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# HPLC METHOD FOR THE ESTIMATION OF ISRADIPINE IN PHARMACEUTICAL DOSAGE FORMS

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## ABSTRACT

A reverse phase high pressure liquid chromatographic method (HPLC) has been developed for the estimation of isradipine in its capsule dosage forms using RP–$C_{18}$ column. The mobile phase (methanol, water) was pumped at a flow rate of 1 mL/min in the ratio of 70 : 30 and the eluents 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. The mean recovery of the drug from the solution containing 20 or 40 µg/mL was 99.66 ± 1.20% 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, HPLC

## INTRODUCTION

Isradipine is calcium channel blocker[1,2] and chemically it is 3,5–pyridinedicarboxylic acid–4 (4–benzofurazanyl)–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 dosages 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 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 (Bondapak, 5 µm particle size) was used. The HPLC system was equipped with software Millennium 32.

## Preparation of stock solution of internal standard

Ethamsylate was used as internal standard for HPLC estimation of isradipine. About 100 mg of ethamsylate was accurately weighed, transferred to 100.0 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). 10.0 mL of stock – I solution (1000 µg/mL) were diluted to 100.0 mL with methanol to give a stock solution containing 100 µg/mL (Stock – II). 1.0 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 100.0 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. 10 mL of stock – I solution (1000 µg/mL) were diluted to 100 mL with methanol to give a stock solution containing 100 µg/mL (Stock – II).

## Chromatographic conditions

Methanol, acetonitrile and acetate buffer[6] were filtered before use through 0.2 µm membrane filter. The flow rate of the mobile phase was maintained at 1 mL/min in the ratio of 70 : 30 (methanol : water). The column temperature was maintained at 40°C and concentration of drug was detected by UV detector at 290 nm. The data was acquired, stored and analyzed with the software millennium 32.

## Procedure

From stock – I solution of isradipine 0.01 – 1 mL quantities of solution were transferred to 10 mL volumetric flasks. To this solutions, 1 mL of ethamsylate (internal standard) containing 1000 µg/mL was added and volume was made up to 10 mL with methanol to get 1, 2, 4, 6, 8, 10, 20, 40, 80, 100, 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 of 1 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 isradipine in pharmaceutical dosage forms.

### Assay of isradipine capsules

Twenty capsules (containing 5 mg) were weighed, finely powdered and an accurately weighed sample of capsules equivalent to 10 mg of isradipine was placed in a 100 mL volumetric flask. 70.0 mL of methanol was added and flask was allowed to stand for 5 hours 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 along with appropriate volume of ethamsylate (internal standard) solution and made up to volume with methanol to give an expected concentration 100 μg/mL of isradipine and 10 μg of ethamsylate (inernal 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 (20 or 40 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 = (s.d/mean) x 100].

### Accuracy

The accuracy of HPLC assay method was assessed by adding known amount (20 or 40 μg) of the drug to drug solution of known concentration (20 μg/mL) along with 10 μg internal standard 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 samples 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 formations. 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 per cent recovery.

## RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerably 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 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 min and 2.6 min, respectively (Fig. 1). Each sample was injected 5 times

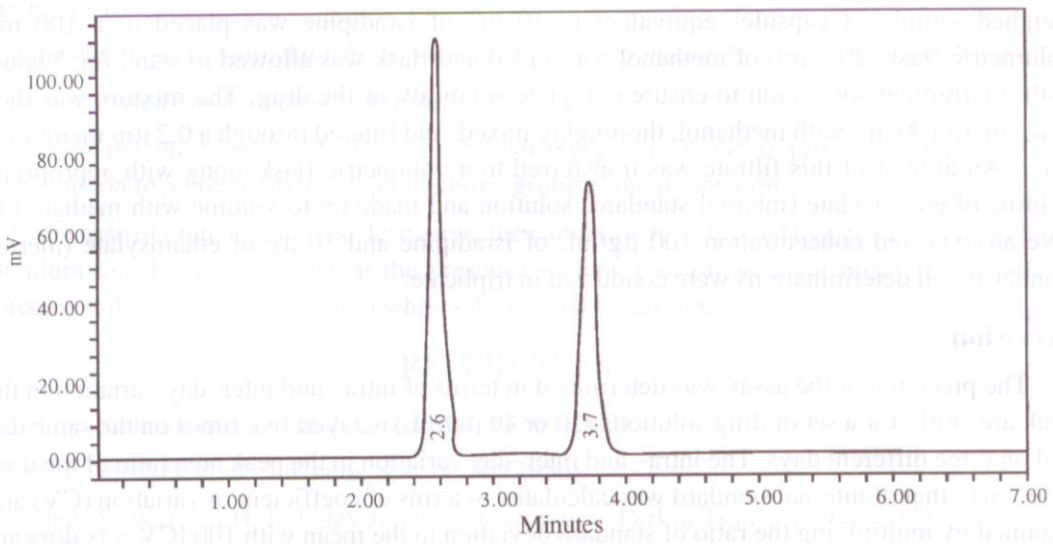


**Figure 1. A typical chrommatogram for isradipine**

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 set up as above were calculated, and the average values for five such determinations are shown in Table 1.

**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 (%) |
|---|---|---|
| 1 | 0.6410 | 1.16 |
| 2 | 0.796 | 1.52 |
| 4 | 1.108 | 2.32 |
| 6 | 1.420 | 0.78 |
| 8 | 1.731 | 1.28 |
| 10 | 2.043 | 0.98 |
| 20 | 3.601 | 2.31 |
| 40 | 6.716 | 1.67 |
| 80 | 12.948 | 0.67 |
| 100 | 16.064 | 1.52 |

Regression equation (from 1 to 100 μg/mL): $Y = 0.485 + 0.155\ X$ ($r = 0.9999$)

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 1 – 100 µg/mL. The regression of isradipine concentration over its peak area ratio was found to be Y = 0.485 + 0.155 X (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).

The proposed HPLC method also validated for intra – and inter – day variation. When the solutions containing 20 or 40 µg/mL of isradipine along with 10 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 2).

**Table 2. Precision of the proposed HPLC method**

| Isradipine concentration (µg/mL) | Concentration of Isradipine (µg/mL) found on | | | |
|---|---|---|---|---|
| | Intra – day | | Intra – day | |
| | Mean (n = 5 ) | CV (%) | Mean (n = 5) | CV (%) |
| 20 | 20.09 | 1.89 | 20.14 | 2.50 |
| 40 | 40.12 | 1.25 | 40.09 | 1.80 |

Thus results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (20 or 40 µg) was added to a known amount of drug solution (20 µg), there was a high recovery (99.66 ± 1.20%) of isradipine (Table 3) indicating that the proposed method is highly accurate.

**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 | 20.03 ± 0.06 | 100.15 ± 0.3 |
| 40 | 39.89 ± 0.08 | 99.72 ± 1.20 |

The HPLC method, developed in the present study has also been used to quantify isradipine in capsule dosage forms. Isradipine (containing 5 mg of drug) were analyzed as procedures described above. The average drug content was found to be 97.8% of the labeled amount (Table 4).

**Table 4. Mean (± s.d) amount of Isradipine in capsule dosage forms by proposed HPLC method**

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

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 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 (98.31 ± 1.20%) indicating that the proposed procedure for the determination of isradipine in the capsule dosage forms is highly accurate.

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