International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 2, Suppl 4, 2010

Research Article

FORCED DEGRADATION STUDY TO DEVELOP & VALIDATE STABILITY INDICATING RP-LC METHOD FOR QUANTIFICATION OF NICARDIPINE HCL IN BULK AND TABLET FORMULATION

TUSHAR GADKARI[1, 2], PRANAV CHANDRACHOOD[1], RASIKA TORANE[1], SHAHAJI TELE[2], NIRMALA DESHPANDE[1], JYOTI SALVEKAR*

Department of Chemistry, S P College, Pune 431 030, Maharashtra, India, T R Ingle Research Laboratory, S P College, Pune 431030, Maharashtra, India, Sai Advantium Pharma Ltd., International Biotech Park, Hinjewadi Phase-II, Pune 411057, Maharashtra, India. Email: tvg5@rediffmail.com

Received: 26 July 2010, Revised and Accepted: 29 August 2010

ABSTRACT

A forced degradation study of nicardipine in bulk and in its tablet form was conducted under the conditions of hydrolysis, oxidation and photolysis in order to develop a rapid and sensitive stability indicating LC-UV method for quantification of nicardipine. Nicardipine was found stable in acidic buffer upto 48 hrs while in alkaline buffer found degraded.The drug and its degradation products were optimally resolved on C18 column with mobile phase composed of acetonitrile-ammonium acetate (100 mM) (70:30, v/v).

A 150 mm, 5 µ ODS column was used for rapid separation. Quantification was achieved by UV detection at 237 nm, on the basis of peak area. Forced degradation studies were performed on a bulk sample of nicardipine using 0.1 M hydrochloric acid, 0.1M sodium hydroxide, 30 % hydrogen peroxide, heat (70 °C), and photolytic degradation. The method was linear in the range of 0.3 – 100 µg mL^{-1} nicardipine concentration. Excellent recoveries (99.5 – 100.6 %) proved that the method was sufficiently accurate. The LOD and LOQ were found to be 130 ng mL^{-1} and 393 ng mL^{-1} respectively. Nicardipine found unstable in alkaline condition.

Keywords: Nicardipine, Stability indicating assay, Forced degradation, LC-PDA.

INTRODUCTION

Nicardipine is a calcium antagonist of the dihydropyridine class and has been widely used for the treatment of hypertension and angina pectoris [1].Nicardipine belongs to the class of blood pressure reducing medications called calcium channel blockers (CCBs). These medications block the movement of calcium into the smooth muscle cells surrounding the arteries of the body. Since calcium promotes contraction of muscles, blocking calcium entry into the muscle cells relaxes the arterial muscles and causes the arteries to become larger. This lowers the blood pressure, which reduces the work that the heart must do to pump blood to the body. Reducing the work of the heart lessens the heart muscle's demand for oxygen and thereby helps prevent angina in patients with coronary artery disease. FDA approved nicardipine in December 1988[2].

Several analytical methods for nicardipine were reported including spectrophotometry [3-6], volammetry [7-9], high performance liquid chromatography [10-14], liquid chromatography-mass spectrometry [15-18], and capillary electrophoresis [19].A study of forced degradation, degradation kinetics and photo stability of nicardipine were also reported in literature [20-22].

The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products requires stress testing to be performed to elucidate the inherent stability characteristics of the active substance [23-24]. Hydrolytic, oxidative, thermal and photolytic stability should be determined. The aim of the present work was to develop validate and apply LCMS compatible stability indicating HPLC method for determination of nicardipine. Because no method has been reported as LCMS compatible stability indicating assay in the literature, it was thought necessary to develop a simple, rapid method for stability studies, which can be further used for identification of degradation product by LCMS.

This paper describes selective and rapid analytical method. The sample preparation and mobile phase preparation steps were made simpler which do not required any pH adjustment. The total run time of method per sample was 10.0 min.

MATERIALS AND METHODS

Nicardipine was extracted from tablets. Purified water was obtained from Milli-Q Water purification system. Methanol (HPLC grade), Acetonitrile (HPLC grade) were purchased from Qualigens (India). Hydrochloric acid and sodium hydroxide both certified grades were purchased from spectrochem (India). Ammonium acetate (AR grade) purchased from Qualigens (India). All reagents were used as received without further purification.

Extraction of nicardipine from tablets

Nicardipine tablet (20 mg) was crushed to powder and sonicated with 100 mL methanol for 5 min. The solution was filtered through 0.45 µm membrane filter then lyophilized for 12 h. The solid nicardipine obtained was approximately 99.9 % pure.

Chromatographic and mass spectrometric system

The HPLC system (LC2010, Shimadzu Corporation, Kyoto, Japan) consisted of low-pressure gradient quaternary pump, Auto sampler, column-oven and photo diode array detector (SPD M20A). LCSolution workstation was used for data acquisition. The analytical column used for the study was C18, 150 mm length, 4.6 mm ID and 5 µm particle size (YMC ODS A, Japan). The data were collected and processed using LCMS solution software.

The mobile phase consisted of 100 mM ammonium acetate and acetonitrile (30:70) (*v/v*). Prior to analysis the mobile phase was degassed using a Millipore vacuum pump. The detector was set at 237 nm. The runtime was set for 10 min. The flow rate was maintained to 1.2 mL min^{-1}. The column oven temperature was maintained to 30 °C. The injection volume was 10 µL.

Forced degradation study

Acid induced hydrolysis

The reaction was initiated by adding 5.0 mL of nicardipine (1.0 mg mL^{-1}) to 100 mL volumetric flask containing 0.1 M hydrochloric acid. The volume made to 100 mL with 0.1 M hydrochloric acid. The degradation was carried out in thermostatically controlled water bath, protected from light.

Alkali induced hydrolysis

The reaction was initiated by adding 5.0 mL of nicardipine (1.0 mg mL^{-1}) to 100 mL volumetric flask containing 0.1 M sodium hydroxide. The volume made to 100 mL with 0.1 M sodium hydroxide. The degradation was carried out in thermostatically controlled water bath, protected from light.

Effect of hydrogen peroxide

The reaction was initiated by adding 5.0 mL of nicardipine (1.0 mg mL^{-1}) to 100 mL volumetric flask containing 30 % hydrogen peroxide. The volume made to 100 mL with 30 % hydrogen peroxide. The degradation was carried out in thermostatically controlled water bath, protected from light.

Effect of light

Nicardipine (50 mg, accurately weighed) was dissolved in 100 mL methanol. The solution was exposed to light from UV lamp for 24 hrs.

Effect of heat

The thermal stability of nicardipine was studied by heating it, both as powder and in methanolic solution (without pH adjustment) to 70 °C for 7 days.

Standard solution and calibration

Stock solution was prepared by dissolving 25 mg nicardipine in 25 mL methanol. Standard solutions were prepared by dilution of stock solution with mobile phase to furnish concentrations in the range 0.1 - 100 µg mL^{-1}. Triplicate 10 µL injections of each solution were chromatographed under the conditions described above. Peak areas were plotted against the corresponding concentrations to obtain calibration plot.

RESULTS AND DISCUSSION

Chromatographic conditions

The choice of chromatographic conditions selected was based on symmetry of peak shape and reduction of chromatographic analysis time. The chromatographic separation was achieved using a mobile phase containing a mixture of aqueous 0.1 M ammonium acetate and acetonitrile in the ratio (30:70, *v/v*). The column temperature (30 °C) has improved the peak shape of nicardipine. In optimized conditions, nicardipine and its degradation product were separated with a resolution greater than 2(Fig 1). The system suitability results were given in Table 1 and the developed LC method was found to be specific for nicardipine and its degradation products.

Table 1: System- suitability report

Compound (*n*=3).	t_R	R_S	N	T
Nicardipine	3.8	13.0	3972	1.11

n =3 determinations; t_R = Retention time in minutes; Rs = USP Resolution; ;T = USP tailing factor; N = No. of theoretical plates

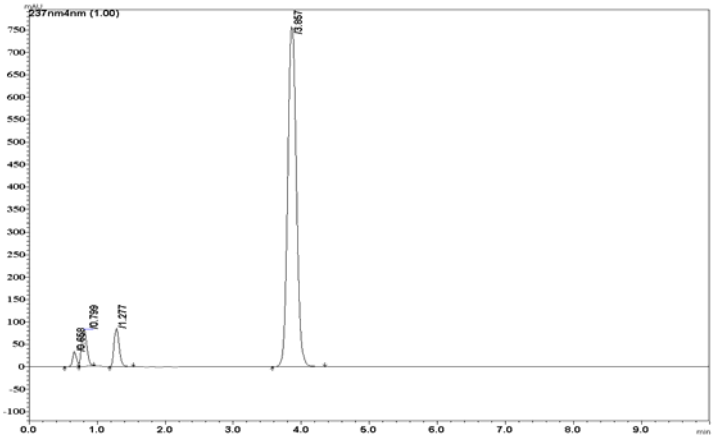

Fig. 1: Representative chromatogram obtained from alkaline solution of nicardipine after 48 h. Peaks: 3.8 min= nicardipine

Stability indicating assay

Degradation was not observed for nicardipine sample during stress conditions like heat and acid except in alkaline buffer and UV light. Nicardipine was degraded in alkaline buffer. Peak purity test results confirmed nicardipine peak is homogeneous in all the stress conditions tested. The mass balance of nicardipine in test samples was close to 100% and moreover, the unaffected assay of nicardipine in presence of degradation product confirms the stability indicating power of the method.

The non-interference of excipient peaks with nicardipine confirms the specificity of the developed method in formulation samples. The summary of forced degradation studies is given in Table 2.

Table 2: Summary of forced degradation study of nicardipine

Stress condition	Time	Assay (%)	Mass balance	Remarks
Acid hydrolysis	48 h	99.92	99.90	no degradation
Base hydrolysis	48 h	84.5	99.00	degradation observed
Oxidation	48 h	92.3	99.90	degradation observed
Thermal	7 days	99.96	99.89	no degradation
UV (254 nm)	48 h	69.52	99.88	degradation observed

Validation of Method

Linearity

The linearity of nicardipine standards was evaluated by analyzing a set of standards ranging from 40.0-to 60.0-µg mL^{-1}. The calibration curve parameters of nicardipine showed a linear relationship between peak area and concentration. The mean correlation coefficient, slope and intercept values were 0.9999, 7157, and 282 respectively.

Precision

The percentage RSD of assay of nicardipine during assay method precision study was within 1 %. The percentage RSD of assay results obtained in intermediate precision study was within 1 % confirming good precision of the method.

Range

The calibration range was established by consideration of the practical range necessary, in accordance with the concentration of

nicardipine in pharmaceutical product, to give accurate, precise and linear results.

Limits of detection and quantification

In accordance with ICH recommendations [23], the approach based on the standard deviation of the response and the slope of the calibration plot was used for determination of limits of detection and quantification. The limit of detection and quantification of nicardipine was 130 and 393 ng mL^{-1} for 10 µL injection volume respectively.

Accuracy

Percentage recovery of nicardipine in bulk drug samples was ranged from 99.5 to 100.6 %. The excellent recovery obtained suggests the accuracy of the method is good.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, percent organic strength, column temperature), the resolution between nicardipine and its degradation product was greater than 2, illustrating the robustness of the method.

Solution stability and mobile phase stability

The % RSD of assay of nicardipine during solution stability and mobile phase stability experiments was within 1 % RSD. No significant change was observed in the content of enicardipine during solution stability and mobile phase experiments. The solution stability and mobile phase stability experiments data confirm that nicardipine sample solutions and mobile phase used during assay were stable for at least 48 h.

CONCLUSION

Comparing with the analytical methods reported previously, the proposed LC-PDA method enables simple, accurate, reproducible and fast quantitative analysis of nicardipine in presence of degradation products. The method has been successfully applied to stability study. Nicardipine was found unstable in alkaline media but stable in acidic media. The optimum stability of nicardipine was at pH 1-7.

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