



A Validated Stability-Indicating HPLC Related substances method for Carmustine in bulk drug

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ABSTRACT

A rapid, sensitive reversed phase stability-indicating high-performance liquid chromatographic (HPLC) assay method was developed and validated for quantitative determination of carmustine in bulk drugs and the degradation products generated from forced degradation. A gradient, reversed phase HPLC method was developed to separate the drug from the degradation products, using an YMC ODS-A C18 (250mm x 4.6) mm, 5 μ m column and the Mobile phase-A 0.01 M Potassium dihydrogen phosphate in water, adjust PH 3.2 with orthophosphoric acid and mobile phase-B methanol was used as mobile phase. The detection was carried out at wavelength 230 nm and 205 nm. The chromatographic resolution between its degraded products was found to be greater than two. The carmustine was subjected to stress conditions of acid, base, oxidation (30 % H₂O₂), thermal degradation and photolytic degradation. The degradation was observed for carmustine in alkali, acid and in thermal hydrolysis. The mass balance was close to 100 in all the stress conditions. The degraded products were well resolved from main peak. The developed method was validated with respect to linearity, accuracy, recovery, precision, system suitability, selectivity, robustness and forced degradation studies prove the stability indicating ability of the method.

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Introduction

1, 3-bis(2-chloroethyl)-1-nitrosourea. Carmustine is a highly lipophilic nitrosourea compound which undergoes hydrolysis in vivo to form reactive metabolites. These metabolites cause alkylation and cross-linking of DNA and RNA.

Other biologic effects include inhibition of DNA repair and de-novo purine synthesis[1]. Nitrosoureas generally lack cross-resistance with other alkylating agents. We are gratified to report a stability indicating HPLC method for the analysis and separation of drugs from the degradation products formed under ICH suggested. It felt necessary to develop a stability indicating LC method for analysis of Carmustine. There is no method found for Validation and stability determination of Carmustine having degradation study. Carmustine or BCNU (bis-chloroethylnitrosourea) is a mustard gas-related β -chloro-nitrosourea compound used as an alkylating agent in chemotherapy[2].

As a dialkylating agent, BCNU is able to form interstrand crosslinks in DNA which prevents DNA replication and DNA transcription. It is used in the treatment of several types of brain cancer (including glioma, glioblastoma multiforme, medulloblastoma and astrocytoma), multiple myeloma and lymphoma (Hodgkin's and non-Hodgkin). BCNU is sometimes used in conjunction with alkyl guanine transferase (AGT) inhibitors, such as O⁶-benzylguanine. The AGT-inhibitors increase the efficacy of BCNU by inhibiting the Direct Reversal pathway of DNA repair, which will prevent formation of the interstrand crosslink between the N¹ of guanine and the N³ of cytosine[3-4].

Experimental

Material and reagents

Carmustine bulk drug was made available from Sun Pharmaceutical Ltd. India (purity 99.3). Potassium dihydrogen phosphate, Orthophosphoric acid, Triethylamine were obtained from Qualigens fine chemicals, India Limited. Ethanol, methanol, hydrochloric acid, sodium hydroxide were obtained from Rankem laboratories, India. All chemicals and reagent were used as HPLC grades, Milli-Q-Water was used throughout the experiment.

Chromatographic Conditions

A chromatographic system Waters consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven and UV detector. The chromatographic column of 250 mm length and internal diameter of 4.6 mm filled with Octadecyl silane YMC ODS-A C18 stationary phase with particle size 5 micron and pore size 100Å was used. The instrumental settings were a flow of 1 ml/min. The HPLC gradient was kept as T/ min and %B: composition of mobile phase-B 0/10,15/10,55/75,62/10,70/10. The column was maintained at 30°C, the injection volume was 20 μ l. The diluent used was ethanol.

Mobile Phase

The mobile phase containing 0.01 M Potassium dihydrogen phosphate in water, adjust PH 3.2 with orthophosphoric acid as mobile phase-A and methanol as mobile phase-B.

Preparation of Standard stock solutions

Standard solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluents used for the standards and sample was ethanol.

A 50-mg sample of carmustine standard was accurately weighed, transferred in a 50-mL volumetric flask, and dissolved with the diluent (1000 $\mu\text{g mL}^{-1}$). Further 1.0 ml above solution transferred in a 100-mL volumetric flask, and dissolved with the diluent (10 $\mu\text{g mL}^{-1}$).

A 50-mg sample of Impurity-A standard was accurately weighed, transferred in a 50-mL volumetric flask, and dissolved with the diluent (1000 $\mu\text{g mL}^{-1}$). Further 1.0 ml above solution transferred in a 100-mL volumetric flask, and dissolved with the diluent (10 $\mu\text{g mL}^{-1}$).

Sample solution

A 50-mg sample was accurately weighed, transferred in a 50-mL volumetric flask, and dissolved with the diluent (1000 $\mu\text{g mL}^{-1}$).

Selectivity

Selectivity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradants, matrix etc. The selectivity of the developed LC method for carmustine was carried out in the presence of its degradation products[5-6]. Stress studies were performed for carmustine bulk drug to provide an indication of the stability indicating property and selectivity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (0.5 N Hydrochloric acid) Fig-4, alkali (0.2N NaOH) Fig-5, thermal degradation (60 °C for 10 days) Fig-6 to evaluate the ability of the proposed method to separate carmustine from its degraded products. For heat study, study period was 10 days where as for acid and alkali 2.0 hour. Related substances studies were carried out for stress samples against carmustine reference standard and the mass balance (% purity + % sum of all impurities + % sum of all degraded products) was calculated.

Results and discussion

Method Development

The primary target in developing this LC method is to achieve determination of carmustine and impurity-A in bulk drugs under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. Taken in to account the instability of carmustine in strong acidic and basic media, a mobile phase with weakly acidic or neutral pH value is preferred. The optimal pH value was found to be 3.2. To achieve this number of stationary phase like C8, C18, CN and NH₂ were employed and different combination of mobile phases were employed. In C18 stationary phase using ammonium acetate and phosphate buffer at different pH the resolution between carmustine, and impurity-A was achieved but broad peak shape of carmustine was obtained having tailing factor about 2.6. To minimize tailing effect, further NH₂ and CN columns were tried but it has been observed that (tailing factor 2.5) but resolution between carmustine and impurity-A decreased and in case of CN stationary phase peak shape of carmustine was improved but peak of impurity-A was eluted at, 5.5 and 20.1 respectively.

Finally used high carbon loading, double end capped C18 (YMC ODS-A C18, 25-cm) column. Mobile phase was selected in terms of its components and proportions. This work began with a binary mixture of acetonitrile and 10mM dipotassium hydrogen phosphate in the ratio of 50:50 (v/v) at different pH 8.0 to pH 5.5. It was observed that at 50% aqueous composition containing dipotassium hydrogen phosphate pH-8.0 peak of carmustine and impurity-A was eluted at 40.1 and 21.3

respectively, while at pH 5.5 of mobile phase resolution between carmustine and impurity-A was good but run time was increased. Finally a mobile phase consisting 0.01 M Potassium dihydrogen phosphate in water, adjust PH 3.2 with orthophosphoric acid as mobile phase-A and methanol as mobile phase-B, which produces good resolution and reasonable retention and acceptable for drug the chromatographic analysis time was 70 min. A typical chromatogram for a standard solution is shown in Fig 2 and 3. The retention time is 12.36 for impurity-A and 36.35 for Carmustine, respectively.

Figure 1. Chemical structure of Carmustine

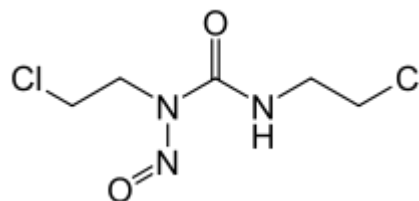


Figure 2. A chromatogram of the Carmustine diluted standard

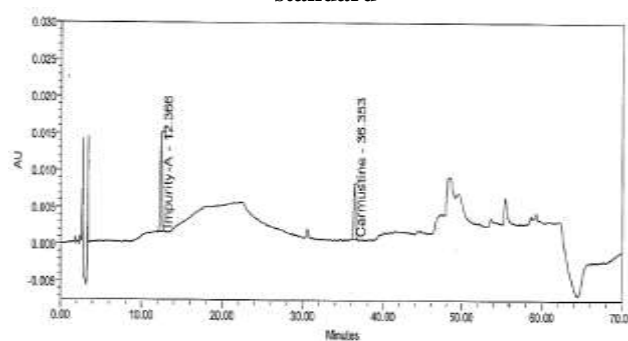
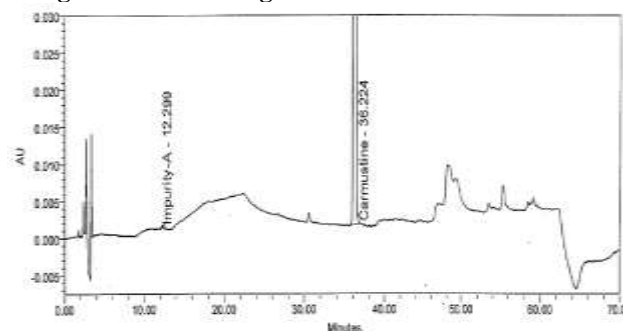


Figure 3. Chromatogram of untreated Carmustine



Method Validation

Precision

The system precision is measure of the method variability that can be expected for given analyst performing the analysis and was determined by performing five replicate analysis of the same working solution. The obtained relative standard deviation (R.S.D.) for carmustine and impurity-A was 2.12% and 1.94%, respectively[7-8]. The intraday precision of developed LC method was determined by preparing the samples from same batches in nine determinations with three concentrations and three replicate each. The R.S.D. of the related substances results, expressed as percentage, was used to evaluate the method precision. The obtained R.S.D. values were 2.32% for carmustine and 2.20% for impurity-A. The results indicated good precision of the developed method. The results are shown in Table I.

Accuracy (Recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by

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Table I
Summary of Forced degradation results

Stress condition	Time	Assay of active Substance %	Remarks
Acid Hydrolysis (0.5 N HCl)	2.0 Hrs	87.22	Degradation
Base Hydrolysis (0.025 N NaOH)	2.0 Hrs	76.81	Degradation
Thermal (60°C)	10 days	93.44	Degradation

Table II
System suitability reports

Compound (n=3)	Retention Time	% RSD	USP tailing	Theoretical plates
Carmustine	36.22	2.11	1.04	6233

Table III
Results of the LOD and LOQ

Name	%LOD	%LOQ
Impurity-A	0.010	0.033
Carmustine	0.011	0.066

Table IV
Results of the Linearity study and Precision

Ingredient	Precision (% RSD)	Linearity (µg/ml)	Slopes* (n= 3)	Coefficients of correlations
Carmustine	2.12	50-150	4008	0.9999
Impurity-A	1.94	50-150	3807	0.9998

*Standard deviation shown in parentheses

Table V
Results of the Recovery Tests for the Carmustine

Level of Addition (%)	Amount added (n = 3) (ppm)	% Recovery*	% Average recovery [^]
50	50	99.88	99.97
100	100	100.12	100.03
150	150	100.02	100.11

* RSD shown in parenthesis.

[^] Average recovery = the average of three levels, nine determinations

Table VI
Results of robustness study

Sr. No.	Parameters	Variations	Resolutions between Impurity-A and base degraded product
1	Temperature	1. at 25 °C	7.21
		2. at 35 °C	7.68
2	Flow rate	1.0.8 ml/min	6.02
		2.1.2 ml/min	6.94
3	pH	1.3.0	5.7
		2.3.4	5.3