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Analytical Development Procedure for Determination of Assay in Finished Product of Bendamustine Hydrochloride in Bendamustine Hydrochloride

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Abstract

The present study was conducted to develop and validate an analytical procedure for the determination of Bendamustine Hydrochloride in Pharmaceutical Formulations. The analytical test attribute Bendamustine Hydrochloride was evaluated as per the guidelines of ICH Q2 (R1). The method was validated for the determination of Assay in finished product of Bendamustine Hydrochloride Injection and the method validation parameters were evaluated for the analytical test attribute Bendamustine Hydrochloride meets the acceptance criteria. The results obtained were within the specified limits thus, this method was used for the determination of Assay in finished product of Bendamustine Hydrochloride Injection (6mg/mL) and the samples were analyzed for test item concentration by High Performance Liquid Chromatography.

Keywords Bendamustine Hydrochloride, Validating the Assay, High Performance Liquid Chromatography, ICH Q2 (R1)

Introduction

The validation of analytical procedures is done in order to assure that drug formulations are prepared in a most efficient and cost effective manner. In this context, Assay procedures are intended to measure the analyte present in a given sample. The assay represents a quantitative measurement of the major component(s) in the drug product.

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 first 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 drug has also demonstrated clinical activity in breast cancer [7-8] and small-cell lung cancer.

In this regard and view of the need for a suitable analytical HPLC method for routine analysis of Bendamustine Hydrochloride in formulations. Attempts were made to develop simple, precise and accurate analytical method for estimation of Bendamustine Hydrochloride and extend it for their determination in formulation.

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The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found.

To demonstrate the accuracy of assay test method, drug substance is spiked quantitatively in to placebo from 50% to 150% of working concentration of test concentration at each level with triplicate preparation and analyzed using the test method. The result for Bendamustine HCl is tabulated in the below table. Typical chromatogram of Accuracy at 100 % level for is exhibited below.

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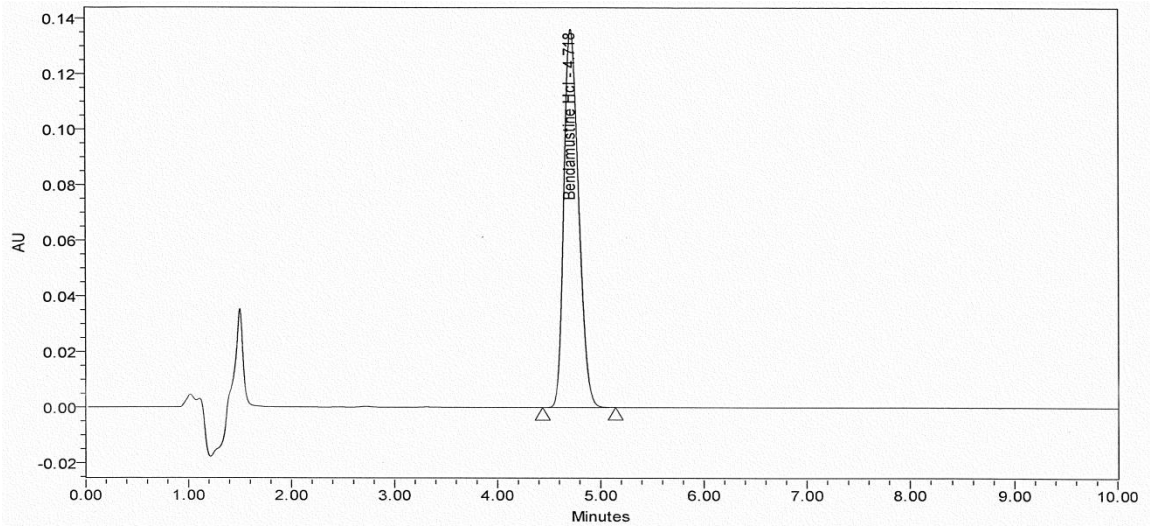
Results of Accuracy for Bendamustine HCl

Accuracy Level	Sample #	Amount (mg added)	Amount(mg found)	% Recovery	Average % Recovery	% RSD
50 %	1	47.235	47.515	100.6	100.5	0.1
	2	47.329	47.578	100.5		
	3	47.207	47.399	100.4		
100 %	1	94.479	94.468	100.0	99.4	0.9
	2	94.329	94.114	99.8		
	3	94.404	92.880	98.4		
150 %	1	141.367	139.762	98.9	99.1	0.3
	2	141.432	140.186	99.1		
	3	141.301	140.407	99.4		
% Recovery for 9 levels						99.7
% RSD for 9 levels						0.8

Chromatogram of Accuracy at 100% Level



Acceptance Criteria:

- % Recovery at each level and overall % recovery should be between 98.0 and 102.0 for Bendamustine HCl.
- The % RSD at each level and overall recovery should not be more than 2.0.

Conclusion:

The results are well within the acceptance criteria; hence the method is accurate for its intended use.

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The Range of the Analytical method is considered from Linearity, Precision and Accuracy of the method.

Based on the Linearity, Method precision and Accuracy data Range of the method is 50 to 150% of test concentration.

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The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study is performed by analyzing the standard at different conditions. The results obtained with altered conditions are compared against results obtained under normal chromatographic conditions.

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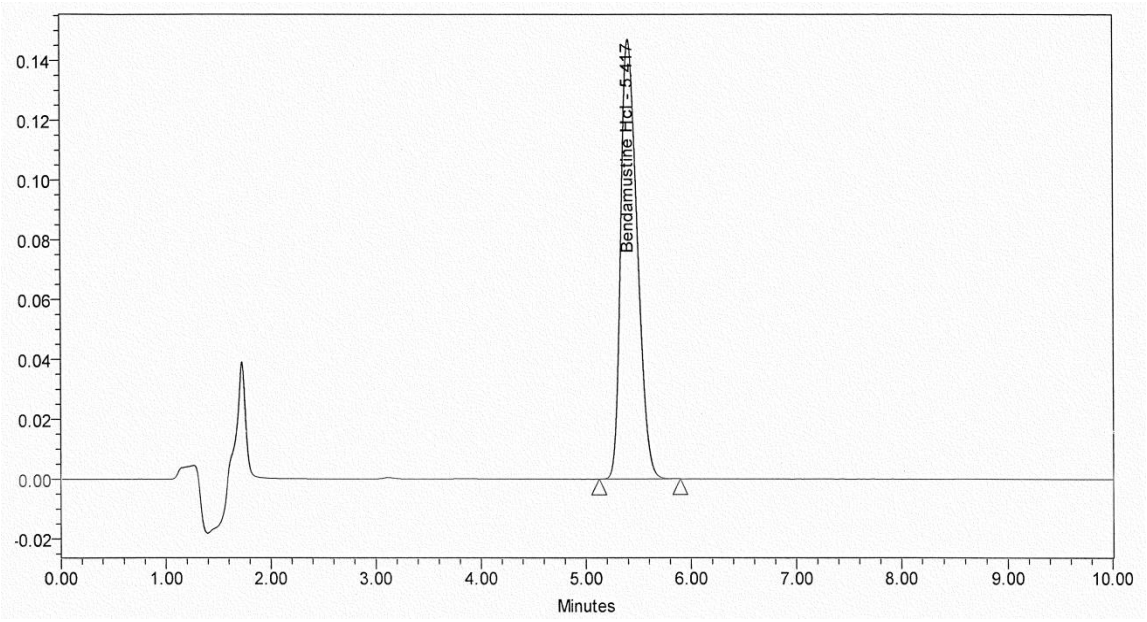
1. Variation in Flow Rate (± 0. 2 mL/min.)

The standard was carried out by varying the flow rate of mobile phase to 1.3 mL/min. and 1.7 mL/min. in place of actual flow rate 1.5 mL/min. The results are summarized in the below table. Typical chromatogram of Robustness for variation in flow rate (0.8 mL/min and 1.2 mL/ min) is exhibited below.

Results of robustness -Variation in flow rate for Bendamustine

Injection #	Flow Rate 1.3 mL/min.		Actual Flow Rate 1.5 mL/min.		Flow Rate 1.7 mL/min.	
	RT	Area	RT	Area	RT	Area
1	5.417	1604526	4.734	1439882	4.170	1245237
2	5.417	1608707	4.730	1420316	4.169	1243739
3	5.417	1610521	4.729	1426127	4.169	1245069
4	5.417	1601094	4.726	1418839	4.169	1249676
5	5.418	1608861	4.726	1427917	4.169	1242068
Mean	NA	1606742	NA	1426616	NA	1245158
% RSD	NA	0.2	NA	0.6	NA	0.2
Tailing factor	1.2		1.2		1.1	
Theoretical Plates	5598		5299		4842	

Chromatogram of Robustness for variation in flow rate (1.3 mL/min)



Chromatogram of Robustness for variation in flow rate (1.7 mL/min)

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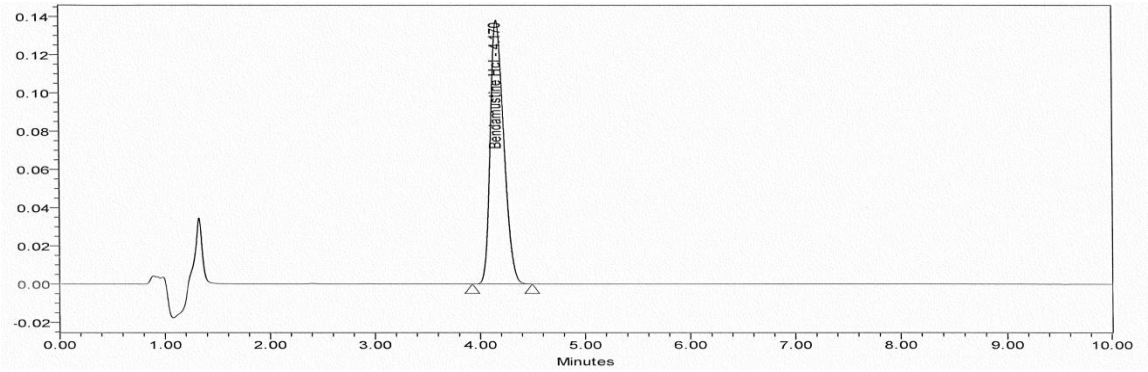


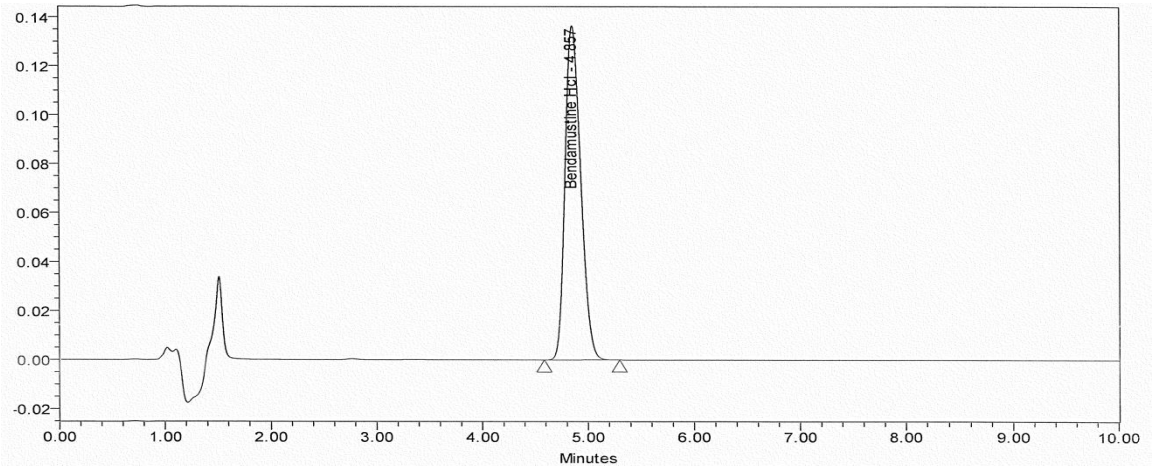
2. Variation in Column Oven Temperature (± 2°C)

The standard was carried out by varying the column oven temperature of 23°C and 27°C in place of actual column oven temperature 25°C. The results are summarized. The results are summarized in the below table. Chromatogram of Robustness for variation in Column Oven Temperature (23°C and 27°C) is exhibited below.

Results of Robustness-Variation in Column oven Temperature for Bendamustine

Injection #	Column Oven Temperature 23°C		Actual Column Oven Temperature 25°C		Column Oven Temperature 27°C	
	RT	Area	RT	Area	RT	Area
1	4.857	1412117	4.734	1439882	4.571	1421139
2	4.857	1405844	4.730	1420316	4.570	1422116
3	4.857	1392908	4.729	1426127	4.568	1423122
4	4.857	1403670	4.726	1418839	4.567	1421483
5	4.857	1406250	4.726	1427917	4.567	1420494
Mean	**NA**	1404158	NA	1426616	NA	1421671
% RSD	**NA**	0.5	NA	0.6	NA	0.1
Tailing factor	1.2		1.2		1.2	
Theoretical plate	4999		5299		5314	

Chromatogram of Robustness for variation in Column Oven Temperature (23°C)



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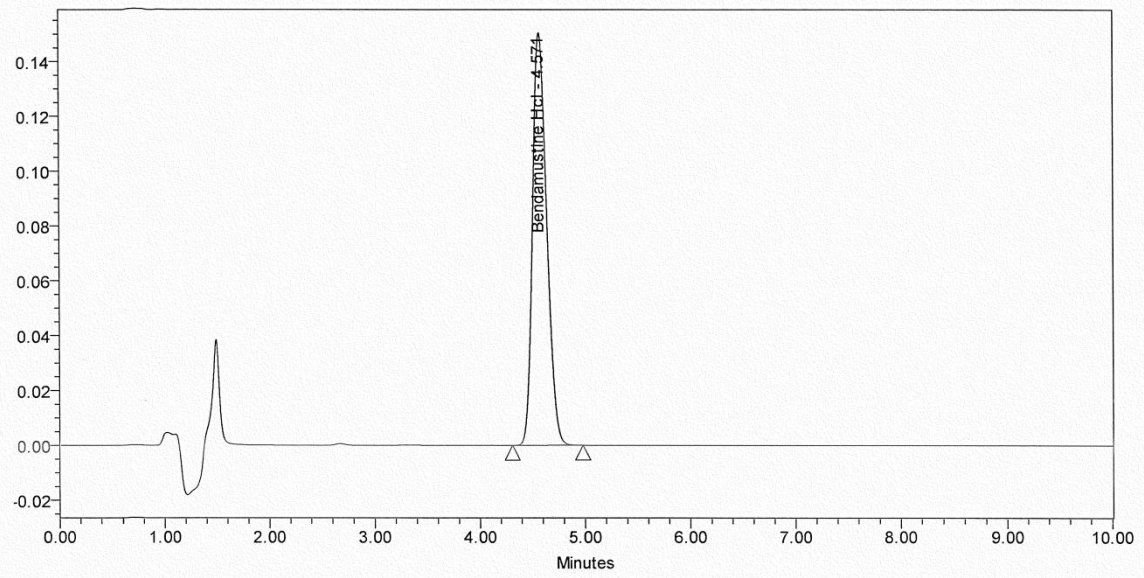
Chromatogram of Robustness for variation in Column Oven Temperature (27°C)



3. Variation in Organic composition (142.5 and 157.5)

The standard was carried out by varying the mobile phase organic composition of 68:32 and 72:28 in place of actual Mobile phase organic composition 70:30. The results are summarized.

The results are summarized in the below table. Chromatogram of Robustness for variation in the Organic composition (68:32 and 72:28) is exhibited below.

Results of Robustness-Variation in organic composition for Bendamustine Hydrochloride

Injection #	Mobile phase composition (68:32)		Actual Mobile phase composition (70:30)		Mobile phase composition (72:28)	
	RT	Area	RT	Area	RT	Area
1	3.731	1436831	4.734	1439882	6.454	1392300
2	3.731	1413689	4.730	1420316	6.454	1386460
3	3.731	1409539	4.729	1426127	6.455	1390738
4	3.731	1415929	4.726	1418839	6.456	1387169
5	3.732	1423610	4.726	1427917	6.456	1391037
Mean	**NA**	1420460	NA	1426616	NA	1389541
% RSD	**NA**	0.8	NA	0.6	NA	0.2
Tailing factor	1.2		1.2		1.2	
Theoretical plates	5603		5299		4568	

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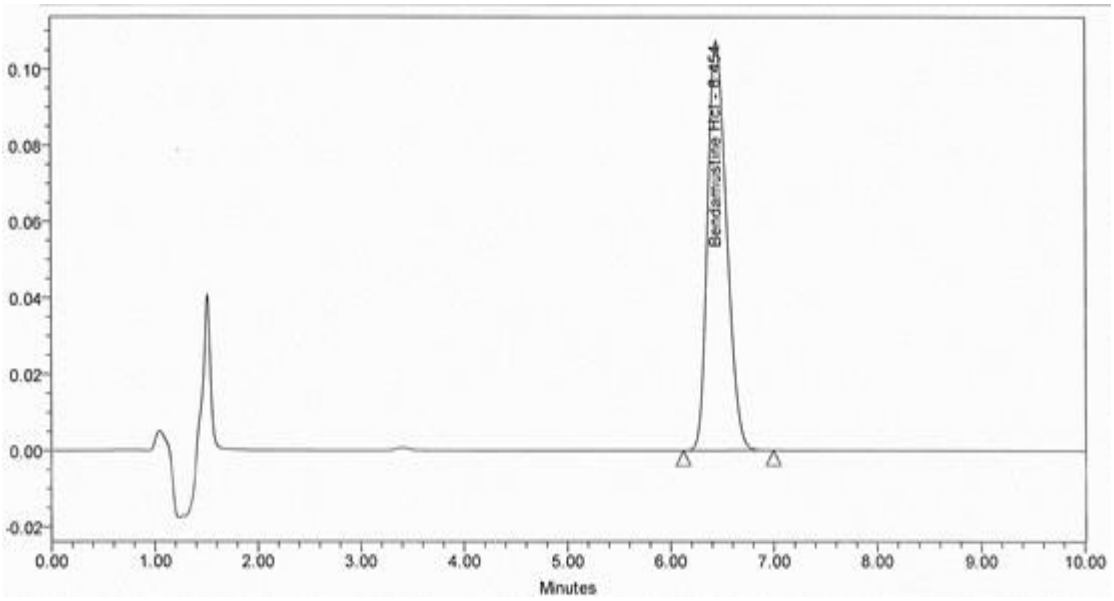
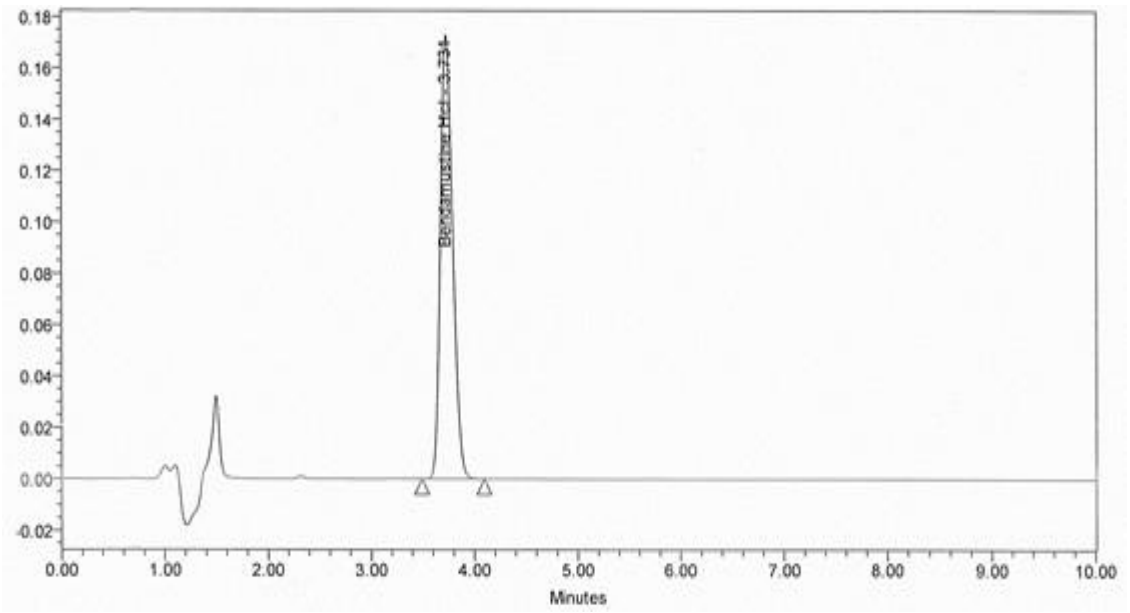
Chromatogram of Robustness for variation in Organic composition (68:32)



Chromatogram of Robustness for variation in Organic composition (72:28)



Acceptance Criteria:

The System suitability defined in test procedure should meet in each condition.

- 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%.

Results of Robustness-Variation in organic composition for Bendamustine Hydrochloride

Injection #	Mobile phase composition (68:32)	Actual Mobile phase composition (70:30)	Mobile phase composition (72:28)

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	RT	Area	RT	Area	RT	Area
1	3.731	1436831	4.734	1439882	6.454	1392300
2	3.731	1413689	4.730	1420316	6.454	1386460
3	3.731	1409539	4.729	1426127	6.455	1390738
4	3.731	1415929	4.726	1418839	6.456	1387169
5	3.732	1423610	4.726	1427917	6.456	1391037
Mean	**NA**	1420460	NA	1426616	NA	1389541
% RSD	**NA**	0.8	NA	0.6	NA	0.2
Tailing factor	1.2		1.2		1.2	
Theoretical plates	5603		5299		4568	

Conclusion:

The system suitability meets for each altered conditions. The results obtained with altered conditions are comparable with the results obtained with normal conditions. The robustness result indicates that the test method is robust enough as demonstrated by altering the Flow rate (± 0.2 mL/min.), column temperature (±0.2°C) and organic composition (±2% of absolute).

Stability of Analyte in Solution

Stability of analyte in solution is evaluated for the standard and sample solutions. The standard and sample solutions are prepared and analyzed as per the analytical procedure. A portion of these solutions were preserved at room temperature and refrigerator (2-8°C) analyzed at different time intervals from the time of preparations.

The results are calculated from initial versus over a period of time. The results are summarized in the below Table A and Table B

Table A Stability of Standard and Sample Solution at Room Temperature

Time Interval	Room Temperature			
	Standard		Sample	
	% Assay	% Difference	% Assay	% Difference
Initial	94.8	NA	101.6	NA
24 hours	95.1	-0.3	103.1	-1.5
48 hours	95.9	-1.2	102.8	-1.2

Table B Stability of Standard and Sample Solution at Refrigerator (2-8°C)

Time Interval	Refrigerator (2-8°C)			
	Standard		Sample	
	% Assay	% Difference	% Assay	% Difference
Initial	94.8	NA	101.6	NA
24 hours	93.7	1.2	100.8	0.8
48 hours	93.6	1.3	102.3	-0.7

Acceptance Criteria:

- % Difference of Bendamustine HCl assay obtained from standard solution at each time point should not be more than ± 2.0 from the initial assay.

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- % Difference of Bendamustine HCl assay obtained from sample solution at each time point should not be more than ± 2.0 from the initial assay.

Conclusion:

The data indicates that the standard and sample solutions are stable up to 48 hours at room temperature and refrigerator (2-8°C) conditions.

Filter variability

Filter variability is evaluated for the sample solution. The sample solution was prepared and analyzed as per the analytical procedure. A portion of these solutions were filtered through 0.22µm PVDF filter and 0.22µm nylon filter and analyzed as per methodology. The results are summarized in the below Table.

Filter variability results

Type of filter	% Assay	% Difference
Unfiltered	100.8	NA
0.22µm PVDF filter	99.8	1.0
0.22µm nylon filter	99.9	0.9

Acceptance criteria

- % Difference of Bendamustine HCl assay obtained from unfiltered sample solution and filtered sample solutions should not be more than ± 2.0.

Conclusion:

The data indicates that the both the filters i.e. PVDF and nylon filters are suitable for preparation of sample solution.

System suitability of overall validation study

The System suitability is an integral part of analytical procedure. The tests are based on the concept that the equipment, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The system suitability results are tabulated inthe below Table.

System Suitability of Overall Validation Study

Parameter	% RSD	Tailing Factor	Theoretical plates
System precision	0.6	1.2	5299
Method Precision	0.6	1.2	5299
Filter variability	0.6	1.2	5299
Specificity(interference)	0.7	1.1	5575
Specificity(Forced degradation Physical conditions)	0.3	1.1	3922
Linearity	0.6	1.2	5299
Intermediate precision	0.2	1.1	4576
Accuracy	0.3	1.2	5026
Robustness (Flow variation 1.3 mL/min)	0.2	1.2	5598
Robustness (Flow variation 1.7 mL/min))	0.2	1.1	4842
Robustness (Temp variation 23°C)	0.5	1.2	4999
Robustness (Temp variation 27°C)	0.1	1.2	5314
Robustness (Low organic)	0.2	1.2	5603
Robustness (High organic)	0.7	1.2	4568
Stability of analyte in solution initial	0.4	1.1	4625

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Parameter	% RSD	Tailing Factor	Theoretical plates
Stability of analyte in solution 24 hours	0.6	1.1	4793
Stability of analyte in solution 48 hours	0.3	1.1	4646
Specificity(Forced degradation Chemical conditions)	0.2	1.1	3996
Minimum	0.1	1.1	3922
Maximum	0.7	1.2	5603
Average	0.4	1.2	4960

Acceptance Criteria:
System suitability criteria should meet during overall validation studies, otherwise needs to be justified. Report minimum, maximum and average values of system suitability parameters.

- 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 results for system suitability are well within the acceptance criteria; hence the given chromatography system is acceptable for its intended use.

References:

1. Balfour, J. A., K. L. Goa. Bendamustine. Drugs, 61, 2001, 631-640.

2. Cheson, B. D., M. J. Rummel. Bendamustine: rebirth of an old drug. J. Clin. Oncol, 27, 2009, 1492-1501.

3. Reck, M., B. Haering, G. Koschel, E. Kaukel, J. von Pawel, U. Gatzemeier. Chemotherapie des fortgeschrittenen nicht-kleinzelligen und kleinzelligen Bronchialkarzinoms mit Bendamustin--EinePhase-II-Studie. Pneumologie, 52, 1998, 570-573.

4. Avendaño, C., J.C. Menéndez, in: Medicinal Chemistry of Anticancer Drugs, Amsterdam, Elsevier, 2008, 139-1.

5. Gandhi, V. Metabolism and mechanisms of action of bendamustine: rationales for combination therapies. Semin. Oncol., 29, 2002, 4-11.

6. Leoni, L. M., J. A. Hartley. Mechanism of action: the unique pattern of bendamustine-induced cytotoxicity. Semin. Hematol., 48, 2011, S12-23.

7. Sharma, G., S. Anabousi, C. Ehrhardt, M. N. Ravi Kumar. Liposomes as targeted drug delivery systems in the treatment of breast cancer. J. Drug Target., 14, 2006, 301-310.

8. von Minckwitz, G., M. Linder, S. Loibl. The reawakening of bendamustine - also in breast cancer? Onkologie, 32, 2009, 462-463.