a change of phase from solid to vapor without passing through the liquid phase. The most common application of pharmaceutical freeze drying is in the production of injectable dosage forms, the process is also used in the production of diagnostics and, occasionally, for oral solid dosage forms where a very fast dissolution rate is desired. Freeze drying of pharmaceutical solutions to produce an elegant stable powder has been a standard practice employed to manufacture many marketed pharmaceutical injectable products. There are several characteristics of the freeze dry process that make it desirable over other drying methods. Freeze drying as a practical commercial process was introduced around the time of the Second World War and found its first application in preservation of blood plasma, followed by manufacture of penicillin and other antibiotics. Application of freeze drying has continued to grow to include vaccines, steroids, vitamins, and a wide range of diagnostic products. The relative importance of freeze drying in pharmaceutical science will continue to expand with the development of the next generation of therapeutic agents from discovery research through clinical trials, FDA approval, and market introduction. Many of these new products are proteins that are chemically or physically unstable in aqueous solution and depend on maintenance of the proper secondary, tertiary, and even quaternary structure for biological activity. Formulation and manufacture of injectable dosage forms of these agents present a challenge to pharmaceutical scientists, and freeze drying will be a necessary tool for the development of these products.

Development of lyophilization cycle for formulation of the product

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique and interdependent processes; freezing, primary drying and secondary drying.

Development of Lyophilization cycle.

Table No. 1: The lyophilization parameters considered in process development

Stage of Process	Freezing	Primary Drying	Secondary Drying
Critical parameters	Freezing set temperature	Primary drying Set temperature Vacuum during Primary drying	Secondary drying set temperature Vacuum during secondary drying
Critical parameters during process	Shelf Temperature Product temperature	• Shelf Temperature • Product temperature • Vacuum vs temperature	• Shelf Temperature • Product temperature • Vacuum vs temperature

Freeze drying microscope:

Introduction to Freeze-drying Microscopy [FDM]:

Freeze-drying Microscopy is a modification of temperature-controlled microscopy in which the sealed temperature controlled stage is connected to a vacuum pump in order to generate a low-pressure system.

Freeze-drying Microscopy allows us to study the freeze-drying behavior of a formulation in micro-scale under controlled temperature and pressure. The main purpose of Freeze-drying Microscopy is to study morphological changes in the drying product as a function of temperature using a microscope. At a temperature characteristic for each formulation, the dried product will undergo first structural changes (onset of collapse, Toc). Product temperatures during primary drying must then be controlled below Toc to achieve optimum final product quality attributes.

Method of Analsyis: required quantity of the sample was injected. The sample was frozen at 10°C/min to -70°C and held for 2 minutes. Vacuum was initiated, and the temperature was slowly increased with 10°C/min through the collapse temperature. Freezing initiated temperature (in figure 1) and Collapse temperature (Tc) (in figure 2) was determined from

images displaying the first signs of structural changes within the dried layer. The solution of the drug was prepared in the concentration of 15 mg/mL. The solution was filled into vials. After filling, the vials were half stoppered with lyophilized stoppers and loaded into lyophilizer. Different lyophilization cycle were tried to optimize the cycle which gives stable and uniform cake.

Lyophilized Formulation trial using alone water for injection [Aqueous based formulation]:

Selection of suitable excipients to develop the Lyophilized dosage form: Based on the pack insert evaluation of globally available Bendamustine hydrochloride for injection, the excipients are chosen.

The possibility of Bendamustine HCl in water for injection was evaluated in order to check the feasibility and degradation profile of Bendamustine HCl for Injection in presence of 100% water. Bulk solution was prepared as per the formula mentioned in the below table.

Table No. 2: Composition details of formulation trial using 100% water for injection [aqueous based formulation] Trial 1

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Water for Injection	USP	q.s to 1 mL
4.	Nitrogen	NF	-

Brief Manufacturing Procedure:

1. Dispensed the required quantity of ingredients as per the above composition table. The required quantity (about 30%) of Water for injection was collected into the mixing vessel and maintain the temperature of the WFI at 2°C to 8°C and also purge with filtered nitrogen and remaining water for injection was kept a side for final volume make up. Temperature: 7.2°C Weighed quantity of Mannitol was added to mixing vessel under stirring. Stirred for about 5

mins. In another vessel collected the above mannitol solution and added Bendamustine Hydrochloride was under stirring to form uniform slurry. Added slurry (Step 4) under stirring. Stir the solution for 15 minutes a clear solution was obtained. Temperature: 5.6°C The solution was made to 100% using remaining WFI having temperature of2°C – 8°C from step 2.

Temperature: 5.0°C

Table No.: 3 Lyophilization cycle

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum(m.bar)
Freezing	-50	150	360	NA
Primary Drying	-25	80	500	0.250
	-11	40	360	0.250
	0	40	260	0.200
	25	30	200	0.200
	35	30	280	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 44.30 Hrs.

Note: After the completion of lyophilization cycle, the stoppering was done and then sealed. All the vials had satisfactory cake appearance.

B. Lyophilized Formulation trial using co-solvent system comprising ethanol & water for injection [solvent based formulation]

The possibility of Bendamustine HCl in water for injection with ethanol as a co-solvent was evaluated in order to check the feasibility and degradation profile of Bendamustine HCl for Injection in presence of ethanol as the degradation in water is very rapid. Bulk solution was prepared as per the formula mentioned in the below table.

Table No. 4: Composition details of formulation trial using co-solvent system comprising ethanol & water for injection [solvent based formulation] Trial 2

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Ethanol [anhydrous]	USP/BP/Ph. Eur.	0.5 mL
4.	Water for Injection	USP	q.s to 1 mL

Brief Manufacturing Procedure:

1. Dispensed the required quantity of ingredients as per the above composition table.

2. Required quantity (about 30%) of Water for injection was collected into the mixing vessel and maintained the temperature of the WFI at 2°C to 8°C and also purged with filterednitrogen and remaining water for injection was kept a side for final volume make up. Temperature: 7.2°C. Weighed quantity of Mannitol was added to mixing vessel under stirring. Stirred for about 5 mins. In another vessel collected required quantity 25 mL of ethanol [anhydrous] and added Bendamustine Hydrochloride under stirring to form uniform slurry. Add slurry (Step 4) under stirring to Step 3.Stirr the solution for 15 minutes a clear solution was obtained. Temperature: 5.6°C. The solution was made to 100% using remaining WFI having temperature of 2°C –8°C from step 2.Temperature: 5.0°C

Note: The temperature of the solution was maintained at 2°C-8°C and continuous nitrogen was sparged throughout the process. Then the solution was filled in vials (fill volume 6.66 mL) and then loaded into the lyophilizer as per the recipe mentioned in the below table.

Table No.: 5 LYOPHILIZATION CYCLE

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum(m.bar)
Freezing	-50	150	360	NA
Primary Drying	-25	80	500	0.250
	-11	40	360	0.250
	0	40	260	0.200
	25	30	200	0.200
	35	30	280	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 44.30 Hrs.

Note: After the completion of lyophilization cycle, the stoppering was done and then sealed. All the vials had a satisfactory cake appearance.

D. Lyophilized Formulation trial using co-solvent system comprising acetone & water for injection [solvent based formulation]

The possibility of Bendamustine HCl using co-solvent system comprising acetone & water for injection [solvent based formulation] was evaluated in order to check the feasibility and degradation profile of Bendamustine HCl for Injection in presence of acetone in order to minimize the degradation of Bendamustine HCl.

Table No. 6: Composition details of Formulation using co-solvent system comprising acetone & water for injection [solvent-based formulation] Trial 3

S. No.	**Raw Materials**	**Pharmacopoeial Status**	**mg/mL**
1.	Bendamustine Hydrochloride	IH	15 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	**25.52 mg**
3.	Acetone	USP/BP/Ph. Eur.	0.5 mL
4.	Water for Injection	USP	q.s to 1 mL

Brief Manufacturing Procedure:

The bulk solution was prepared as per the above procedure described under the formulation containing ethanol and water for injection. In this case, instead of adding ethanol, acetone was added.

Table No. 7: Lyophilization cycle

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum(m.bar)
Freezing	-50	150	360	NA
Primary Drying	-25	80	500	0.250
	-11	40	360	0.250
	0	40	260	0.200
	25	30	200	0.200
	35	30	280	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 44.30 Hrs.

Note: After the completion of the lyophilization cycle, the stoppering was done and then sealed. All the vials had satisfactory;y cake appearance.

Formulation trial using co-solvent system comprising tertiary butyl alcohol & water for injection [solvent based formulation]

The possibility of Bendamustine HCl using co-solvent system comprising tertiary butyl alcohol & water for injection [solvent based formulation] was evaluated in order to check the feasibility and degradation profile of Bendamustine HCl for Injection in presence of tertiary butyl alcohol in order to minimize the degradation of Bendamustine HCl. The Bulk solution was prepared as per the formula mentioned in the below table.

Table No.:8 Composition details of formulation trial using co-solvent system comprising tertiary butyl alcohol & water for injection [solvent based formulation] Trial 4

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary Butyl alcohol	USP/BP/Ph. Eur.	0.5 mL
4.	Water for Injection	USP	q.s to 1 mL
5.	Nitrogen	NF	-

Brief Manufacturing Procedure:

The bulk solution was prepared as per the above procedure described under the formulation containing ethanol and water for injection. In this case, instead of adding ethanol, tertiary butyl alcohol was added. **Note:** The temperature of the solution was maintained at 2°C-8°C and continuous nitrogen was sparged throughout the process

Table No. 9: LYOPHILIZATION CYCLE

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum(m.bar)
Freezing	-50	150	360	NA
Primary Drying	-25	80	500	0.250
	-11	40	360	0.250
	0	40	260	0.200
	25	30	200	0.200
	35	30	280	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 44.30 Hrs. Note: After the completion of lyophilization cycle, the stoppering was done and thensealed. All the vials had satisfactory cake appearance.

Tertiary butanol ratio optimization through holdtime study:

Based on the above study of finding suitable solvent for yielding better control on the impurity profile, a solvent system comprising water and tertiary butyl alcohol was finalized. The various ratios of solvent system comprising water and tertiary butyl alcohol was attempted and the details are given below.

Table No. 10: The ratio of TBA: Water and process temperature and hold time evaluation

Sl. No.	Trial Number	TBA:WATER	Process Temperature & Hold time evaluation
1	Trial 5	0.2:0.8	2 to 8°C and hold time evaluation at 2 to 8°C
2	Trial 6	0.3:0.7	2 to 8°C and hold time evaluation at 2 to 8°C
3	Trial 7	0.4:0.6	2 to 8°C and hold time evaluation at 2 to 8°C
4	Trial 8	0.5:0.5	2 to 8°C and hold time evaluation at 2 to 8°C

Note: The details of the above batches are given below Trial with TBA: WATER is 0.2:0.8 [Trial 5]

Table No. 11: Composition details of TBA: WATER [0.2:0.8] Bulk solution as per below table. Trial 5

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary butanol	IH	0.2 mL/mL
4.	Water for Injection	USP	q.s to 1 mL

Brief Manufacturing Procedure:

Dispensed the required quantity of ingredients as per the above composition table. Required quantity (about 30%) of Water for injection was collected into the mixing vessel and maintained the temperature of the WFI at 2°C to 8°C and also purge with filtered nitrogen and remaining water for injection was kept a side for final volume make up. Temperature: 7°C Weighed quantity of Mannitol was added to the mixing vessel under stirring. Stirred for about 5 mins. In another vessel collected required quantity 10 mL of Tertiary butanol was taken and added Bendamustine Hydrochloride under stirring to form uniform slurry. Add slurry under stirring to Step 3.Stirr the solution for 15 minutes a clear solution was obtained. Temperature: 5°C. The solution was made to 100% using remaining WFI having a temperature of2°C – 8°C of step 2. Temperature: 5°C. **Note:** The temperature of the solution was maintained at 2°C-8°C and continuous nitrogen was sparged throughout the process. The Solution was also stored in the room temperature to check the impact oftemperature on assay & RS and in the refrigerated sample.

I. Trial with TBA: WATER is 0.3:0.7 Composition [Trial 6]

Table No. 12: Composition details of TBA: WATER [0.3:0.7] Bulk solution

S. No.	Raw Materials	PharmacopoeialStatus	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary butanol	IH	0.3 mL/mL
4.	Water for Injection	USP	q.s to 1 mL

Brief manufacturing procedure: as described above in 0.2:0.8 ratio experiment. The parameters observed during this manufacturing are given below

Temperature of the solution was observed 3°C [after the addition of Bendamustine Hydrochloride] Temperature of the solution was observed 5°C [after the 100% makeup]

II. Trial with TBA: WATER is 0.4:0.6 Composition: [Trial 7]

Table No. 13: Composition details of TBA: WATER [0.4:0.6] Bulk solution

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary butanol	IH	0.4 mL/mL
4.	Water for Injection	USP	q.s to 1 mL

Brief manufacturing procedure: as described above in 0.2:0.8 ratio experiments.Tt

he parameters observed during this manufacturing are given below Temperature of the solution was observed 4°C [after the addition of Bendamustinehydrochloride]

The Temperature of the solution was observed 5°C [after the 100% makeup]

III. Trial with TBA: WATER is 0.5:0.5 Compositions: [Trial 8]

Table No. 14: Composition details of TBA: WATER [0.5:0.5] Bulk solution

S. No.	Raw Materials	Pharmacopoeial Status	Mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary butanol	IH	0.5 mL/mL
4.	Water for Injection	USP	q.s to 1 mL

The parameters observed during this manufacturing are given below Temperature of solution was observed 6°C [after the addition of Bendamustine Hydrochloride] Temperature of the solution was observed 4°C [after the 100% makeup]

General note on the usage of TBA in the formulation

The toxicity of TBA is low. According to the ICH Guidelines, solvents are divided into three different categories: class 1, class 2 and class 3 solvents, with class 1 indicating extremely high toxicity, and class 3 indicating very low toxicity. Although not listed in the ICH Guidelines for Residual Solvents, TBA is likely to fall in the category of a Class 3 solvent based on its similarity of LD50 toxicity data for other Class 3 solvents. As per ICH, Class 3 solvent is said to have PDEs of 50 mg or more per day, Assuming a TBA content of 0.5%w/w a patient may take up to 10 g of solid dispersion, since the maximally allowed dose for Class 3 solvents is 50 mg/day. Obviously, toxicity is not an issue. Water for Injection and *tertiary*-Butyl Alcohol are co-solvents that would be used in the compounding process,

Formulation of Lyophilized Bendamustine Hydrochloride for Injection Formulation with 1:1 Ratio of Water: Tertiary Butyl Alcohol. Lab Scale batch of Bendamustine Hydrochloride for Injection 25 mg/vial

Table No. 15: Composition details Lab scale batch of Bendamustine HCl FOI 25mg/vial Trial 9

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary Butyl alcohol	IH	0.5 mL
4.	Water for Injection	USP	q.s to 1 mL

Brief manufacturing procedure: The above composition of bulk solution was manufactured as described in the earliertrials.

Fill volume and lyophilization: 1.66 mL of the above bulk solution in 8 mL amber vial, partially stoppered and then lyophilized.

Table No. 16: LYOPHILIZATION CYCLE:

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum (m.bar)
Freezing	-50	90	200	NA
Primary Drying	-25	40	300	0.250
	-11	40	140	0.250
	0	40	120	0.200
	25	30	200	0.200
	35	30	300	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 31 Hrs.

Note: After the completion of the lyophilization cycle, the stoppering was done and then sealed. All the vials had satisfactory cake appearance.

Lab Scale batch of Bendamustine Hydrochloride for Injection 100 mg/vial

Table No. 17: Composition details Lab scale batch of Bendamustine HCl FOI 100 mg/vial. Trial 10

S. No.	Raw Materials	Pharmacopoeial Status	mg/mL
1.	Bendamustine Hydrochloride	IH	15.00 mg
2.	Mannitol[PFG]	USP/BP/Ph. Eur.	25.52 mg
3.	Tertiary Butyl alcohol	IH	0.5 mL
4.	Water for Injection	USP	q.s to 1 mL

Brief manufacturing procedure:

The above composition of bulk solution was manufactured as described in the earliertrials. **Fill volume and lyophilization:** 6.66 mL of the above bulk solution in 20 mL amber vial, partially stoppered and then lyophilized.

Table No. 18: LYOPHILIZATION CYCLE

Stage	Temperature (°C)	Ramp (mins)	Soak (mins)	Vacuum(m.bar)
Freezing	-50	150	360	NA
Primary Drying	-25	80	500	0.250
	-11	40	360	0.250
	0	40	260	0.200
	25	30	200	0.200
	35	30	280	0.100
Secondary Drying	45	30	300	0.05

Total cycle time: 44.30 Hrs. Note: After the completion of lyophilization cycle, the stoppering was done and thensealed. All the vials had a satisfactory cake appearance.

Table No.: 19: Lyophilized Formulation trial using alone water for injection [Aqueous based formulation]

Sl. No.	**Test Parameters**	**Results**
1	Description	White to off-white lyophilized cake
2	Reconstitution time	2 min 50 seconds
3	pH [Reconstituted solution]	3.02
4	Water content by KF	1.78% w/w
5	Assay by HPLC	96.4%
6	Related substances by HPLC	Imp A:3.84%Imp B:0.16%Imp C:0.04% Highest UNK Imp:0.31% Total Imp: 4.52%

Table No.: 20: Lyophilized Formulation trial using co-solvent system comprising ethanol & water for injection [solvent based formulation]

Sl. No.	Test Parameters	Results
1	Description	White to off-white lyophilized powder
2	Reconstitution time	1 min 45 seconds
3	pH [Reconstituted solution]	3.18
4	Water content by KF	1.36% w/w
5	Assay by HPLC	100.4%
6	Related substances by HPLC	Imp A:2.84%Imp B:0.13%Imp C:0.03% Highest UNK Imp:0.11% Total Imp: 3.18%

Table No.: 21: Lyophilized Formulation trial using co-solvent system comprising acetone & water for injection [solvent based formulation]

Sl. No.	Test Parameters	Results
1	Description	White to off-white lyophilized powder
2	Reconstitution time	1 min 10 seconds
3	pH [Reconstituted solution]	3.18
4	Water content by KF	1.24% w/w
5	Assay by HPLC	100.4%
6	Related substances by HPLC	Imp A:1.82%Imp B:0.23%Imp C:0.08% Highest UNK Imp:0.11% Total Imp: 2.24%

Table No.: 22: Lyophilized Formulation trial using co-solvent system comprising tertiary butyl alcohol & water for injection [solvent basedformulation]

Sl. No.	Test Parameters	Results
1	Description	White to off-white lyophilized powder
2	Reconstitution time	50 seconds
3	pH [Reconstituted solution]	3.09
4	Water content by KF	0.86% w/w
5	Assay by HPLC	100.4%
6	Related substances by HPLC	Imp A:0.81%Imp B:0.13%Imp C:0.02% Highest UNK Imp:0.09% Total Imp: 1.14%

Tertiary butanol ratio optimization through hold timestudy:

Table No.: 23: Physical and chemical evaluation of aqueous Bendamustine Hydrochloride Formulations containing different concentrations of Water: Tertiary Butyl Alcohol

Sl. No.	Formulation codes	Description		pH		Assay		Related substances	
		Initial	24 Hrs	Initial	24 Hrs	Initial	24 Hrs	Initial	24 Hrs at 2 to 8
1	CSF1 [100% water]	#	#	2.72	2.84	88.42 %	85.42 %	Imp A:9.82% Imp B:0.25% Imp C:0.12% Highest UNK Imp:0.08% Total Imp:11.26%	Imp A:10.14% Imp B:0.28% Imp C:0.17% Highest UNK Imp:0.10% Total Imp:12.18%
2	CSF2 [02TBA:08Water]	#	#	2.84	2.81	99.62 %	99.12 %	Imp A:2.45% Imp B:0.08% Imp C:0.12% Highest UNK Imp:0.08% Total Imp:2.86%	Imp A:3.01% Imp B:0.10% Imp C:0.16% Highest UNK Imp:0.05% Total Imp:3.98%
3	CSF3 [03TBA:07Water]	#	#	2.79	2.86	98.56 %	97.52 %	Imp A:1.95% Imp B:0.04% Imp C:0.16% Highest UNK Imp:0.12% Total Imp:2.34%	Imp A:2.05% Imp B:0.08% Imp C:0.16% Highest UNK Imp:0.14% Total Imp:3.74%
4	CSF4 [04TBA:06Wat	#	#	2.94	2.88	98.56 %	97.23 %	Imp A:1.38% Imp B:0.08%	Imp A:1.88% Imp B:0.14%

	er]							Imp C:0.12% Highest UNK Imp:0.11% Total Imp:1.61%	Imp C:0.18% Highest UNK Imp:0.14% Total Imp:2.41%
5	CSF5 [05TBA:05Water]	#	#	2.85	2.91	97.58 %	96.42 %	Imp A:0.89% Imp B:0.02% Imp C:0.14% Highest UNK Imp:0.04% Total Imp:1.04%	Imp A:0.99% Imp B:0.04% Imp C:0.16% Highest UNK Imp:0.08% Total Imp:1.94%

CSF: Co solvent formulation, # is clear colorless solution, Imp: Impurity, UNK:unknown,

Table No.: 24, Lab scale batch lyophilization cycle recipe of Bendamustine Hydrochloride for Injection 25 mg/vial

Sl. No.	Test Parameters	Results
1	Description	White to off-white lyophilized powder or cake
2	Reconstitution time	35 seconds
3	pH [Reconstituted solution]	3.02
4	Water content by KF	1.15% w/w
5	Assay by HPLC	99.6%
6	Related substances by HPLC	Imp A:0.86%Imp B:0.11%Imp C:0.04% Highest UNK Imp:0.05%Total Imp: 1.17%
7	Tertiary butyl alcohol content	898 ppm

Table No.: 25, Lab scale batch lyophilization cycle recipe of Bendamustine Hydrochloride for Injection 100 mg/vial

Sl. No.	Test Parameters	Results
1	Description	White to off-white lyophilized powder or cake
2	Reconstitution time	45 seconds
3	pH [Reconstituted solution]	3.12
4	Water content by KF	0.85% w/w
5	Assay by HPLC	98.6%
6	Related substances by HPLC	Imp A:0.91%Imp B:0.12%Imp C:0.02% Highest UNK Imp:0.06%Total Imp: 1.21%
7	Tertiary butyl alcohol content	928 ppm

Table No.: 26 Evaluation of Indian market samples of Bendamustine HCl forinjection 5mg/vial

Sl. No.	Test Parameters	Results		
1	Brand names	**ABC**	**MNO**	**XYZ**
2	Description	$	$	$
3	Reconstitution time	80 seconds	92 seconds	74 seconds
4	pH [Reconstituted solution]	3.12	3.45	3.29
5	Water content by KF	1.85% w/w	1.76% w/w	1.91% w/w
6	Assay by HPLC	98.6%	98.24%	97.26%
7	Related substances by HPLC			
	Impurity A	1.42%	1.68%	1.94%
	Impurity B	0.08%	0.09%	0.12%
	Impurity C	Not detected	0.02%	0.01%
	Single max impurity	0.18%	0.19%	0.21%
	Total impurities	1.75%	2.06%	2.34%
8	Tertiary butyl alcohol content	1128 ppm	1211 ppm	963 ppm

$: White to off white lyophilized powder

Table No.: 27 Evaluation of Indian market samples of Bendamustine HCl for injection 100 mg/vial

Sl. No.	Test Parameters	Results		
1	Brand names	**ABC**	**MNO**	**XYZ**
2	Description	$	$	$
3	Reconstitution time	90 seconds	85 seconds	88 seconds
4	pH [Reconstituted solution]	3.09	3.18	3.18
5	Water content by KF	1.75% w/w	1.96% w/w	1.51% w/w
6	Assay by HPLC	98.6%	97.98%	97.25%
7	Related substances by HPLC			
	Impurity A	1.62%	1.87%	2.04%
	Impurity B	0.06%	0.12%	0.16%
	Impurity C	0.02%	0.04%	0.03%
	Single max impurity	0.09%	0.16%	0.19%
	Total impurities	1.85%	2.26%	2.45%
8	Tertiary butyl alcohol content	1312 ppm	1141 ppm	1063 ppm

$: White to off white lyophilized powder

Stability Study of Optimized Formulation: Bendamustine hydrochloride for injection 100 mg/vial

Table No.: 28 Optimization/Stability batch lyophilization cycle recipe of Bendamustine Hydrochloride for Injection 100 mg/vial

Sl. No.	Test Parameters	Results						
		Initial	**40/75 1M**	**40/75 2M**	**40/75 3M**	**40/75 6M**	**25/60 3M**	**25/60 6M**
1	Description	$	$	$	$	$	$	$
2	Reconstitution time [in seconds]	45	50	42	45	55	50	50
3	pH [Reconstituted solution]	3.12	3.08	3.15	3.09	3.12	3.05	3.16
	Water contentby							

4	KF [% w/w]	0.85	0.91	1.19	1.08	0.96	1.09	1.12
5	Assay by HPLC [in %]	98.6	99.24	99.12	99.63	98.91	99.24	99.32
6	Related substances by HPLC	0.91%	1.04%	1.08%	0.98%	0.90%	1.08%	0.89%
		0.12%	0.13%	0.14%	0.09%	0.11%	0.14%	0.12%
	Imp C	0.02%	0.04%	0.02%	0.05%	0.02%	0.04%	0.04%
		0.06%	0.04%	0.05%	0.08%	0.03%	0.05%	0.04%
	Total Imp:	1.21%	1.31%	1.38%	1.24%	1.10%	1.39%	1.17%
7	Tertiary butyl alcohol content [in ppm]	928	889	931	842	911	898	908

$: White to off white lyophilized powder, HUNK: Highest Unknown impurity.

Lyophilization Trial with Water Alone:

The lyophilized formulation containing water alone has much higher content of % impurity A and unknown impurity in related substances tests and the assay test parameter was also found less. However, the other tested parameters were found satisfactory. Hence in order to control the degradation due to water, the bulk formulation was tried using ethanol, acetone and Tertiary Butyl alcohol would betried.

Lyophilization Trials with Co-Solvent System:

Hence, lyophilized formulation trial using co-solvent system comprising ethanol & water for injection was tried but the content of % impurity A was much higher though assay test parameter and other test parameters were found satisfactory. An attempt was also made to check the possibility of controlling the % impurity A by using acetone: water as co-solvent system. When, lyophilized Formulation trial using co-solvent system comprising acetone & water was tried, again % content of impurity A was higher in this case also but however, the other tested parameters were found satisfactory. Lyophilized formulation trial using co-solvent system comprising tertiary butyl alcohol & water for injection trial gave a very well controlled content of % impurity A and other impurities also. The assay test parameter and other test parameters were found satisfactory. Hence the co-solvent system comprising the tertiary butyl alcohol and water is finalized for further developmental activity. While developing lyophilized dosage form, an attempt was made to develop lyophilized which is solvent free containing only water, however, there was significant amount of hydrolytic impurity [impurity A] was observed indicating the need of co-solvent in the lyophilized formulation trials of bendamsutine hydrochloride. Based on the results of lyophilized formulation when attempted with 100% water, study was carried in order to have one such solvent which gives better stability and also an acceptable to human consumption use by safety and efficacy perse.

CONCLUSION:

In conclusion, the overall results of this study has shown clearly that the usage of tertiary Butyl alcohol as a co-solvent system in the bulk solution stage is able to yield the better stable dosage form of Bendammustine Hydrochloride. The present research work was designed to develop a stable injectable dosage form of anti-neoplastic drug candidate called Bendamsutine Hydrochloride. Since, the Bendamustine Hydrochloride is very sensitive to

water hydrolysis and the controlling the impurity levels by designing a co-solvent system in the bulk formulation and subsequentremoval of co solvent through lyophilization system in the present research work was focused.

During preformulation study, it was confirmed that the active substance is Bendamustine hydrochloride.

Also the objective of the research work was to find out the feasibility of developing liquid formulations of Bendamsutine hydrochloride. It was concluded that there is no feasibility of developing aqueous formulations due to severe degradation of hydrolytic impurity in the aqueous environment.

Further to the unsatisfactory results of aqueous formulation attempts, nonaqueous trials of Bendamustine hydrochloride formulations were tried and yielded better results when compared to aqueous formulations of Bendamustine hydrochloride. But there is a scope to work further on fine tuning control of impurities in non-aqueous formulations of Bendamustine Hydrochloride.

While developing lyophilized dosage form, attempt was made to develop lyophilized which is solvent free containing only water, however, there was significant amount of hydrolytic impurity [impurity A] was observed indicating the need of co-solvent in the lyophilized formulation trials of bendamsutine hydrochloride.

Based on the results of lyophilized formulation when attempted with 100% water, study was carried in order to have one such solvent which gives better stability and also acceptable to human consumption use by safety and efficacy perse.

After having identified tertiary butyl alcohol as identified solvent, an optimization trials of Bendamustine hydrochloride bulk formulation with co-solvent approach with different ratios of Water: Tertiary butyl alcohol was attempted. From this study, it was concluded that bulk formulation having 1:1Water: Tertiary butyl alcohol gave satisfactory results.

Further, based on the optimization trial, a lyophilized formulation having 1:1 ratios ofWater: Tertiary butyl alcohol was finalized.

Further, the critical aspect of the research study was the development of lyophilization cycle development,

Process Step: Freezing Process design criteria:

- Collapse temperature of product initiation at: - 26.2°C.

The shelf temperature in the freezing was initiated at -50°C to ensure the complete freezing of the formulation components. The product temperatures of less than - 34°C shall be attained by gradually cooling the shelf from room temperature to -50 °C. **Process step: primary drying:**

Process Design Criteria:

The shelf temperature in primary drying was ramped up from –34°C to +42°C in Twelve steps [both in the case of 25 mg/vial & 100 mg/vial]. The heating rate during early steps of this rampup was kept below 2°C per hour [for both strengths]. This ensures a gradual heating of the product vial the vacuum was maintained at 0.25 mbar such that melt back during the process does not happen.

During ramp-up of shelf temperature from –34°C to +42°C, the chamber pressure was increased from 0.250 mbar to 0.100 mbar. This is done gradually by increasing the chamber pressure in a programmed manner. This increase in chamber pressure is necessary to ensure that product does not melt back during the process.

Process Step: Secondary DryingProcess Design Criteria:

Secondary drying of the product was set at +42°C. The chamber pressure was maintained at 0.100mbar. During secondary drying there will be rapid loss of free moisture from the product. Hence, a low chamber vacuum would ensure that moistureis removed efficiently.

After the completion of the lyophilization cycle, stoppering was done by backfilling the chamber with 0.2 µm filtered nitrogen.

After developing the lyophilization cycle, the cycle parameter of 25 mg/vial strength and 100 mg/vial strength was applied to lab scale and optimization stability batches.

The lyophilized dosage forms were characterized for the required critical parameters like description, reconstitution time, description of reconstituted solution, water content, pH, assay and related substances and tertiary butyl alcohol content test. The tested parameters were found satisfactory and also much comparable to the marketed available formulations of

Indian market.

Measurement of tertiary butyl content in the lyophilized product was also in line with the marketed product. From the photostability study, it was concluded that ambercolor vial is suitable for Bendamustine hydrochloride formulations.

Short time reconstitution solution stability were assessed and it was found that the lyophilized drug product after reconstitution again forma hydrolytic impurity [impurity A] when the lyophilized product is reconstituted for the required amount of time. However, the similar analytical trend was observed in the marketed reference product in the reconstitution solution study assessment.

Physiological solution compatibility study proven that the drug product is compatible to the diluents mentioned in the pack insert and also at the concentration of 0.2 mg/mL and 0.6 mg/mL as per the recommended time points in the pack insert.

Considering the above all points, it was concluded that lyophilization technique was adopted to develop a stable form of Bendamustine hydrochloride which has the following potential advantages:

lyophilization technique was adopted which has the following potential advantages: • Rapid and easy dissolution of reconstituted product.

- Enhanced product stability in dry state.
- Removal of water and solvent without excessive heating of the product.
- Ease of processing a liquid, which simplifies aseptic handling in the commercialization scale

Finally it is concluded that the lyophilized formulation when the bulk formulation of Bendamustine Hydrochloride was formulated using 1:1 water: tertiary butyl alcohol is a suitable to increase the stability of formulation.

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