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HPLC Method for the Estimation of Nicorandil in Pharmaceutical Dosage Forms

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A reverse phase high pressure liquid chromatographic method (HPLC) has been developed for the estimation of nicorandil in its tablet dosage forms using RP-C18 column. The mobile phase (acetonitrile and 0.02 M potassium dihydrogen orthophosphate) was pumped at a flow rate of 0.8 mL/min in the ratio 40 : 60, and the eluents were monitored at 254 nm. The intra- and inter-day variation was found to be less than 2.5% showing high precision of the assay method. The mean recovery of the drug from the solutions containing 4 or 6 μg/mL was 99.83 ± 0.2% indicating high accuracy of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining nicorandil in bulk drug samples or in tablet dosage forms.

Key Words: Estimation, Nicorandil, HPLC.

INTRODUCTION

Nicorandil chemically is N-(2-hydroxy ethyl) nicotinamide nitrate. It is a potassium channel activator and is used in the treatment of angina pectoris[1,2]. So far only one HPLC method has been reported for the estimation of nicorandil[3]. The present study describes the determination of nicorandil in bulk drug samples and pharmaceutical dosage forms by using RP-C18 column with UV detection. Owing to the widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating nicorandil. The aim of this study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of nicorandil either in bulk drug samples or in pharmaceutical dosage forms.

EXPERIMENTAL

Nicorandil and metronidazole were gift samples from M/s Sun Pharmaceutical Industies Ltd, Ahmedabad, India and M/s Alkem Laboratories, Mumbai, India respectively. Acetonitrile and water used were of HPLC grade (Qualigens). All other reagents (potassium dihydrogen orthophosphate, methanol) used in the study were of AR grade (Qualigens).

A gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and RP-C18 column (250 mm × 4.6 mm I.D.;

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particle size 5 μm; Merck Lichrospher, USA) was used. The HPLC system was equipped with the software "Class-VP series version 5.03 (Shimadzu)".

Preparation of stock solution of internal standard: Metronidazole was used as internal standard for HPLC estimation of nicorandil. About 100 mg of metronidazole was accurately weighed, transferred to 100 mL volumetric flask, dissolved in methanol and made up to volume with methanol so as to give a stock solution of 1000 μg/mL (Stock-I). 5 mL of this stock solution was diluted to 100 mL with 0.02 M potassium dihydrogen orthophosphate to give 50 μg/mL solution (Stock-II). 1 mL of stock-II solution is added to standard nicorandil sample solutions.

Preparation of stock solutions of nicorandil: About 100 mg of nicorandil was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved in water and the solution was made up to volume with water. Each mL of this stock solution (Stock-I) contained 1000 μg of nicorandil. 10 mL of stock-I solution (1000 μg/mL) were diluted to 100 mL with 0.02 M potassium dihydrogen orthophosphate to give a stock solution containing 100 μg/mL (Stock-II).

Chromatographic conditions: Both acetonitrile and 0.02 M potassium dihydrogen orthophosphate were filtered before use through 0.2 μm membrane filter. The flow rate of the mobile phase was maintained at 0.8 mL/min in the ratio of 40 : 60 (acetonitrile : 0.02 M potassium dihydrogen orthophosphate). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector at 254nm. The data were acquired, stored and analyzed with the software "Class-VP series version 5.03 (Shimadzu)".

Procedure: From stock-II solution of nicorandil, 0.02 to 2 mL quantities of solution were transferred to 10-mL volumetric flasks. To these solutions 1 mL of metronidazole (internal standard) containing 50 μg/mL was added and volume was made up to 10 mL with 0.02 M potassium dihydrogen orthophosphate to get 0.2, 0.5, 1, 2, 4, 6, 8, 10 and 20 μg/mL. The standard solutions, prepared as above, were filtered through 0.4 μm membrane filter and the filtrate was injected five times into the column at a flow rate of 0.8 mL/min. The ratio of drug peak area to that of internal standard for each of the drug concentration was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. This regression equation was used to estimate the amount of nicorandil in pharmaceutical dosage forms.

Assay of nicorandil tablets: Ten tablets of nicorandil (containing 10 mg) were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 1 mg of nicorandil was placed in a 100 mL volumetric flask. 70 mL of water was added and the flask allowed to stand for 5 h with intermittent sonication to ensure complete solubility of the drug. The mixture was then made up to 100 mL with water, thoroughly mixed, and filtered through a 0.2 μm membrane filter. An aliquot of this filtrate was transferred to a volumetric flask along with appropriate volume of metronidazole (internal standard) solution and made up to volume with mobile phase to give an expected concentration 10 μg/mL of nicorandil and 5 μg/mL of metronidazole (internal standard). All determinations were conducted in triplicate. The same procedure was used to estimate the concentration of nicorandil in another commercial brand of nicorandil tablets.

Precision: The precision of the assay was determined in terms of intra- and inter-day variation in the peak area ratio for a set of drug solutions (10 or 20 μg/mL) assayed five times on the same day and on three different days. The intra- and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of coefficient of variation (CV), and obtained by multiplying the ratio of standard deviation to the mean with 100 [CV = (SD/mean) × 100].

Accuracy: The accuracy of HPLC assay method was assessed by adding known amount (4 or 6 μg) of the drug to a drug solution of known concentration (2 μg /mL) along with 5 μg internal standard solution and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (10 or 20 μg/mL) was added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of nicorandil in tablet formulations. In both the cases, the recovery studies were replicated five times. The accuracy was expressed in terms of the recovery, and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 100 so as to give the per cent recovery.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The goal of this study was to develop a rapid and sensitive HPLC method for the analysis of nicorandil in bulk drug samples and its tablet formulations using the most commonly employed RP C-18 column with UV detection.

The run time was set at 7 min and the retention times for nicorandil and internal standard (metronidazole) were 4.5 min and 3.7 min respectively (Fig. 1). Each sample was injected 5 times and the retention times of the drug and internal standard were same. The ratios of peak area of nicorandil to peak area of internal

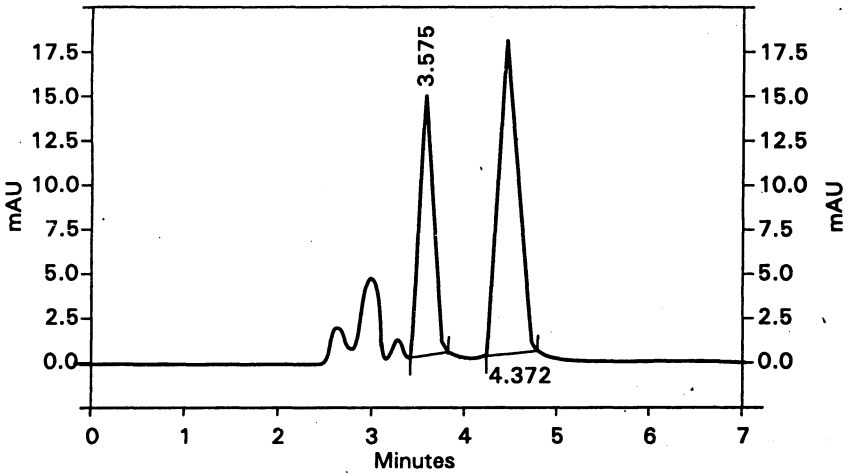

Fig. 1. A typical chromatogram for nicorandil

standard for different concentrations set up as above were calculated, and the average values for 5 such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (2.49%). When the concentration of nicorandil and its respective peak area ratios were subjected to regression analysis by least squares method, a good linear relationship (r = 0.9998) was observed between the concentration of nicorandil and the respective peak areas ratio in the range 0.2–20 μg/mL. The regression of nicorandil concentration over its peak area ratio was found to be Y = 0.0121 + 0.1389X (where Y = ratio of peak area of drug to that of internal standard, X = concentration of nicorandil). This regression equation was used to estimate the amount of nicorandil either in tablet formulations or in validation study (precision and accuracy).

TABLE-1
CALIBRATION OF THE HPLC METHOD FOR THE ESTIMATION OF NICORANDIL

Concentration of nicorandil (μg/mL)	Mean (±s.d) peak-area ratio (n = 5)	C.V. (%)
0	0	0
0.2	0.034	1.11
0.5	0.076	1.31
1	0.152	1.05
2	0.306	1.98
4	0.581	1.76
6	0.842	1.28
8	1.127	2.04
10	1.375	2.49
20	2.801	1.78

Regression Equation (from 0.2 to 20(g/ mL):
Y = 0.0121 + 0.1389X (r = 0.9998)

The proposed HPLC method was also validated for intra- and inter-day variation. When the solutions containing 10 or 20 μg/mL of nicorandil along with 5 μg/mL of metronidazole were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area ratio for five replicate injections was found to be less than 1.89%. Also, the inter-day variation (3 days and five injections) was found to be less than 2.5% (Table-2). Thus, the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (4 or 6 μg) was added to a known amount of drug solution (2 μg), there was a high recovery (99.97 ± 0.2%) of nicorandil (Table-3) indicating that the proposed method is highly accurate.

The HPLC method, developed in the present study, has also been used to quantify nicorandil in tablet dosage forms. Nicorandil tablets (containing 10 mg

of the drug) were analyzed as per the procedure described above. The average drug content was found to be 99% of the labeled amount (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in the tablet formulation did not interfere with the estimation of the drug by the proposed HPLC method. A known amount of the drug solution was added to the powder sample of the tablet dosage form and subjected to the estimation of the drug by the proposed method. There was a high recovery of nicorandil (98.47 ± 1.31%) indicating that the proposed procedure for the determination of nicorandil in the tablet dosage forms is highly accurate.

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Nicorandil concentration (μg/mL)	Concentration of nicorandil (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	C.V. %)	Mean (n = 5)	C.V. (%)
10	10.09	1.89	10.14	2.50
20	20.12	1.25	20.09	1.80

TABLE-3
RECOVERY OF NICORANDIL

Amount of drug added (μg)	Mean (±s.d.) amount (μg) found (n = 5)	Mean (±s.d.) % of recovery (n = 5)
4	4.03 ± 0.06	100.75 ± 0.3
6	5.99 ± 0.08	99.83 ± 0.2

TABLE-4
MEAN (±s.d) AMOUNT OF NICORANDIL IN TABLET DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the tablet	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AA	10	9.89 ± 1.04	98.9 ± 0.99
BB	10	9.94 ± 0.67	99.4 ± 1.20

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