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# Development and Validation of a Reverse Phase HPLC Method for the Analysis of Isradipine in Pharmaceutical Dosage Forms

R. NAGESWARA RAO*, P. NAGARAJU, C. SRINIVASULU
P. RADHA KRISHNAMURTHY, D. SIREESHA and V. SUMNATH REDDY
*Department of Pharmaceutical Chemistry*
*College of Pharmaceutical Sciences, Berhampur*
*AR/PO Mohuda, Dist. Ganjam-760 002, India*
*E-mail: nageshpharma@rediffmail.com*

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 in a mobile phase consisting of methanol : water in the ratio of 70 : 30 v/v. The mobile phase was pumped at flow rate of 1 mL/min and the eluents were monitored at 290 nm. The calibration curve was linear in the range of 1–100 μg/mL. The intra- and inter-day variation was found to be less than 2% showing high precision of the assay method. The mean recovery of the drug from the solution containing 20 μg/mL was 99.75 ± 1.10% 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 and pharmaceutical dosage forms.

**Key Words: Isradipine, Reversed phase HPLC, Dosage forms.**

## INTRODUCTION

Isradipine is calcium channel blocker[1,2] and chemically 3,5-pyridinedicarboxylic acid-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl methyl-1-methyl ethyl ester. A few analytical methods have been reported for the estimation of isradipine in pharmaceutical dosage forms. Some of the methods utilize TLC and gas chromatography, and the process is considered tedious. Other reported methods such as spectrophotometry and HPLC[3–5] are not accurate. The HPLC methods using the most commonly available columns and detectors like UV are preferred. The present study describes the determination of isradipine in bulk drug samples and pharmaceutical dosage forms by using RP-$C_{18}$ column with UV detector.

## EXPERIMENTAL

Isradipine was a gift sample from Pfizer Pharmaceuticals Ltd., Mumbai, India. Methanol and water were of HPLC grade (Qualigens). An isocratic HPLC (Waters India, USA) with a single Waters-510 pump, Waters-486 tunable absorbance

detector and RP-$C_{18}$ column (Bondapak, 5 μm particle size) was used. The HPLC system was equipped with software Millennium-32.

The contents of the mobile phase, methanol and water in the ratio of 70 : 30 v/v were filtered before use through a 0.4 μm membrane filter and degassed for 30 min.

The components of the mobile phase were pumped from the solvent reservoir to the column at a flow rate of 1 mL/min that yielded column back pressure 140–150 kg/cm$^2$. The column temperature was maintained at 40°C. The eluents were monitored at 290 nm. Prior to the injection of the drug solutions, the column was calibrated for at least 30 min with the mobile phase flowing through the system.

**Procedure**

The solutions were prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. Stock solution of the drug was prepared by dissolving 100 mg of isradipine in 100 mL volumetric flask containing 70 mL of methanol, sonicated for about 20 min and then made up to volume with methanol. Working standard solution of isradipine was prepared by suitable dilution of the stock solution with methanol. Five sets of isradipine were prepared in methanol at concentrations of 1, 2, 4, 6, 8, 10, 20, 40, 60, 80, 100 μg/mL. Each of the samples (20 μL) was injected five times into the column and the peak area of the drug was recorded.

**Assay of Isradipine in capsules:** 20 capsules were weighed, finely powdered and an accurately weighed sample of powdered capsules equivalent to 100 mg of isradipine was placed in a 100 mL volumetric flask. 70 mL of methanol was added, shaken well and the flasks allowed to stand for 4 h with intermittent sonication to ensure complete solubility of drug. The mixture was then made up to volume with methanol, thoroughly mixed and then filtered through a 0.4 μm membrane filter. An aliquot of the filtrate was transferred to a volumetric flask and made up to volume with methanol to give an expected concentration of 20 μg/mL of isradipine. 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 for a set of drug solutions on three different days (n = 5). The intra- and inter-day variation in the peak area of drug solution (20 or 40 μg/mL) was calculated in terms of coefficient of variation (CV) and obtained by multiplying the ratio of standard deviation to the mean with 100 [CV = ± s.d./mean × 100].

**Accuracy:** The accuracy of the HPLC assay method was assessed by adding known amount (20 or 40 μg) of the drug to a drug solution of known concentration (20 μg/mL) and subjecting the samples to the proposed HPLC method. Also, known amount of drug solution (20 or 40 μg/mL) was added to the volumetric flask containing the powder sample of the capsule formulation with known amount of drug. The drug was estimated as in the procedure described above for the estimation of isradipine in the capsule 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 concentrarion with 100, so as to give the per cent recovery.

## RESULTS AND DISCUSSION

The run time of the method was set at 10 min and isradipine appeared on the chromatogram at 3.3 min (Fig. 1). When the same drug solution was injected 5 times, the retention time of the drugs was same. The peak area of isradipine was calculated and the averages of five such determinations were given in Table-1. When the concentration of isradipine and its respective peak area were subjected to regression analysis by least square method, a high correlation coefficient was observed ($r = 0.9999$) in the range of 1–100 μg/mL only. The regression of isradipine concentration over its peak area was found to be $Y = 68213 + 30220X$ where 'Y' is the peak area and 'X' is the concentration of isradipine. This regression was used to estimate the amount of isradipine either in capsule formulation or in validation study.

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

| Concentration of isradipine (μg/mL) | Peak area | CV (%) |
|---|---|---|
| 1 | 86498 | 1.18 |
| 2 | 127859 | 1.62 |
| 4 | 185581 | 2.12 |
| 6 | 243302 | 0.57 |
| 8 | 316034 | 1.82 |
| 10 | 368746 | 0.31 |
| 20 | 662355 | 0.99 |
| 40 | 1329573 | 1.82 |
| 60 | 1846790 | 0.92 |
| 80 | 2474008 | 0.64 |
| 100 | 3101226 | 0.76 |

Regression equation (from 1 to 100 μg/mL): $Y = 68213 + 30220\ X$ ($r = 0.9999$)

Proposed HPLC methods were also validated for intra- and inter-day variation. When the solutions containing 20 or 40 μg/mL of isradipine were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area of the drug for five replicate injections was found to be less than 2.5%. Also, the inter-day variation (3 days and five injections) was found to be less than 2% (Table-2). Thus the results have shown that the proposed HPLC method is highly reproducible. When a known amount of drug solution (20 or 40 μg) was added to a known concentration of drug solution (20 μg/mL), this was a high recovery ($99.75 \pm 1.10\%$) of isradipine (Table-3) indicating the high accuracy of the proposed method.

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 (%) |
| 20 | 19.32 | 0.62 | 19.81 | 1.34 |
| 40 | 41.03 | 1.23 | 40.83 | 1.98 |

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) |
|---|---|---|
| 20 | 19.95 ± 0.21 | 99.75 ± 1.10 |
| 40 | 39.65 ± 0.42 | 99.13 ± 1.20 |

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 the drug) were analyzed as per the procedure described above. The average drug content was found to be 96% 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 drug by the proposed HPLC method.

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

| Brand of the capsule | Labelled amount (mg) | Observed amount (mg) | Purity (%) |
|---|---|---|---|
| AAA | 5 | 4.83 ± 0.09 | 96.60 ± 1.12 |

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