INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

VALIDATION OF RP-HPLC ANALYTICAL METHOD FOR ESTIMATION OF CARMUSTINE IN BULK AND LYOPHILIZED VIALS

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ARTICLE INFO

Article history
Received 20/09/2017
Available online
03/10/2017

Keywords
Lyophilized Vials,
Acetonitrile,
Pda Detector,
Regression Coefficient And
Quality Control Tool.

ABSTRACT

Carmustine is an anti-neoplastic agent. A reverse phase high-performance liquid chromatographic (RP-HPLC) assay method was developed by slightly modifying the USP method and validated for quantitative determination of carmustine in bulk drug and in lyophilized vials. The column utilized for the estimation of assay was 4.6 mm X 15-cm, 5-µm Packing L1 (C18) and the mobile phase employed was a mixture of acetonitrile and water in the ratio of 3:7. The detection was carried out at a wavelength of 200 nm with PDA detector. The flow rate is 1.5 mL per minute at a set temperature of 4-6°C with a total run time of 15 minutes. The linearity range was studied ranging from 25-150% with regression coefficient value of 0.999 and the method precision for estimation of assay was below 1.0% RSD. During the forced degradation studies, the drug substance was found to be degraded in all stress conditions because of the nature of the drug substance and the purity angle was found to be less than the purity threshold in all stress conditions. The degradation products were well separated from the main assay peak. The prepared stock solutions of test and standard were found to be stable up to 24hrs at refrigerated condition. Finally it can be concluded that the validated RP-HPLC method can be successfully used as a quality control tool for the estimation of drug substance in the bulk drug and in lyophilized vials.

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*Please cite this article in press as **Yaswanth Allamneni** et al. Validation of RP-HPLC Analytical Method for Estimation of Carmustine in Bulk and Lyophilized Vials. Indo American Journal of Pharmaceutical Research.2017:7(09).*

INTRODUCTION

Carmustine belongs to the category of alkylating agent which can be used for treatment of several types of brain cancer (including glioma, glioblastoma multiform, medulloblastoma and astrocytoma), multiple myeloma and lymphoma (Hodgkin's and non-Hodgkin)[1]. Carmustine alkylates and cross-links DNA during all phases of the cell cycle, resulting in disruption of DNA function, cell cycle arrest, and apoptosis[2]. This agent also carbamoylates proteins, including DNA repair enzymes, resulting in an enhanced cytotoxic effect. Carmustine is highly lipophilic and crosses the blood-brain barrier readily.

The IUPAC name for Carmustine is 1, 3-bis (2-chloroethyl)-1-nitrosoureas and its empirical formula is $C_5H_9C_{12}N_3O_2$. The chemical structure of carmustine was provided in Figure 1. Vitthal D. Dhakane et al.[3] has been developed and validated a stability indicating HPLC method for estimation of related substances for carmustine in bulk drug. RP-HPLC analytical method for carmustine estimation has been developed as per USP monograph. The developed method has been validated in accordance with ICH Q2 (R1) requirement. The developed method was validated with respect to the validation parameters like system suitability, specificity, precision (method precision and intermediate precision) and robustness because the base route of the method was designed from USP.

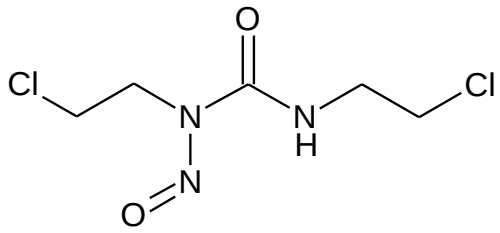

Fig. 1: The chemical structure of Carmustine[1]

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile, hydrochloric acid, sodium hydroxide, hydrogen peroxide were procured from Merck Specialities Private Limited. Carmustine related gift samples were provided by SP Accure Labs Private Limited.

Instruments and Equipments Details

HPLC with PDA detector (Make: Waters, Model No.: 2998 PDA 2695 pump), Electronic Balance (Make: Mettler-Toledo, Model No.: XS-205 dual range), Laboratory precision oven (Make: Tempo, Model No.: TI-128C S/G), pH meter (Make: Mettler-Toledo, Model No.: FEP20 FIVE Easy Plus PH), Flexible Poly Urethane Isolator (Make: PFI systems Limited, Model No.: IB821), Powered air purifying respirator (Make: BLS Italy, Model No.: JS 3025 PAPR SGE2600 CE) and Hypersil C18 Column (Make: Thermofisher).

Validation of assay method[3, 6, 10]

Standard solution preparation

Weigh accurately about 15 mg of Carmustine reference standard into a10 mL volumetric flask, dissolve and dilute to volume with acetonitrile and mix well. The concentration of the bulk solution is 1.5 mg/mL.

Sample stock solution preparation

Weigh accurately about 15 mg of Carmustine sample or equivalent dosage form into a 10 mL volumetric flask, dissolve and dilute to volume with acetonitrile and mix well. The concentration of the bulk solution is 1.5 mg/mL. The concentration of the bulk solution is 1.5 mg/mL. These solutions are prepared in low-actinic refrigerated glassware.

Preparation of mobile phase

A mixture of acetonitrile and water in the ratio of 3:7 for isocratic pumping system.

Chromatographic conditions

Table 1: Chromatographic conditions for the validation assay method.

S. No.	Parameter	Condition/Set value
1.	Column	4.6 mm X 15-cm, 5-µm Packing L1 (C18)
2.	Mobile phase	A mixture of acetonitrile and water in the ratio of 3:7
3.	Flow rate	1.5 mL/minute
4.	Run time	15 minutes
5.	Column temperature	4-6°C
6.	Injection volume	10 µL
7.	Detection wavelength	UV at 200 nm
8.	Diluent	Acetonitrile
9.	Pump mode	Isocratic

System suitability

To verify the analytical system is working properly and can give accurate and precise results, the system suitability parameters are to be set. Inject separately each solution into the HPLC.

Specificity:

Specificity was established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of Carmustine with respect to acid, base, peroxide, thermal and photolytic degraded samples, respective blank solutions.

Precision:

Method precision (Repeatability):

Precision of the method was demonstrated through the %RSD of known Carmustine by injecting six sample preparations of the "Carmustine API".

Intermediate Precision:

The Intermediate precision was carried out by performing six replicate analyses of Carmustine API by different analyst, on different HPLC systems, different column than that used for repeatability test. The %RSD of the data for the repeatability and intermediate precision results were used for evaluation purpose.

R**obustness:**

The data from analysis of the sample preparation after storing the sample for 12 hrs, 24 hrs and 48 hrs at refrigerator temperature was used to establish the solution stability.

RESULTS AND DISCUSSION

System suitability

Table 2: System suitability results.

S. No.	Name of the solution	Measuring Parameter with acceptance criteria	Results
1.	System suitability solution	Tailing factor for the Carmustine Peak from System suitability chromatogram should be NMT 1.9.	Tailing factor for Carmustine : 0.9
2.	Standard solution	The relative standard deviation of peak areas of Carmustine peaks from five replicate injections of system suitability solution should be NMT 2.0%.	%RSD Carmustine**:** 0.5

From the above results, the system is suitable for analysis.

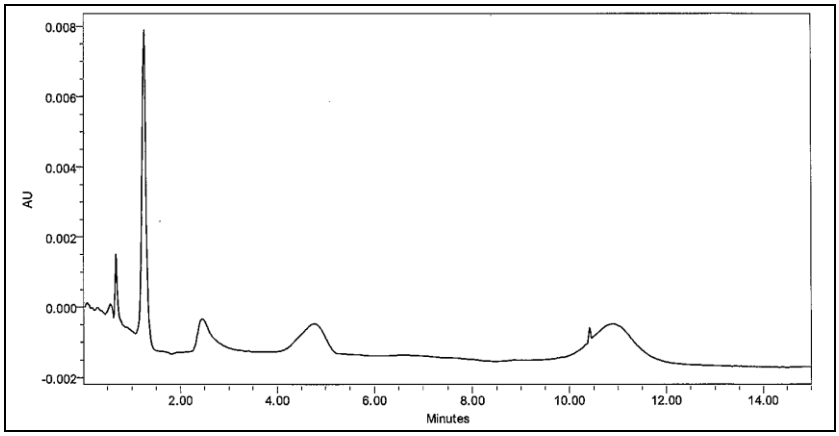

Fig. 2: Typical chromatogram of Blank (diluent) for Carmustine assay method.

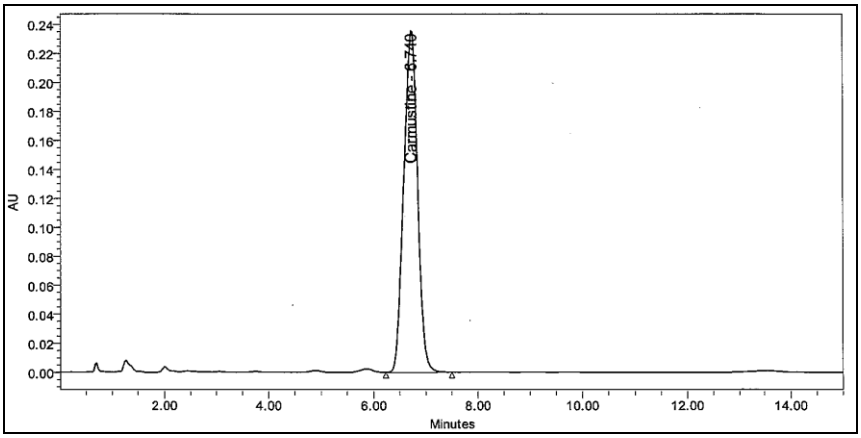

Fig. 3: Typical chromatogram of Standard for Carmustine assay method.

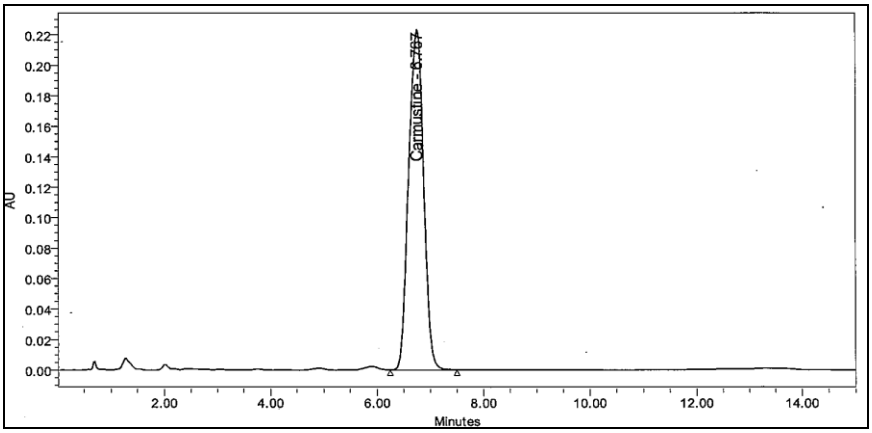

Fig. 4: Typical chromatogram of Sample for Carmustine assay method.

Specificity

Table 3: Specificity results.

S. No.	Measuring Parameter with acceptance criteria	Results
1.	The chromatogram of Blank should not show any peak at the retention time of Carmustine	No Blank peaks observed at the retention time of Carmustine
2.	Purity angle should be less than Purity Threshold	Purity angle is less than purity threshold in all the stress conditions.

Table 4: Forced degradation results.

S. No.	Sample Treatment	Purity angle	Purity threshold	% Degradation
1.	Acid degradation (3N HCl)	0.064	0.308	19.9
2.	Base degradation (0.02N NaOH)	0.065	0.309	19.8
3.	Peroxide degradation (10% H_2O_2)	0.100	0.416	Nil
4.	Heat degradation (Vacuum oven at 80°C, 4 hrs)	0.085	0.372	21.8
5.	Light degradation (UV at 254nm, 6 hrs)	0.080	0.356	18.0

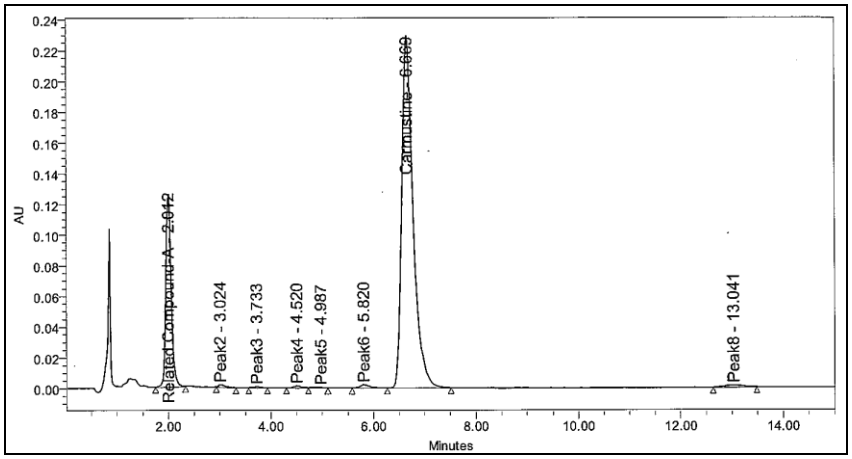

Fig. 5: Typical chromatogram of Carmustine during acid degradation studies.

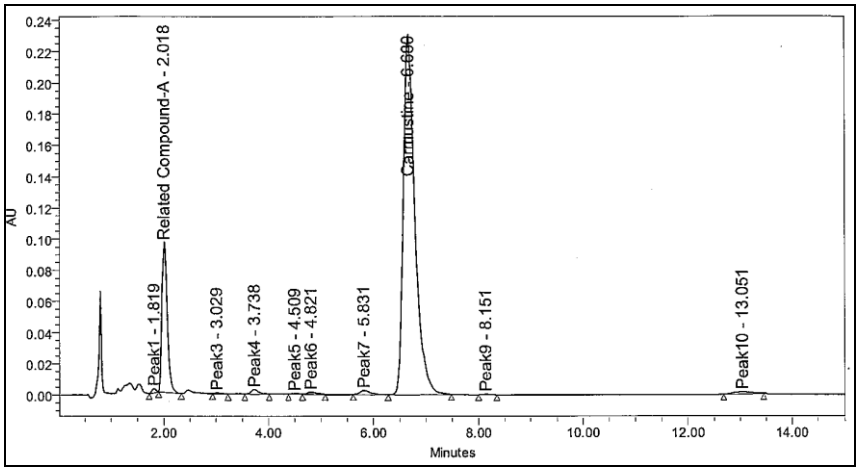

Fig. 5: Typical chromatogram of Carmustine during base degradation studies.

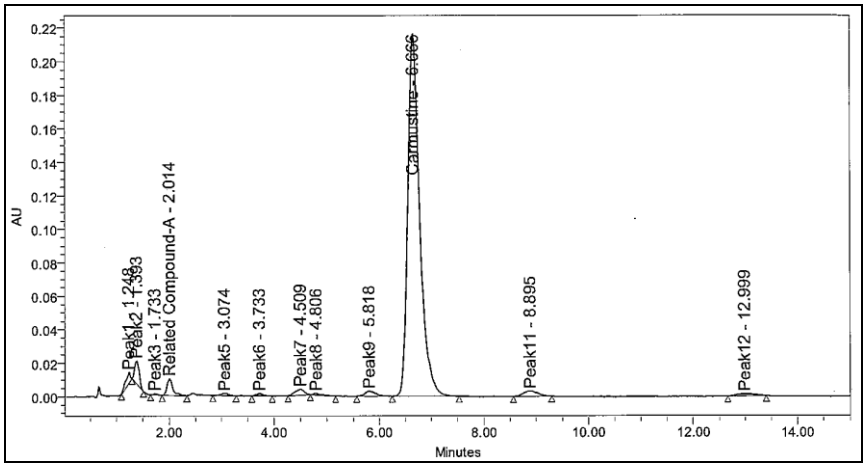

Fig. 6: Typical chromatogram of Carmustine during thermal degradation studies.

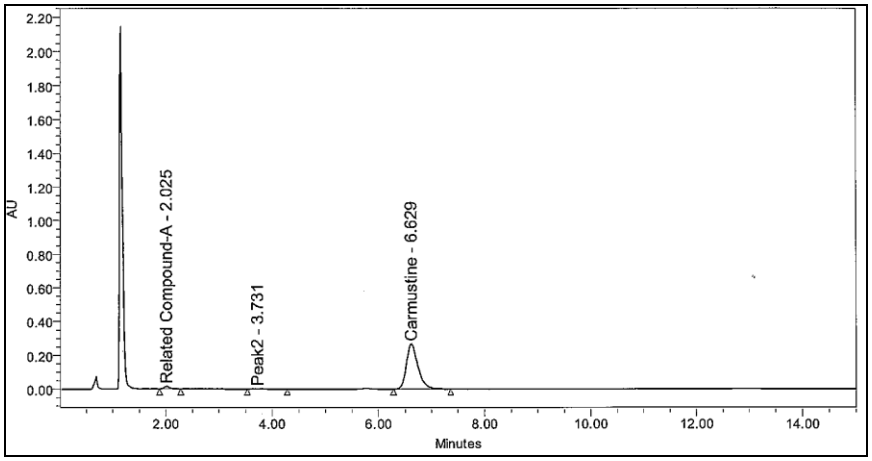

Fig. 7: Typical chromatogram of Carmustine during oxidative degradation studies.

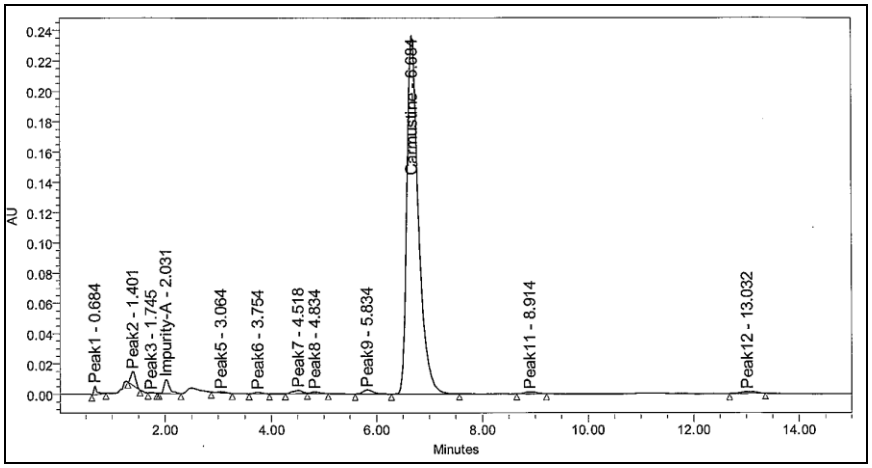

Fig. 8: Typical chromatogram of Carmustine during light degradation studies.

Diluent and mobile phase does not show any interference at the retention time corresponding to the peak of carmustine. The blank solutions (acid, alkali and peroxide) does not show any interference at the retention time corresponding to the peak of carmustine.

Precision

Table 5: Repeatability (method precision) and intermediate precision results.

S. No.	Sample name	% Carmustine
Method precision		
1.	Sample solution-1	99.7
2.	Sample solution-2	99.2
3.	Sample solution-3	99.6
4.	Sample solution-4	99.2
5.	Sample solution-5	99.9
6.	Sample solution-6	99.1
	Mean	99.5
	%RSD	0.3
Intermediate precision		
7.	Intermediate precision sample-1	100.7
8.	Intermediate precision sample-2	100.2
9.	Intermediate precision sample-3	100.3
10.	Intermediate precision sample-4	100.9
11.	Intermediate precision sample-5	100.6
12.	Intermediate precision sample-6	100.4
	Cumulative % RSD	0.6

From the above results, this study proves that the method is repeatable and precise. The proposed method is rugged for the variabilities like two different instrument, different columns, two different analysts on two different days.

Robustness (Solution stability)

Table 6: Solution stability results (sample solution).

S. No.	Time intervals	% Carmustine	Difference (%)
1.	Initial	100.6	Not applicable
2.	12 hours	100.2	0.4
3.	24 hours	99.6	1.0
4.	48 hours	100.1	0.5

Table 7: Solution stability results (standard solution).

S. No.	Time intervals	Similarity factor
1.	Initial	Not applicable
2.	12 hours	1.00
3.	24 hours	1.00
4.	48 hours	1.00

For the sample solution, % difference in carmustine assay was found to be less than 1.0% under refrigerated condition for 48 hrs. The similarity factor value for standard solution was found to be less than 1.05 under refrigerated condition for 48 hrs. Test solutions are stable for 48 hrs under refrigerated condition.

CONCLUSIONS

The results are found to be complying with the acceptance criteria for each of the parameter. The validated method is found to be Specific, Linear, Precise, Accurate, Robust and Rugged for the estimation of Carmustine in Carmustine API and lyophilized vials. Hence it is concluded that the Assay method is found to be valid in terms of reliability, precision, accuracy and specificity, suitable for chemist-to chemist and day-to-day for routine analysis as well as for stability analysis. In the forced degradation studies, it was concluded that the main peak is well separated from the degradation products. The validated HPLC method can be adopted for the estimation of drug substance as a quality control tool in the bulk and lyophilized vials. The future scope of this work will be extended to develop a single stability indicating HPLC method which will be used for estimation of the assay and related substances of carmustine in the bulk.

CONFLICT OF INTEREST

The author declared that there is no conflict of interest.

ACKNOWLEDGEMENTS

Authors wish to give thanks to Management of SP Accure Labs Private Limited, Hyderabad, India for constant support and given research laboratory to carry out this project work. We also acknowledge the help provided by our colleagues in completion of the project.

LIST OF ABBREVIATIONS

RP-HPLC	:	Reverse phase high performance liquid chromatography
PDA	:	Photodiode array detector
RSD	:	Relative standard deviation
DNA	:	Deoxyribonucleic acid
IUPAC	:	International union of pure and applied chemistry
USP	:	United states pharmacopeia
ICH	:	International conference on harmonization
µm	:	Micrometer
Cm	:	Centimeter
UV	:	Ultraviolet
mL	:	Milliliter
µL	:	Microliter
API	:	Active pharmaceutical ingredient
HCl	:	Hydrochloric acid
NaOH	:	Sodium hydroxide

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