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Stability indicating HPLC method for the determination of Dacarbazine in pharmaceutical dosage form

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ABSTRACT

A simple, rapid, and precise stability indicating HPLC method is developed for the quantitative determination of dacarbazine in pharmaceutical dosage form. The chromatographic separation of dacarbazine was achieved with an agilent eclipse XDB C18, 150 x 4.6 mm, 5µ particle size analytical column using buffer and acetonitrile taken in 96:4%v/v and the response was detected at 323nm by using PDA detector. The retention time was found to be 4.333. Dacarbazine drug substance was exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analyzed by the proposed method. Peak homogeneity data of dacarbazine is obtained by photodiode array detector in the stressed sample chromatograms, demonstrating the specificity of the method for its estimation in presence of degradation product. The described method shows excellent linearity over a range of 25–150 µg/mL. The correlation coefficient for dacarbazine was found to be 0.9999. The relative standard deviation for six measurements in two sets of dacarbazine in injection is always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination and stability study of dacarbazine in pharmaceutical preparations.

Keywords: Dacarbazine, Cancer, HPLC method, Analysis

INTRODUCTION

Dacarbazine (DCZ) is an antineoplastic chemotherapy drug (fig. 1). It works by causing a chemical reaction that damages the DNA in a cell and there by inhibits protein synthesis and results in cellular death. DCZ is used in the treatment of various cancers like malignant melanoma, Hodgkin's lymphoma, sarcoma and islets cell carcinoma of the pancreas. Stability-testing is an important part of the process of drug product development. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and enables recommendation of storage conditions, retest periods, and shelf-life to be established. The two main aspects of drug product that play an important role in shelf-life determinations are assay of active drug and degradation product generated during stability study.

Literature review reveals that there are few proposed methods for the determination of DCZ and its metabolites in plasma[1], HPLC estimation of DCZ and its photolytic degradation product in plasma and urine[2], LC methods for the estimation of DCZ on an undervitized silica[3], Isocratic HPLC method for the determination of DCZ and its related impurities[4], LC/MS/MS method for the determination of DCZ in plasma[5], an electro analytical method for the estimation[6] and induced- photosensitivity reaction of DCZ[7]. However among all the above described literature, no article described the forced degradation carried out under thermolytic, photolytic, acid/base

hydrolytic, and oxidative stress conditions and also none of the reported analytical procedures describes a method for the estimation of DCZ in pharmaceutical dosage forms. In the present study, attempts were made to develop a rapid, economical, precise, and accurate HPLC method for the estimation of the dacarbazine and its degradation products [8-11].

MATERIALS AND METHODS

Chemicals and reagents

DCZ standard was obtained from Taj Pharmaceuticals Ltd (Mumbai, India). Orthophosphoric acid, Triethylamine and Acetonitrile (HPLC grade) were obtained from Rankem Chemicals (Mumbai, India). The drug product of DCZ (DABAZ powder for injection) (Intas Pharmaceuticals Ltd. Gujarat, India) with a label claim (100mg/?ml) was purchased from the market. The 0.45µ nylon filter was obtained from advanced micro devices (Ambala Cantt, India).Triple distilled water (Millipore water) was used throughout the experiment. Other chemicals used were analytical or HPLC grade.

Chromatographic conditions

The chromatographic system used was an Waters 2695 model, which comprised a degasser, quaternary pump, auto injector, column compartment, and photodiode array detector, and the system was controlled through Empower 2 software. Agilent Eclipse XDB C18 (150 x 4.6 mm, 5µ.) column maintained at 30°C column oven temperature and a mobile phase flow rate of 1.0 mL/min. The mobile phase composed of Buffer and Acetrnitrile was taken in 96:4%v/v. The buffer used in mobile phase contains 1 ml of Conc. Ortho phosphoric acid solution in to a 1000ml beaker add about 800ml of milli-Q water and make up to final volume with Water add 2ml of triethylamine, then pH was adjusted to 2.4 with dil.OPA solution and filtered through a 0.45-µm nylon filter before being degassed in ultrasonic bath prior to use. Measurements were made with injection volume 10 µL and PDA detection at 323 nm. For the analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained directly from the spectral analysis report by using the previously mentioned software.

Standard stock solution

Standard solution was prepared by dissolving the drug in the diluent and diluting it to the desired concentration. Diluent used for the standard and sample preparation was composed of methanol and water in the ratio of 50:50 (v/v).

DCZ standard stock solution

About 10 mg sample of DCZ (99.95%) was transferred in to a 10-mL volumetric flask and diluted to volume with diluent.

Calibration curve solutions

The solutions which were used for the preparation of the calibration curve were in the concentration range of 25–150 µg/mL.

Preparation of sample

Five tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 100mg of DCZ was transferred in to a 100-mL volumetric flask. To this flask, 70mL of diluent was added, and the solution was sonicated for 25min with intermittent shaking. Then the volume was made up with diluent and centrifuged at 10,000 rpm for 10 min. The centrifuged solution was filtered through a 0.45-µm filter. From the filtered solution, 0.2 mL of solution was transferred into a 10-mL volumetric flask and diluted to volume with diluent.

Procedure for forced degradation study of drug substances

Forced degradation of drug substance and the drug product was carried out under thermolytic, photolytic, acid/base hydrolytic and oxidative stress conditions. The ICH guideline states the minimum desired exposure as 200 Wh/m2, which corresponds to a change in absorbance of 0.5 AU of quinine actinometer at 400 nm. This change was observed in 24 h of irradiation. A second photolytic stress test experiment with greater irradiation time of 48 h was carried out. After the degradation, solution was diluted to achieve concentration of 100 µg/mL of DCZ.

Acidic degradation

To 1 ml of stock solution, 1ml of 1N Hydrochloric acid was added and refluxed for 60mins at 60^0c .The resultant solution was diluted to obtain 100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali degradation

To 1 ml of stock solution, 1 ml of 0.1N sodium hydroxide was added and refluxed for 60mins at 60^0c. The resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Oxidative degradation
To 1 ml of stock solution, 1 ml of 10% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 60 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal degradation
The standard drug solution was placed in oven at 105^0c for 60mins to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100 µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

UV-short degradation
The photochemical stability of the drug was also studied by exposing the 100 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation
Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to 100 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions
The chromatographic method was optimized by using different stationary phases like C18, C8, CN and different mobile phases containing buffers like phosphate, ammonium acetate, and triethylamine with different pH (2–5), and organic modifier (acetonitrile) were used. Finally, the chromatographic separation was achieved using Agilent Eclipse, XDB C18, (150 x 4.6 mm, 5µ) column. Changing the composition of mobile phase optimized the chromatographic method. To develop a stability-indicating method assessing the effect of change of proportion, the pH of mobile phase was maintained at 2.4 and the drug was well-resolved from degradation products at mobile phase composition of buffer–acetonitrile (96:4, v/v). From the development studies, it was determined that ortho phosphoric acid in milli-Q water and then pH adjusted to 2.4 with triethylamine and acetonitrile in the ratio of 96:4 (v/v), the flow rate of mobile phase 1.0 mL/min, and column temperature 30°C was optimal. The analyte had adequate retention, peak shape, less tailing and the chromatographic analysis time was less than 8 min. In optimized conditions DCZ and its degradation product were well-separated. Typical retention time of DCZ was about 4.34min.

Though conditions used for forced degradation were attenuated to achieve degradation in the range of 2–10%, this could not be achieved in the case of acid even after exposure for prolonged duration. During the initial forced degradation experiments, it was observed that photolytic degradation was a fast reaction for DCZ. Oxidative degradation was a fast reaction for DCZ, and almost complete degradation occurred when 30% H_2O_2 solution is used. For drug substances, diluted NaOH(0.1 N) and 1% H_2O_2 was used to achieve 2–10% degradation. Table 1 indicates the extent of degradation of DCZ under various stress condition. Chromatographic peak purity data was obtained from the spectral analysis report, and a peak purity value greater than 990 indicates a homogeneous peak. The peak purity values for analyte peak, were in the range of 999–1000 for drug substance and in the range of 998–1000 for dosage form, indicating homogeneous peaks and thus establishing the specificity of assay method. Fig. 2 shows the blank chromatogram (a), chromatogram of DCZ standard solution (b), the chromatogram of DCZ market sample(c), and fig. 3 shows the chromatograms of degraded DCZ by various exposures.

METHOD VALIDATION

Specificity
Photodiode array detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak. The purity angel was less than the purity threshold for drug substance as well as drug product, which shows that the peaks of analyte was pure and also that formulation excipients and degradation product were not interfering with the analyte peak. The results are shown in Table 2.

Calibration and linearity
Linearity the method was tested from 25 to 150% of the targeted level of the assay concentration (DCZ 100 µg/mL) for analyte. Standard solutions contained 25–150 µg/mL of DCZ. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area against the concentration of the drugs. The equations of the calibration curves for DCZ obtained was $y = 34222x + 8396.9$. In the DCZ determination, the calibration

graphs were found to be linear in the aforementioned concentrations with correlation coefficients 0.9998. Relative standard deviation (%RSD) for slope of DCZ was 0.836. The results are shown in Table 3.

Precision

The repeatability of the method was studied by determining the concentrations of DCZ six times. The average assay% for six determinations was 100.19%. For DCZ the %RSD of assay values was 0.3975. The results of the precision study indicate that the method is reliable (%RSD < 2). Intermediate precision of the method was determined by analyzing the samples six times on different days by different chemists using different analytical columns of the same make and different HPLC systems. The assay results of chemist 2 for DCZ was 99.42%. The %RSD of assay value was 0.106, for DCZ. The results are shown in Table 4.

Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 50, 100 and 150% of labeled amount of the drug (100mg DCZ) in tablets. Three replicate samples of each concentration level were prepared and the %recovery at each level (n = 3), and mean% recovery (n=9) were determined (Table 2). The recovery samples were prepared as per the procedure mentioned earlier. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for DCZ ranged from 99.31 to 101.08%. The average recovery of three levels (nine determinations) for DCZ was 100.17(0.636) with% RSD shown in parenthesis. The results are shown in Table 5.

Robustness

The robustness of a method is the ability of method to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered, and chromatographic changes were evaluated. The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on retention of DCZ, it was changed to 0.1 units from1.0 to1.1mL/min and 0.9mL/min. The effect of column temperature on resolution was studied at 28°C and 32°C instead of 30°C, while other mobile phase components were held constant. The effect of mobile phase composition on retention of DCZ was studied with buffer–acetonitrile at 98:2 (v/v) and 94:6 (v/v). The effect of buffer pH on retention of DCZ was studied at pH 2.2 and 2.6. At all conditions it was found that no significant change in the retention time of DCZ was observed.

Determination of limits of quantification and detection

The limit of detection (LOD) and limit of quantitation (LOQ) for DCZ was determined at a signal-to-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ for DCZ were 0.451 and 1.367 µg/mL , respectively, for 10-µL injection volume.

Solution stability

The stability of the standard solution was tested at intervals of 24 and 48 h. The stability of solutions was determined by comparing results of area%, resolution, and peak purity of DCZ. The area% values were within 0.5 % after 48 h. The results indicate that the solutions were stable for 48 h at ambient temperature as there was no formation of any unknown peak and solution remains stable. The RSD of peak area% was 0.37%, peak angel was less than the purity threshold.

Assay

The Content of DCZ in the DABAZ powder for injection was found by the proposed method and the percentage assay value was found to be 100.19±0.398. The results were shown in Table-6.

CONCLUSION

The isocratic reversed phase-LC method developed for analysis of DCZ in its pharmaceutical preparations is precise, accurate, and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is a stability indicating, separates degradation product, and can be conveniently used by quality control department to determine the assay of pharmaceutical preparations and also stability samples.

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Table1. RESULTS OF FORCED DEGRADATION STUDIES

Stress Condition	% Degradation	Peak Purity
Acid	15.96	999.387
Alkaline	13.41	999.843
Oxidation	20.48	999.429
Thermal	4.05	999.628
UV	52.32	999.508
Neutral	2.72	999.916

Table 2. SYSTEM SUITABILITY PARAMETERS (n=6)

Parameters	Found	Acceptable limits
USP theoretical plates	4500	N>2000
USP tailing factor	1.23	T<2.0
Repeatability (t_R)	0.77	RSD<2%
Repeatability (peak area)	0.8	RSD<2%

Table 3. STATISTICAL DATA OF CALIBRATION CURVES (n = 3)

Parameters	Results
Linearity range	25-150µg/ml
Regression equation	y = 34222x + 8396.9
Relative standard deviation of slope (%)	0.836
Correlation coefficient (r^2)	0.999

TABLE 4. RESULTS OF INTERMEDIATE PRECISION

DCZ	Set 1(n=6)	Set 2(n=6)
Mean assay (%)	100.16	100.92
%RSD	0.93	0.90
F-test(calc value/critical value)	2.121/6.188	2.534/6.291
t-test(calc value/critical value)	0.600/2.326	1.891/2.306

TABLE 5. RESULTS OF RECOVERY TESTS

Name of the drug	Amount of drug in dosage form(µg/ml)	Amount of pure drug added(µg/ml)	Total found(µg) (Mean±SD) (n=3)	RSD%	Recovery of pure drug added (%)
DCZ	100	50	50.060±0.198	0.3970	100.121
	100	100	100.572±0.710	0.7061	100.572
	100	150	149.754±1.113	0.7435	99.83

Table 6: ASSAY OF DACARBAZINE DOSAGE FORM

No of samples assayed	Label amount(mg)	Amount found(mg)	%Assay (Mean±SD)	RSD
6	100	100.19	100.19±0.398	0.397

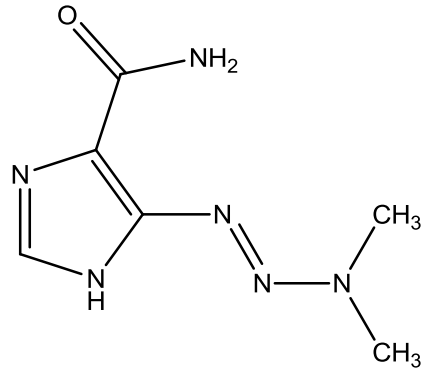

Fig 1. Chemical structure of Dacarbazine

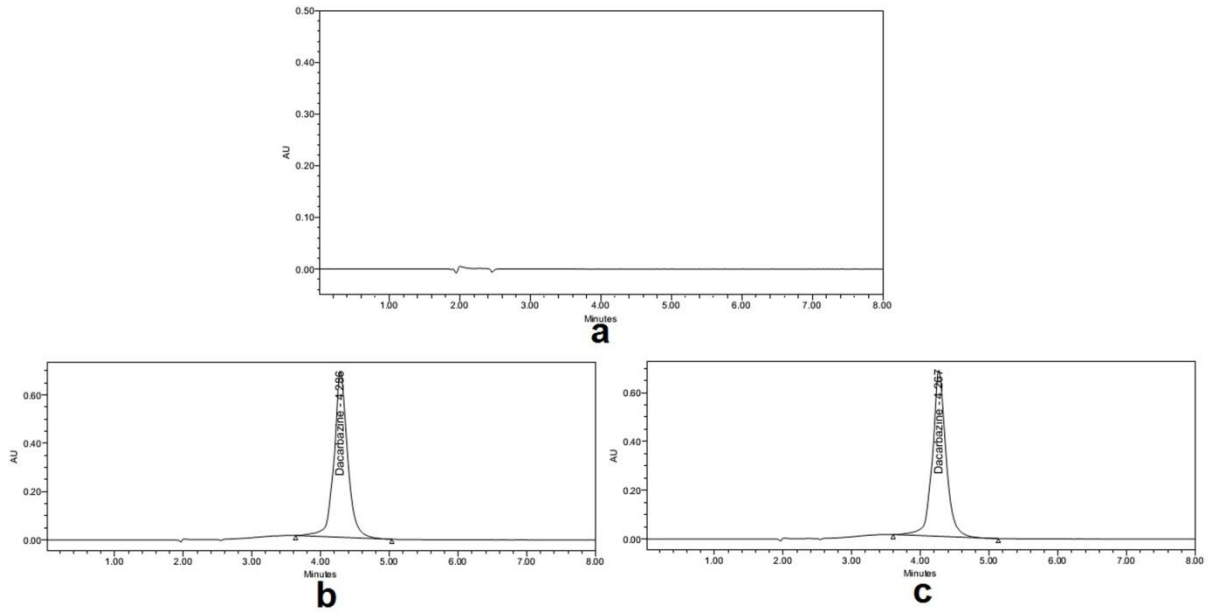

Fig.2.Typical HPLC chromatogram for Blank(a), DCZ standard(b), DCZ market sample(c).

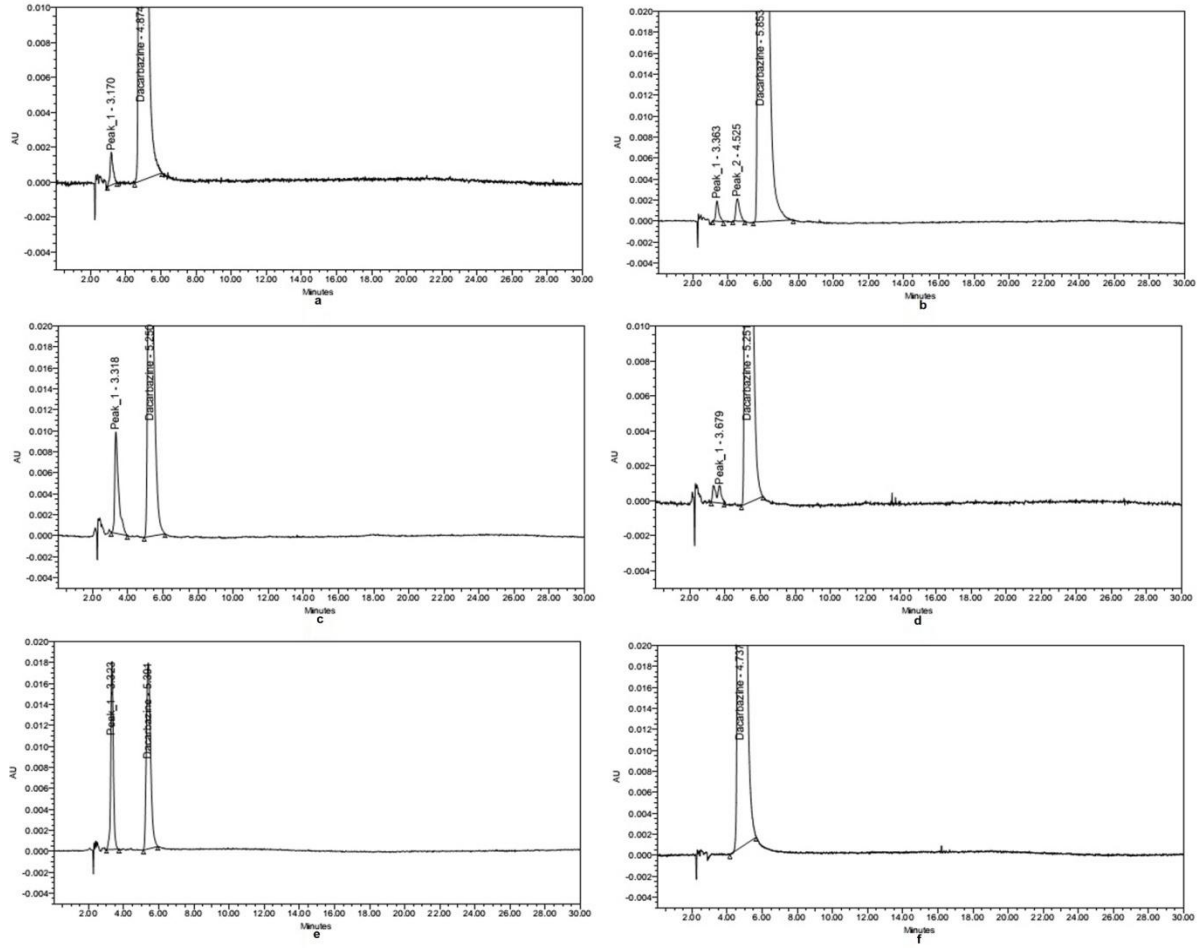

Fig.3. Typical HPLC Chromatograms of acid hydrolysis-degraded drug product (a), base hydrolysis-degraded drug product(b), oxidative degraded drug product(c), dry heated drug product(d), photo-degraded drug product(e) and neutral-hydrolysis degraded drug product(f), respectively.

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