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Development of analytical procedure for the determination of Bendamustine Hydrochloride in Pharmaceutical Formulations

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Abstract
The aim of the method is to develop analytical procedure for the determination of Bendamustine Hydrochloride in Pharmaceutical Formulations. The analytical procedure for determination of Assay in finished product of Bendamustine Hydrochloride in Bendamustine Hydrochloride for injection 25 mg and 100 mg per vial is an In-House procedure.The method shall be validated for the System precision, Method precision and Intermediate precision. The Chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

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I. Introduction

In order promote a good public health, validation of analytical procedures is done to ensure quality, safety and efficacy of therapeutic drugs used for public health. It's very important to determine the content of Active Pharmaceutical Ingredient or drug content in the presence of excipients, Impurities or various inert substances that originate from raw materials, key starting materials, intermediates, byproducts, manufacturing process steps, impurities that are formed during drug excipient interactions, degradation impurities etc but not limited to. The validation of analytical procedures is done in order to assure that drug formulations are prepared in a most efficient and cost effective manner.

Bendamustine hydrochloride is a nitrogen mustard alkylating agent, structurally related to chlorambucil, which has been elaborated in 1962 in the former German Democratic Republic, and since its very clinical introduction in 1969 has been used exclusively in this country up until the reunion of Germany [1-3]. Bendamustine hydrochloride is among the fi rst rationally designed alkylating drugs, whose structure comprises three pharmacophore moieties: the bis-2-chloroethylamine alkylating group, a benzimidazole ring serving as a purine base mimic (suggesting possible antimetabolite effects), and a butyric acid side chain to increase water solubility [4-6]. The rapid degradation of the drug in serum and the extensive liver metabolism impair its cytotoxic action within a short period of time, necessitating application of relatively high doses [7].

Bendamustine bearing the name Treanda is a chemotherapic medication used in the treatment of chronic lymphocytic leukemia, multiple myeloma, and non-hodgkins lymphoma. Bendamustine is a white, watersoluble microcrystalline powder with amphoteric properties. It acts as an alkylating agent causing intra-strand and inter-strand cross-links between DNA bases. After intravenous infusion it is extensively metabolised in the liver by cytochrome p450 [8-12].

II. Experimental

Materials:
The following material (chemicals and reagents) were used for the preparation of solutions.
Chemicals:

Trifluoroacetic acid	:	AR Grade (Merck Ltd)
Acetonitrile	:	HPLC Grade (Merck Ltd)
Hydrochloric acid	:	AR Grade (Merck Ltd)
Water	:	HPLC grade (Milli Q)

Standard:
Bendamustine Hydrochloride Monohydrate Working / Reference standard

Equipment & Chromatographic Conditions:

Equipment	HPLC Equipped with UV detector and an auto sampler or its equivalent
Column	Inertsil ODS-2 (150X 4.6) mm, 5µm
Detection wavelength	235 nm
Flow rate	1.5 mL / min
Injection volume	10 µL
Run time	10 minutes
Column temperature	25°C
Sample Temperature	5°C
Seal wash	Acetonitrile: Water (20:80 %v/v)
Needle wash	Acetonitrile: Water (80:20 %v/v)
Rinse solution	Acetonitrile: Water (80:20 %v/v)

Preparation of Buffer:
Accurately transfer 1 mL of Trifluoroacetic acid in to 1000 mL milliQ water and sonicate for 10 minutes and filter through 0.45µm.

Preparation of Mobile Phase:
Mix and Degas the Buffer and Acetonitrile in the ratio of 70: 30 (% v/v).

Preparation of Diluent: 100% Methanol

Blank: Use diluent as blank

Preparation of Standard Solution:
Accurately weigh and transfer about 26 mg of Bendamustine Hydrochloride Monohydrate Working / Reference standard into a 50 mL volumetric flask, sonicate to dissolve in 10 mL of diluent and make up to the volume with diluent. Further dilute 5 mL of the above solution in to a 50 mL volumetric flask and make up to mark with diluent and mix well.

Preparation of Test Sample Solution:
A) For 25 mg/Vial
Reconstitute 4 test sample Vials with diluent and sonicate to dissolve, pool together in to a 200 mL volumetric flask and make up to the mark with diluent. Filter the solution through 0.22 µm PVDF filter. Further transfer 5 mL of above filtered solution into a 50 mL volumetric flask and dilute up to the mark with diluent and mix well.

B) For 100 mg/Vial
Reconstitute 1 test sample Vial with diluent and sonicate to dissolve and transfer in to a 200 mL volumetric flask and make up to the mark with diluent. Filter the solution through 0.22 µm PVDF filter. Further transfer 5 mL of above filtered solution into a 50 mL volumetric flask and dilute up to the mark with diluent and mix well.

Standard and Samples Used in Method Validation Study
The detail of standard and samples used in validation study is summarized in the below table

Name	Lot / Batch No.	%Purity/%Potency
Bendamustine Hydrochloride	WS/2007/15/01	94.8
Bendamustine Hydrochloride (API)	2007/3/008/13	94.0

Name	Lot / Batch No.	%Purity/%Potency
Dihydroxy ester	2-SHE-41-1	98.55
HP-1	030615E	93.44
Bendamustine Hydrochloride for injection 25 mg/vial	25-PD002	NA
Bendamustine Hydrochloride for injection 25 mg/vial Placebo	25-PD002P	NA
Bendamustine Hydrochloride for injection 100 mg/vial	100-FD005	NA
Bendamustine Hydrochloride for injection 100 mg/vial Placebo	100-FD005P	NA

Injection Sequence

Separately inject each solution into the chromatographic system in the following order.

Diluent (Blank)	1 injection
Standard solution	5 injections
Test sample solution	2 injections
Standard (As Bracketing Standard)	1 injection

III. Results, Discussion & Conclusions

System precision

System precision is assessed from the five replicate injections of the standard preparation from the same vial. The results of system precision are summarized in Table. Typical chromatogram of diluent and standard preparation is exhibited below

Results of System Precision

Injection #	Bendamustine Peak	
	Retention Time	Area
1	4.734	1439882
2	4.730	1420316
3	4.729	1426127
4	4.726	1427917
5	4.726	1418839
Mean	NA	1426616
% RSD	NA	0.6
Tailing factor	1.2	
Theoretical Plate	5299	

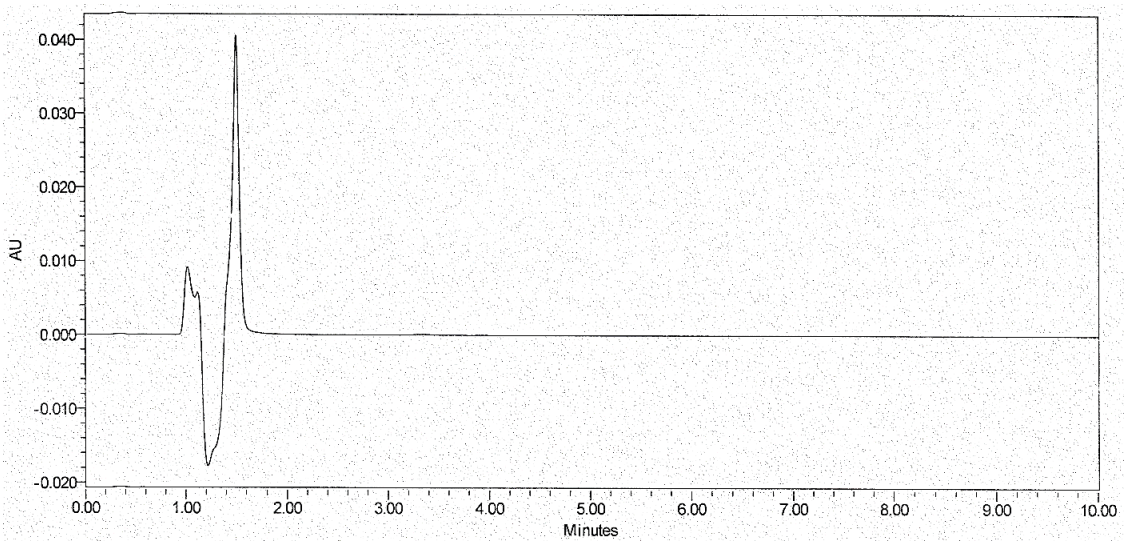
Acceptance Criteria:

- The Tailing factor for Bendamustine peak from first injection of standard solution should be not more than 2.0.
- Theoretical Plates for Bendamustine peak from first injection of standard solution should be not less than 2000.
- The relative standard deviation for Bendamustine peak from five replicate injections of standard solution should be not more than 2.0%.

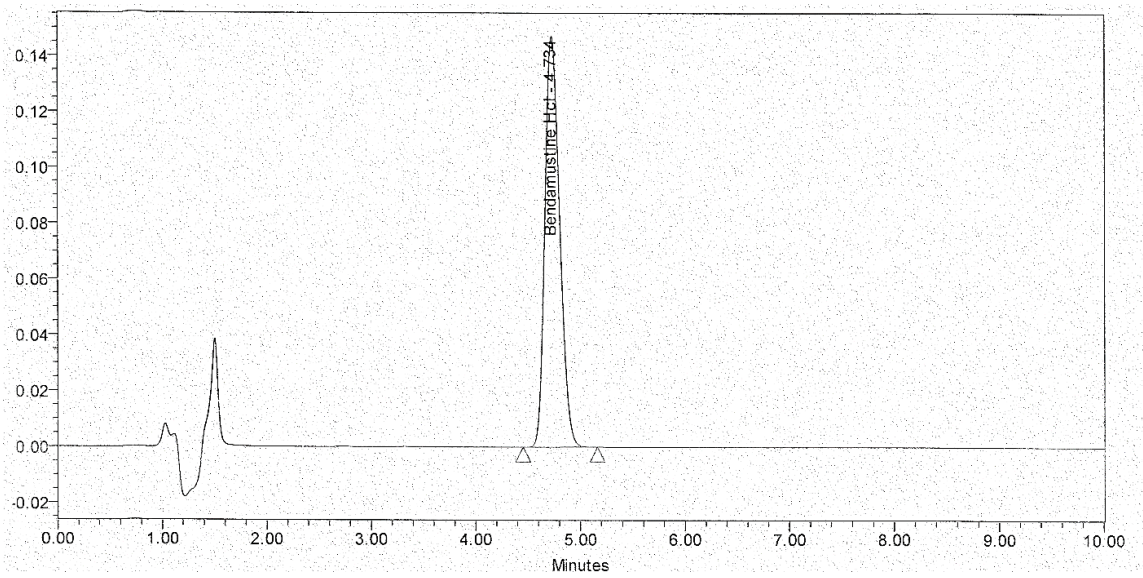
Conclusion:

The result meets the acceptance criteria; hence the analytical procedure is precise with respect to the chromatographic system.

Chromatogram of Diluent (System precision)



Chromatogram of Standard (System precision)



METHOD PRECISION

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample under the prescribed condition. To evaluate the method precision, six individual samples were prepared for Assay and analyzed the samples as per the analytical procedure. The results of method precision for assay are tabulated in the below table. Chromatogram of sample solution of assay is exhibited below.

Results of Method Precision for 25 mg per vial

Sample #	Retention Time (Average)	% Assay
1	4.723	101.4
2	4.722	101.9
3	4.721	102.3
4	4.719	100.9
5	4.717	101.2

Sample #	Retention Time (Average)	% Assay
6	4.716	101.5
Mean	NA	101.5
% RSD	NA	0.5

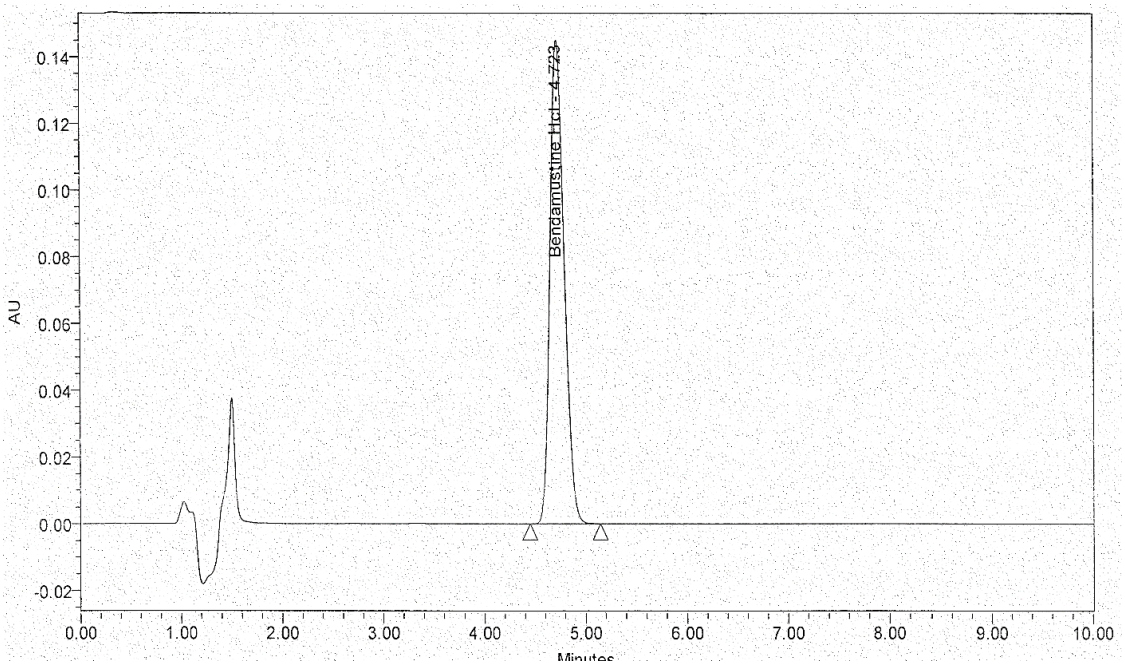
Results of Method Precision for 100 mg per vial

Sample #	Retention Time (Average)	% Assay
1	4.714	101.6
2	4.715	101.9
3	4.714	100.8
4	4.714	101.3
5	4.713	101.4
6	4.713	101.5
Mean	NA	101.4
% RSD	NA	0.4

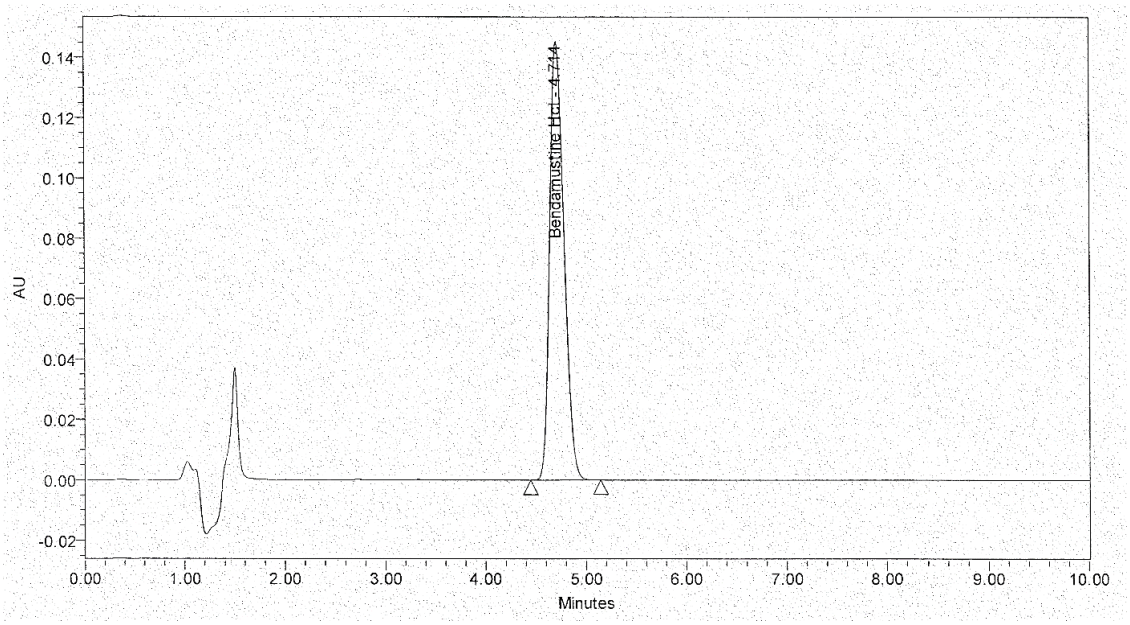
Acceptance Criteria:
The relative standard deviation of assay results obtained from six sample preparations should not be more than 2.0%

Conclusion:
The result meets the acceptance criteria; hence the analytical procedure is precise with respect to chromatographic method.

Chromatogram of sample solution-25 mg per vial (Method precision)



Chromatogram of sample solution-100 mg per vial (Method precision)



INTERMEDIATE PRECISION

Intermediate precision expresses within-laboratories variations such as different days, different analysts, different columns, different equipment's etc. Ruggedness incorporates the concept described under the terms "Intermediate Precision" as defined in USP <1225>. Intermediate precision is established by doing same exercise as system and method precision by different analyst on different days using different column and different equipment. The same Lot/Batch of standard and sample were used within the laboratory.

The results of Intermediate Precision are tabulated inbelow table. Comparison of Method Precision and Intermediate Precision result is summarized in the table below.

Results of Intermediate Precision (Sample Solution)

Sample #	Retention Time (Average)	% Assay
1	4.163	101.0
2	4.170	100.5
3	4.174	100.5
4	4.171	101.6
5	4.174	101.8
6	4.179	101.6
Mean	NA	101.2
% RSD	NA	0.6

Comparison of Method Precision and Intermediate Precision Results

Parameter	Method Precision	Intermediate Precision
Analyst	Analyst 1	Analyst 2
HPLC ID.	EP_QCI_089	EP_QCI_088
Column ID.	HPLCC070	HPLCC009
Column Sr. No.	5GS10114	4GS10139
Comparison of Method Precision and Intermediate precision		
Sample #	**% Assay of Bendamustine HCl**	
	Method Precision	**Intermediate Precision**

1	101.4	101.0
2	101.9	100.5
3	102.3	100.5
4	100.9	101.6
5	101.2	101.8
6	101.5	101.6
Mean	101.5	101.2
% RSD	0.5	0.6
Overall Mean	101.4	
Cumulative % RSD	0.5	

Acceptance Criteria:

1. The relative standard deviation of results obtained from six sample preparations should not be more than 2.0%
2. The cumulative relative standard deviation of method precision and intermediate precision results obtained from twelve sample (6 methods precision and 6 intermediate precision) preparations should not be more than 2.0%.

IV. Conclusion:

The result meets the acceptance criteria and found comparable, indicates that the method is precise and rugged with respect to analyst to analyst, day to day, column to column and equipment to equipment for its intended use.

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