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Reverse Phase HPLC Method for the Estimation of Isradipine in Pharmaceutical Dosage Forms

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A rapid and sensitive high performance liquid chromatographic method was developed for the estimation of isradipine in pharmaceutical dosage forms. Isradipine was chromatographed on a reverse phase C_{18} column using ethamsylate as internal standard in a mobile phase consisting of acetonitrile : methanol : water (40 : 50 : 10 v/v). The mobile phase was pumped at a flow rate of 1 mL/min, and the eluents were monitored at 290 nm. The calibration curve was linear in the range of 2–160 μg/mL. The intra- and inter-day variation was found to be less than 1% showing high precision of the assay method. The mean recovery of the drug from the solution containing 50 μg/mL was 98.38 ± 0.41% 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 isradipine in bulk drug samples or in pharmaceutical dosage forms.

Key Words: Estimation, Isradipine, Reverse phase HPLC.

INTRODUCTION

Isradipine is chemically, 3,5-pyridinedicarboxylic acid-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl methyl, 1-methyl ethyl ester. It is a calcium channel blocker and is used in the treatment of hypertension[1, 2]. 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 widespread use of HPLC in routine analysis, it is important that well validated HPLC methods are to be developed for the estimating isradipine. The aim of the study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of isradipine either 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 Industries Ltd., Bhopal, India respectively. Acetonitrile, methanol and water used were of HPLC

grade (Qualigens). A isocratic HPLC (Waters India, USA) with a single Waters-510 pump, Waters-486 tunable absorbance detector and RP-C_{18} column (Bond-apak, 5 μm particle size) was used. The HPLC system was equipped with the software Millennium-32.

Preparation of Stock Solution of Internal Standard: Ethamsylate was used as internal standard for the 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). 2 mL of the stock solution was diluted to 10 mL with methanol to give a 200 μg/mL solution (Stock-II). 1 mL of stock-II solution was added to standard isradipine sample solution.

Preparation of Stock Solution of Isradipine: About 100 mg of isradipine was accurately weighed and transferred to 100 mL volumetric flask. It was dissolved in methanol and the solution was made up to volume with methanol. Each mL of stock solution (Stock-I) contained 1000 μg of isradipine. 10 mL of this stock solution was diluted to 100 mL with methanol to give 100 μg/mL solution (Stock-II).

Chromatographic Conditions: Methanol, acetonitrile and water were filtered before use through 0.4 μm membrane filter. The flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 40 : 50 : 10 (acetonitrile : methanol : water). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector 290 nm. The data was acquired, stored and analyzed with software Millenium-32.

Procedure

From stock-II solution of isradipine, 0.2–16 mL of solutions were transferred to 10 mL volumetric flasks. To these solutions 1 mL of ethamsylate (internal standard) containing 200 μg/mL was added and volume was made up to volume with methanol to get 2, 4, 6, 8, 10, 20, 40, 60, 80, 160 μg/mL. The standard solutions prepared as above were filtered through 0.4 μm membrane filter and filtrate was injected 5 times into the column at a flow rate of 1 mL/min. The ratio of the drug peak area to that of internal standard for each of the drug concentrations was calculated. The regression of the drug concentration over the ratio of drug peak area to that of internal standard was obtained. The regression equation was used to estimate the amount of isradipine either in pharmaceutical formulation or in validation study.

Assay of Isradipine in Capsules: 20 capsules (each containing 5 mg) were weighed, powdered and an accurately weighed sample of powder equivalent to 50 mg of isradipine was placed in a 100 mL volumetric flask. 70 mL of methanol was added and the 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 through a 10 mL volumetric flask along with appropriate volume of ethamsylate solution and made up to volume with methanol to give an expected concentration of 100 μg/mL of isradipine and 20 μg/mL of ethamsylate (internal standard). All determinations were conducted 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 (50 or 100 μg/mL), on three different days (n = 5). The intra- and inter-day variation in the peak area ratio 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 the HPLC assay method was assessed by adding known amount (50 or 100 μg) of the drug to a drug solution of known concentration (50 μg/mL) along with 20 μg/mL internal standard and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (50 or 100 μg) was added to the volumetric flask containing the powder 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 the cases, the recovery studies was 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 present study was carried out to develop a specific, sensitive, precise and accurate HPLC method for the analysis of isradipine in pharmaceutical capsule dosage forms. The run time of the method was set at 10 min; the retention times of isradipine and ethamsylate (internal standard) were 3.7 min and 2.2 min respectively (Fig. 1). When the same drug solution was injected five times, the retention time of the drug and internal standard was the same. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes fewer amounts of expensive HPLC solvents. Table-1 shows the mean peak area ratios of isradipine solutions for 5 such determinations. When the concentration of isradipine and its respective peak area ratios were subjected to regression analysis by least square method, a high correlation coefficient was observed (r = 0.9999) in the range of 2 to 160 μg/mL. The regression of isradipine concentration over its peak area ratio was found to be $Y = -0.1110 + 0.46215X$, where Y is the peak area ratio and X is concentration of isradipine. The regression equation was used to estimate the amount of isradipine either in capsule formulations or in validation study (precision and accuracy).

The proposed HPLC methods were also validated for intra- and inter-day variation. When the solutions containing 50 or 100 μg/mL of isradipine along with 20 μg/mL of ethamsylate were repeatedly injected in the same day, the coefficient of variation (CV) in the peak area ratio of the drug for five replicate injections was found to be less than 1.9%. Also, the inter-day variation (3 days and 5 injections) was found to be less than 3% (Table-2). Thus the results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (50 or 100 μg) was added to a preanalyzed sample of drug solution (50 μg/mL), there was high recovery (98.38 ± 0.41%) of isradipine indicating that the proposed HPLC method is highly accurate. The HPLC method, developed in the present study, has also been used to quantify Isradipine in capsule dosage forms. Isradipine capsules (containing 5 mg of drug) were analyzed as per the procedure described

above. The average drug content was found to be 97% of the labeled amount (Table-4).

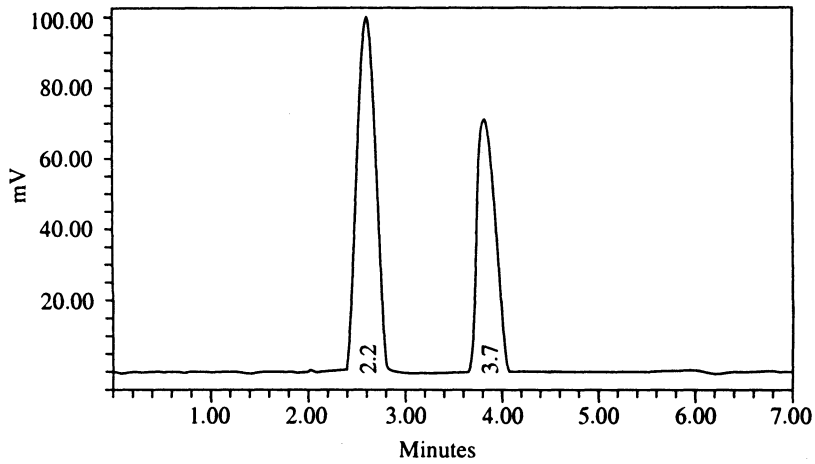

Fig. 1. Typical Chromatogram for isradipine

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

Concentration of isradipine (μg/mL)	Mean (± s.d.) peak area ratio (n = 5)	CV (%)
0	0	0
2	0.7621	1.86
4	1.3822	1.12
6	2.5869	1.23
8	3.5119	1.98
10	4.5628	1.94
20	9.2629	1.86
40	18.5221	1.62
60	27.6162	2.32
80	36.8123	2.16
160	72.8121	1.52

Regression equation (from 2 to 160 μg/ml): $Y = -0.1110 + 0.46215X$ ($r = 0.9999$)

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Isradipine concentration (μg/mL)	Concentration of isradipine (μg/mL) found on			
	Intra-day		Inter-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
50	50.32	0.98	50.06	1.46
100	100.16	1.89	100.21	2.38

TABLE-3
RECOVERY OF ISRADIPINE

Amount of drug added (μg)	Mean (± s.d.) amount (μg) recovered (n = 5)	Mean (± s.d.) % of recovery (n = 5)
50	49.32 ± 0.12	98.64 ± 0.62
100	98.90 ± 0.14	98.90 ± 0.10

TABLE-4
MEAN (± S.D.) AMOUNT OF ISRADIPINE IN CAPSULE DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the capsule	Labelled amount (mg)	Observed amoutn (mg)	Purity (%)
AAA	5	4.85 ± 0.16	97 ± 2.47

No interfering peaks were found in the chromatogram indicating that excipients used in the capsule 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 capsule dosage form and subjected to the estimation of the drug by the proposed method. There was high recovery of isradipine (98.41 ± 0.38%) indicating that the proposed procedure for the determination of isradipine in the capsule dosage form is highly accurate.

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