REPORT FOR DETERMINATION OF BENDAMUSTINE HYDROCHLORIDE API CONCENTRATION LEVELS IN DETOXIFIED PROCESS EFFLUENT

Block : I
Product : Bendamustine Hydrochloride

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APPROVAL PAGE

Signing of this approval page of miscellaneous study report indicates agreement with the methodology and the various factors captured in this report.

	Name	Designation	Sign/Date
Prepared by	Mr. CH. Rambabu	Sr. Executive-PD	02/09/23

	Name	Designation	Sign/Date
Reviewed by	Mr. Girish M Bhandare	Manager-PD	02/09/23
	Mr. Veereshappa	Sr. GM-PD	02/09/23
	Mr. B.K. Mangaraj	Sr. GM–QC	02/09/23
	Mr. Elangovan R.	DGM-EHS	02/09/23
	Mr. N. Prasad Achanta	Manager-QA	04/09/23

	Name	Designation	Sign/Date
Approved by	Mr. N. Saravanan	AGM-QA	04/09/23

1.0 OBJECTIVE:

Purpose of this report is, to evaluate the efficiency of 5 % Sodium hydroxide solution as detoxification agent and to prove that 5 % Sodium hydroxide solution is capable to detoxify the Bendamustine hydrochloride API to not detection levels in process effluent.

2.0 SCOPE:

This report is applicable for estimation of Bendamustine hydrochloride API concentration levels in detoxified process effluent / washings, which is manufactured in I Block at M/s. Shilpa Pharma Lifesciences Limited, Unit-2.

3.0 RESPONSIBILITIES:

3.1 Production department personnel to prepare the report for determination of Bendamustine hydrochloride API concentration levels in detoxified process effluent.

3.2 Production Head or his authorized designee is responsible to review the report.

3.3 QA, QC & EHS department personnel are responsible to review the report.

3.4 QA Head or his authorized designee is responsible to approve of the report.

4.0 ABBREVIATIONS/DEFINITIONS:

4.1 ABBREVIATIONS:

QA	:	Quality Assurance	U-2	:	Unit-2
ETP	:	Effluent Treatment Plant	RPT	:	Report
ADI	:	Acceptable Daily Intake	PC	:	Protocol
EHS	:	Environment, Health & Safety	GEN	:	General
API	:	Active Pharmaceutical Ingredients	PD	:	Production
CR&D	:	Chemical Research and Development	QC	:	Quality Control
HPLC	:	High Pressure Liquid Chromatography			
CAS	:	Chemical Abstracts Service Registry Number			
IUPAC		International Union of Pure and Applied Chemistry			
SAP	:	Systems, Applications and Products in Data Processing			

4.2 DEFINITIONS:

4.2.1 **Detoxification:** Detoxification is a process to remove the toxicity of substance or neutralize the toxicity of the substance.

4.2.2 **API:** Active Pharmaceutical Ingredient (API) (or drug substance): Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an

active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure and function of the body.

4.2.3 **Cytotoxic API:** Cytotoxic drugs (sometimes known as antineoplastic) describe a group of medicines that contain chemicals which are toxic to cells, preventing their replication or growth, and so are used to treat cancer.

5.0 REASON FOR STUDY:

Bendamustine hydrochloride API manufacturing process is validated and is being produced commercially on regular basis. During product development CR&D has established the detoxification procedure and concluded that, 5 % Sodium hydroxide solution is suitable for detoxification of Bendamustine hydrochloride API. Accordingly CR&D detoxification procedure has been adopted for commercial use and 5 % Sodium hydroxide solution is being used for detoxification agent for Bendamustine hydrochloride API prior to send the effluent for further treatment.

As part of continual improvement, challenging study is planned to re-assess the efficiency of detoxification agent (5 % Sodium hydroxide solution) for detoxification of Bendamustine hydrochloride API.

6.0 PROCEDURE:

6.1 General Information's of Bendamustine hydrochloride API:

Name of the API	Bendamustine hydrochloride
Description	White to off white crystalline powder
IUPAC Name	4-[5-[bis (2-chloroethyl)amino]-1-methylbenzimidazol-2-yl] butanoic acid; hydrochloride
CAS Number	3543-75-7
Pharmacological Activity	Antineoplastic Agent, Alkylating
Molecular Formula	C16H22Cl3N3O2
Molecular Weight	394.7 g / mol
ADI Value (µg/kg/day)	1.83
Solubility	Freely soluble in methanol, sparingly soluble in water and insoluble in chloroform.
Detoxification Agent	5 % Sodium Hydroxide Solution.

Handling Precaution	Restrict access to work area. Avoid open handling. Ground and bond all bulk transfer equipment. Minimize dust generation. Use process containment, local exhaust ventilation or perform work under fume hood/fume cupboard. Avoid inhalation and contact with skin, eyes, and clothing. When handling, use appropriate personal protective equipment. Wash hands and any exposed skin after removal of PPE.

6.2 **Analysis Results:**

Detoxified solution samples was analyzed by QC personnel as per procedure define in method of analysis Bendamustine hydrochloride API content (BY HPLC) in section (7.0) and found that, Bendamustine hydrochloride API content was not detected.

Samples Analysis Details			
Sample No.	**Sample from decontamination tank**	**Acceptance Criteria**	**Bendamustine hydrochloride contents in detoxified samples**
Sample-1 (Detoxified Effluent)	Day-1	Not Detected	Not Detected
Sample-2 (Detoxified Effluent)	Day-2	Not Detected	Not Detected
Sample-3 (Detoxified Effluent)	Day-3	Not Detected	Not Detected

7.0 METHOD OF ANALYSIS API CONTENT (BY HPLC):

The effluent samples were analyzed for Bendamustine hydrochloride API concentration levels as per regular Bendamustine hydrochloride API method of analysis. Method of analysis is mentioned below,

Chromatographic conditions:

Instrument	:	HPLC with UV detector and Empower-2 software or equivalent.
Column	:	Zorbax SB-C18 5µ, 250 x 4.6 mm or equivalent.
Flow rate	:	1.0 mL/min.
Wavelength	:	230 nm
Injection volume	:	10 µL
Column temperature	:	30°C ± 2 °C
Sample temperature	:	5 °C

Preparation of Buffer Solution: Transfer 1 mL of Trifluoroacetic acid in 1000 mL volumetric flask containing 500 mL of water, mix well and dilute with water.

Preparation of Mobile Phase-A: Prepare a mixture of Buffer solution and Acetonitrile in the ratio of 90:10 v/v, filter through 0.45µ membrane filter paper and degas.

Preparation of Mobile Phase-B: Prepare a mixture of Buffer solution & Acetonitrile in the ratio of 50:50 v/v, filter through 0.45µ membrane filter paper and degas.

Blank: Methanol

Preparation of Standard Solution:

Weigh accurately and transfer 20 mg of Bendamustine Hydrochloride standard into 20 mL volumetric flask, dissolve and dilute to volume with methanol. Dilute 2 ml of this solution to 100 mL with methanol. Further dilute 2 ml of this solution to 20 mL with methanol.

Preparation of Test Solution:

Weigh accurately and transfer 25 mg of Bendamustine Hydrochloride sample into 25 mL volumetric flask. Dissolve and dilute to volume with methanol.

Solution stability: Standard and sample solutions are stable up to 24 hours at 25°C±2°C and 5°C±3°C temperature.

Gradient Programme:

Time (min)	Mobile phase-A (%) v/v	Mobile phase-B (%) v/v
0	100	0
3	100	0
16	50	50
33	30	70
35	10	90
50	10	90
55	100	0
60	100	0

Procedure: Create sequence as follow and inject the solution according to sequence.

Solution	No. of Injections
Blank	1
Standard solution	6
Blank	1
Test solution	1
Standard solution (Bkt)	1

Examine the blank chromatogram for any extraneous peaks and disregard any corresponding peaks observed in the chromatogram of test preparation.

The Retention time of Bendamustine is about 25.0 minutes.

System suitability parameter:

Relative standard deviation for the peak area response of six injections of standard solution should not be more than 5.0 %.

Calculation: Calculate % of Bendamustine HCl as follow

$$= \frac{A}{B} \times \frac{C}{20} \times \frac{2}{100} \times \frac{2}{20} \times \frac{25}{D} \times \frac{P}{100} \times 100$$

Where,

A : Peak area response of Bendamustine obtained in the Chromatogram of sample solution.

B : Average peak area response of Bendamustine HCl obtained in the chromatogram of reference solution.

C : Weight of Bendamustine HCl in standard solution in mg.

D : Weight of Sample in mg.

P : Purity of Bendamustine HCl standard on as is basis in %w/w.

Reporting: Report as a value in % w/w.

8.0 CONCLUSION:

Based on the analytical results, it is evident that 5 % Sodium hydroxide solution is capable to detoxify the Bendamustine hydrochloride API to not detection levels in 24 hours.

Hence, it is conducted that, the existing practice of using 5 % Sodium hydroxide solution as detoxification agent for the Bendamustine hydrochloride API is adequate and the same can be continued for routine usage.

9.0 DISTRIBUTION RECORD:

S.No.	Department / Block	No. of Copies	Remarks
1.	I	01	----
2.	QA	01	----
3.	QC	01	----
4.	EHS	01	----

10.0 ATTACHMENTS:

10.1 Detoxification and Disposal Procedure : Attachment-I

10.2 Requisition Slip & Results for Analysis of Process Effluent Samples : Attachment-II

10.3 QC Executed Analytical Work Sheets and Chromatograms : Attachment-III