Asian Journal of Chemistry Vol. 18, No. 2 (2006), 813–817

Reversed Phase High Performance Liquid Chromatographic Assay of Isradipine

K.V.S. PRASADA RAO, B. NEELIMA, P. NAGARAJU, G. PRABHAKAR* and JAMEELUNNISA BEGUM†
Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530 003, India

A simple, rapid and precise high performance liquid chromatographic method was developed for the estimation of isradipine in bulk and in pharmaceutical formulations. Isradipine was chromatographed on a reversed phase C18 column using amlodipine as internal standard in a mobile phase consisting of acetonitrile, methanol and water in the ratio of 60 : 30 : 10 v/v. The mobile phase was pumped at a flow rate of 1.0 mL/min, and the eluent was monitored at 290 nm. The calibration curve was linear in the range of 0.5–50 μg/mL. The results were found to be accurate, reproducible and free from interference.

Key Words: HPLC, Estimation, Isradipine, Pharmaceutical formulations

INTRODUCTION

Isradipine (ISR) is a calcium antagonist, chemically, 3,5-pyridine dicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-1-methyl ethyl ester. ISR is a dihydropyridine calcium channel blocker. It binds to calcium channels with high affinity and specificity and inhibits calcium flux into cardiac and smooth muscle. The effects observed in mechanistic experiments *in vitro* and studied in intact animals and men are compatible with this mechanism of action.

The literature survey reveals that a few HPLC[1, 2], spectrophotometric methods[3], gas chromatoghaphy[4] and TLC[5] were reported for its analytical monitoring in either biological fluids or formulations. However, a critical literature survey indicated that no attempt has been made so far for assay of ISR in a single dosage form by HPLC using UV detection. The authors have made attempts in this direction and succeeded in developing a sensitive and precise HPLC method for the determination of ISR in bulk samples and pharmaceutical formulations by using Bondapack C-18 (250 × 4.6 mm, packed with 5 micron) column as stationary phase, solvent combination (acetonitrile : methanol : water, 60 : 30 : 10 v/v) as mobile phase along with amlodipine as internal standard.

* Prof. G. Prabhakar, H. No. 8-60-7/1, 1st Cross Road, Vidyanagar, Visakhapatnam-530 003, India; E-mail: vsprk@yahoo.co.in
† Department of Chemistry, St. Theresa's College for Women, Visakhapatnam-530 003, India.

EXPERIMENTAL

All chemicals used were of analytical or pharmaceutical grade. Water HPLC grade (Qualigens), methanol HPLC grade (E. Merck), acetonitrile HPLC grade (E. Merck) were used.

Quantitative HPLC was performed on a gradient high pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable diode array detector SPD-M10AS VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu). The HPLC system was equiped with the software "Class-VP series (Shimadzu)".

Preparation of standard drug solution: A stock solution (1 mg/mL)of pure drug was prepared by dissolving 100 mg of isradipine and 100 mg of amlodipine (internal standard) separately in 100 mL volumetric flasks containing 70 mL of methanol, sonicated for at least 15 min and then made up to volume with methanol. Daily working standard solutions of isradipine and internal standard were prepared by suitable dilution of the stock solution with appropriate mobile phase.

Chromatographic conditions: The separation was performed on a Bondapack C 18 column (5 μm, 25 cm × 4.6 mm I.d.). A mixture of acetonitrile, methanol and water was used as a mobile phase at a flow rate of 1 mL/min with an operating pressure of 131–133 kg/cm^2. A Rheodyne 7125 injection with a 20 μL loop was used for injection of the samples. Detection was performed at 290 nm. The mobile phase was filtered through a 0.45 μm Millipore membrane filter and degassed. The separation was carried out at room temperature.

Method development: To develop an HPLC method for the analysis of ISR in formulations, different solvent systems were used. The criteria employed for assessing the suitability of a particular solvent system for the drug were cost, time required for analysis, sensitivity of the assay, solvent noise, preparatory steps involved and use of the same solvent system for extraction of the drug from the formulation excipients matrix for estimation of the drug content.

Preparation of standard curve: Compositon and flow rate of the mobile phase were programmed from motor pump and the mobile phase acetonitrile : methanol : water (60 : 30 : 10) was passed through the 0.45 μm membrane filter using Millipore HPLC solvent filtration assembly and delivered at 1.0 mL/min for column stabilization. During this period, the baseline was continuously monitored. The wavelength of detection was selected at 290 nm. The prepared dilutions containing concentrations of isradipine in the range 0.5–50 μg/mL and fixed concentraion (4 μg/mL) of internal standard (amlodipine) were injected into the chromatograph. The peak area ratios to the internal standard were calculated. The stability of the solution of isradipine during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration. Chromatogram parameters, retention time and asymmetry factor were standardized.

Estimation of isradipine from the commercial formulations by the pro-

posed method: Commercially available capsules of isradipine were taken randomly from the Indian market for estimation of total drug content per capsule by the proposed method. 20 Capsules were weighed, contents were thoroughly mixed and aliquot amount (equivalent to 100 mg isradipine) was dissolved in 20 mL of methanol. The weighed amount 100 mg of active ingredient was extracted with methanol and made to get a stock solution of 1 mg/mL. This solution was filtered through a 0.45 μm membrane filter. This solution was further diluted stepwise with mobile phase as under preparation of standard solution different concentrations are required. The area under the curve and the drug content per capsule (on an average weight basis) was calculated. The results are presented in Table-2.

RESULTS AND DISCUSSION

For the determination of isradipine different mobile phases were employed. Initially a mobile phase consisting of acetonitrile : methanol : water in the ratio of 80 : 15 : 5 was tried. Symmetry RP-C18 columns 290 nm were used. Early elution with tailing of peaks was observed in the above condition.The composition of mobile phase was changed to 70 : 20 : 10. Under these conditions broad peaks shape and pronounced tailing was observed. For the same mobile phase, if the ratio was changed to 60 : 30 : 10, isradipine was eluted at around 5.62 min with symmetric peak shape.

A typical chromatogram for isradipine using C18 RP HPLC column with mobile phase, composed for acetonitrile : methanol : water (60 : 30 : 10) at 1.0 mL/min flow rate is shown in Fig. 1. The λ_{max} of detection was fixed at 290 nm so that there was less interference from mobile phase with highest sensitivity according to UV analysis.

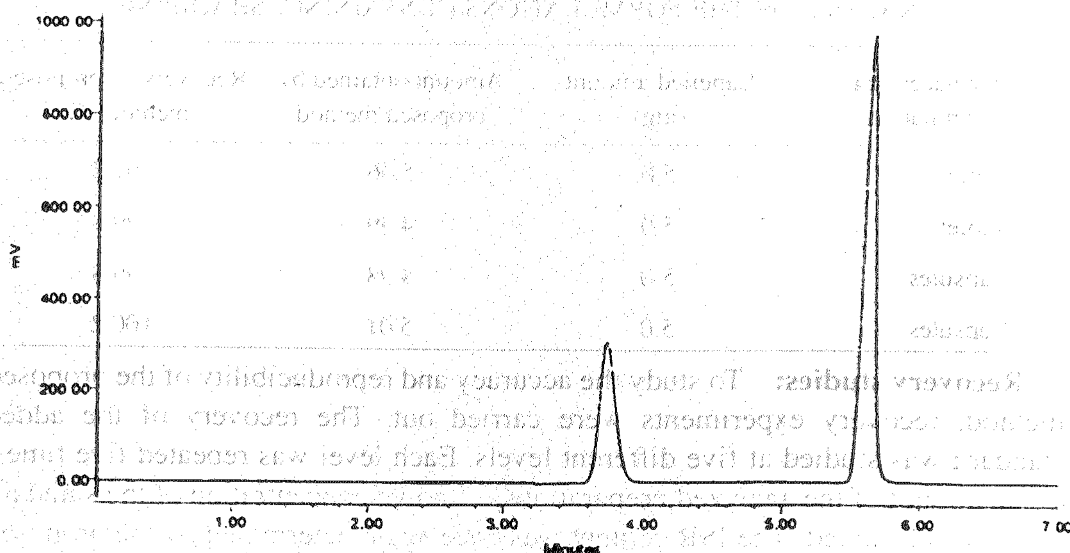

Fig. 1. Model chromatogram for isradipine (ISR)

The peak area ratio to the internal standard *vs.* concentration (μg/mL) was found to be linear. Values obtained for the calibration curve points and their standard deviation, coefficient of variance and standard error are presented in Table-1. The reproducibility of the detector response at each concentration level was examined by carrying out the experiment in triplicate.

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED HPLC METHOD FOR ISR

Parameter	Method
Detection wavelength (nm)	290
Linearity range (μg/mL)	0.5–50
Detection limits (μg/mL)	0.0683
Regression equation (Y = a + bC)	
Slope (b)	0.4514
Standard deviation of slope (S_b)	4.18×10^{-2}
Intercept (a)	–0.1722
Standard deviation of intercept (S_a)	1.028×10^{-1}
Standard error of estimation (S_e)	1.197×10^{-1}
Correlation coefficient	0.9977

The linear regression equation obtained for the proposed methods was $Y = -0.1722 + 0.4514X$ (r = 0.9977) where Y is the peak area ratio to the internal standard and X is the concentration of isradipine.

Precision of the method was studied with five replicates of the standard solution. The results are shown in Table-2. The low values of the relative standard deviation indicate the high precission of the method.

TABLE-2
ANALYSIS OF THE FORMULATIONS CONTAINING ISRADIPINE

Pharmaceutical formulation	Labelled amount (mg)	Amount obtained by proposed method	Recovery of proposed method (%)
Tablet	5.0	5.06	101.2
Tablet	5.0	4.99	99.8
Capsules	5.0	4.98	99.8
Capsules	5.0	5.01	100.2

Recovery studies: To study the accuracy and reproducibility of the proposed method, recovery experiments were carried out. The recovery of the added standard was studied at five different levels. Each level was repeated five times. To an aliquot of the analyzed preparations, a known concentration of the standard solution was added. The ISR content was once again determined by the proposed method. From the amount of the drug present, the per cent recovery was calculated. The results are shown in Table-3.

TABLE-3
RESULTS OF THE RECOVERY ANALYSIS IN DYNACIRC CAPSULE

Amount (mg/tablet)		RSD (%)
Labelled	Found	
2.5	2.51	0.030
2.5	2.49	0.710
5.0	5.02	0.680
5.0	4.98	0.042

Interference studies: The effects of wide range of excipients and other additives usually present in the formulation of ISR in the determination under optimum conditions were investigated. The common excipients like starch, talc, magnesium sterate, methyl and propyl parabens, cellulose derivatives and propylene glycol were added to the sample and injected. They did not disturb the elution or quantification of drug or internal standard. In fact, many have no absorption at this UV maximum.

The proposed method gives a good resolution betwen ISR and釋 internl standard. The total time of analysis is only 6 min. The method was simple, rapid and does not involve complicated sample preparation. High per cent recovery values show that the method is free from interference by the excipients used in the preparation. Hence, the present method can be used for routine quality control.

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(*Received*: 29 November 2004; *Accepted*: 7 November 2005) AJC-4494