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RP-HPLC Estimation of Isradipine in Pharmaceutical Dosage Forms

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A reverse phase high performance liquid chromatographic method has been developed for the estimation of isradipine in its capsule dosage forms using RP-C_{18} column. The mobile phase (methanol, acetonitrile and acetate buffer pH 2.8) was pumped at a flow rate of 1 mL/min in the ratio of 60:30:10 and the eluent were monitored at 290 nm. The intra- and inter-day variation was found to be less than 2 % showing high precision of the assay method when compared with reported result. The mean recovery of the drug from the solution containing 200 or 300 µg/mL was 99.66 ± 1.20 % indicating high precision and accuracy. The proposed HPLC method may be used for determining isradipine in bulk drug samples or in pharmaceutical dosage forms.

Key Words: Estimation, Isradipine, RP-HPLC.

INTRODUCTION

Isradipine is calcium channel blocker[1,2] and is chemically 3,5-pyridine-dicarboxylic-4-(4-benzofurazonyl)-1,4-dihydro-2,6-dimethyl methyl, 1-methyl ethyl ester. So far only two HPLC methods have been reported for the estimation of isradipine[3-5]. The present study describes the determination of isradipine in bulk drug samples and pharmaceutical dosage forms by using RP-C_{18} column with UV detection. Owing to the wide spread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for estimating isradipine. The aim of this study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of isradipine in bulk drug samples or in pharmaceutical dosage forms.

EXPERIMENTAL

Isradipine and ethamsylate were gift samples from M/s Pfizer Pharmaceutical Industries Ltd., Mumbai, India and M/s. Aristo Pharmaceutical

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industries Ltd., Bhopal, India, respectively. Acetonitrile, methanol and water used were of HPLC grade (Qualigens). All other reagents used in this study were of AR grade (Qualigens).

An isocratic HPLC (Waters India, USA) with a single Waters 510 pump, Waters 486 tunable absorbance detector and RP-C_{18} coloumn (Bondapak, 5 mm particle size) were used. The HPLC system was equipped with software Millennium 32.

Preparation of stock solution of internal standard: Ethamsylate was used as internal standard for RP-HPLC estimation of isradipine. About 100 mg of ethamsylate 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). 1 mL of stock-I solution is added to standard isradipine solutions.

Preparation of stock solution of isradipine: About 100 mg of isradipine was accurately weighed and transferred to a 100 mL volumetric flask. It was dissolved in methanol and the solution was made up to volume with methanol. Each mL of this stock solution (Stock-I) contained 1000 µg of isradipine.

Chromatographic conditions: Methanol, acetonitrile and acetate buffer[6] were filtered through 0.2 µm membrane filter before use. The flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 60:30:10 (methanol:acetonitrile:acetate buffer pH 2.8).The column temperature was maintained at 40 ºC and concentration of drug was detected by UV detector at 290 nm. The data was collected, stored and analyzed with the software Millennium 32.

Procedure: From stock-I solution of isradipine 0.5-6 mL quantities of solution were transferred to10 mL volumetric flasks. To this solution, 1 mL of ethamsylate (internal standard) containing 1000 µg/mL was added and volume was made up to 10 mL with methanol to get 50, 100, 200, 300, 400, 450, 500 and 600 µg/mL. The standard solutions, prepared as above, were filtered through 0.4 µm membrane filter and filtrate was injected five times into the column at a flow rate 1 mL/min. The ratio of drug peak area to that of internal standard for each 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 isradipine in pharmaceutical dosage forms.

Assay of isradipine capsules: 20 Capsules (containing 5 mg) were weighed, contents were finely powered and an accurately weighed sample of powder equivalent to 10 mg of isradipine was placed in a 100 mL volumetric flask. 70 mL of methanol was added and flask was 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 methanol, thoroughly

mixed and filtered through a 0.2 μm membrane filter. An aliquot of this filtrate was transferred to a volumetric flask with appropriate volume of ethamsylate (internal standard) solution and made up to volume with methanol to give expected concentration 100 μg/mL of isradipine and 100 μg of ethamsylate (internal standard). All determinations were conducted in triplicate.

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 (100 or 200 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) 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 (200 or 300 μg) of the drug to drug solution of known concentration (200 μg/mL) along with 100 μg internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (200 or 300 μg/mL) was added to the volumetric flask containing the powdered sample of the capsule formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of isradipine in capsule formulations. In both 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 percent recovery.

RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in 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 RP-HPLC method for the analysis of isradipine in bulk drug samples and its capsule formulations using most commonly employed RP-C_{18} column with UV detection.

The run time was set at 10 min and the retention times for isradipine and internal standard (ethamsylate) were 3.7 and 5.6 min, respectively. Each sample was injected 5 times and the retention times of the drug and internal standards were same. The ratios of peak area of isradipine to peak area of internal standard for different concentrations setup as above were calculated and the average values for five such determinations are shown in Table-1. The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient variation (3 %). When the concentration of isradipine and its respective peak area ratios were subjected to regression analysis by least squares method, a good linear relationship

(r = 0.9999) was observed between the concentration of isradipine and the respective peak areas in the range 50-600 µg/mL. The regression of isradipine concentration over its peak area ratio was found to be Y = -1.958 + 0.051X (where Y = ratio of peak area of drug to that of internal standard, X = concentration of isradipine). This regression equation was used to estimate the amount of isradipine either in capsule formulation or in validation study (precision and accuracy).

TABLE-1
CALIBRATION OF THE RP-HPLC METHOD FOR THE ESTIMATION OF ISRADIPINE

Concentration of isradipine (µg/mL)	Mean (± SD) peak area ratio (n = 5)	CV (%)
50	0.629	1.80
100	3.217	1.21
200	8.393	1.32
300	13.569	1.82
400	18.745	1.89
450	21.332	1.78
500	23.920	2.94
600	29.096	1.76

Regression equation (from 50 to 600 µg/mL):
Y = -1.958 + 0.051 X (r = 0.9999).

The proposed RP-HPLC methods also validated for intra- and inter-day variation. When the solutions containing 100 or 200 µg/mL of isradipine along with 100 µg/mL of ethamsylate 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 2 % (Table-2A and 2B) when compared with reported result[3]. The results show that the proposed RP-HPLC method is highly reproducible. When a known amount of drug solution (200 or 300 µg) was added to a known amount of drug solution (200 µg), there was a high recovery (99.66 ± 1.20 %) of isradipine (Table-3) indicating that the proposed method is highly accurate.

TABLE-2A
PRECISION OF THE PROPOSED RP-HPLC METHOD
CONCENTRATION OF ISRADIPINE FOUND ON INTRA DAY

Isradipine concentration (µg/mL)	Concentration of isradipine (µg/mL) found			
	In the method adopted		Reported result*	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
100	100.09	1.89	97.9	0.5
200	200.12	1.25	193.9	4.7

*According to reference no. 3.

TABLE-2B
PRECISION OF THE PROPOSED RP-HPLC METHOD
CONCENTRATION OF ISRADIPINE FOUND ON INTER DAY

Isradipine concentration (µg/mL)	Concentration of isradipine (µg/mL) found			
	In the method adopted		Reported result*	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
100	100.14	2.50	94.1	3.2
200	200.09	1.80	-	-

*According to reference no. 3.

TABLE-3
RECOVERY OF ISRADIPINE

Amount of drug added (µg)	Mean ± SD amount (µg) recovered (n = 5)	Mean ± SD % of recovery
200	200.03 ± 0.06	100.01 ± 0.30
300	298.99 ± 0.08	99.66 ± 1.20

The RP-HPLC method, developed in the present study has also been used to quantify isradipine (5 mg) in capsule dosage forms. The average drug content was found to be 97.8 % of the labelled amount (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in the capsule formulations did not interfere with the estimation of the drug by the proposed RP-HPLC method. A known amount of the drug solution was added to the powder sample of the capsule dosage form and subjected to the estimation of the drug by the proposed method. There was high recovery of isradipine (98.32 ± 1.20 %) indicating that the proposed procedure for the determination of isradipine in the capsule dosage forms is highly accurate.

TABLE-4
MEAN ± SD AMOUNT OF ISRADIPINE IN CAPSULE
DOSAGE FORMS BY PROPOSED RP-HPLC METHOD

Brand of the capsule	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AAA	5	4.89 ± 1.04	97.8 ± 0.99

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