Asian Journal of Chemistry Vol. 20, No. 1 (2008), 530-534

HPLC Method for Estimation of Lercanidipine in Pharmaceutical Dosage Froms

PASUMARTHY N.V. GOPAL*, A.V. HEMAKUMAR† and S.V.N. PADMA‡
Department of Pharmaceutical Chemistry, Sarada College of Pharmaceutical Sciences
Kondakavuru, Narasaraopet Mandal-522 602, India
E-mail: viswanadh16@rediffmail.com

A reverse phase HPLC method has been developed for the determination of lercanidipine for the estimation of the lercanidipine in its tablet dosage forms using RP-C_{18} column. The mobile phase (methanol, phosphate buffer pH 8) was pumped at a flow rate of 1 mL/min in the ratio of 90:10 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 30 µg/mL was 98.7 ± 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 lercanidipine in bulk drug samples or in pharmaceutical dosage forms.

Key Words: Estimation, Lercanidipine, HPLC.

INTRODUCTION

Lercanidipine (LRP) is an anti-hypertensive drug[1,2]. Chemically, LRP is 3,5-pyridine dicarboxylic acid,1,4-dihydro-2,6-dimethy1-4-(3-nitrophenyl)-2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester. LRP is calcium channel blocker, which reduces arterial pressure by inhibiting calcium ion influx into the vascular smooth muscle cells, which results in a decrease in smooth muscle tone and vascular resistance. Few analytical methods like LC-MS[3], voltammetry[4], supercritical fluid chromatography[5] have been reported for the estimation of lercanidipine in pharmaceutical dosage forms. Other reported methods such as spectrophotometry[6] and HPLC[7] are also reported for the estimation of lercanidipine. All the methods are not accurate and the process is considered tedious. The present study describes the determination of lercanidipine 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,

†S.R.M. College of Pharmacy, Kattankulathur-603 203, India.
‡Hindu College of Pharmacy, Guntur-522 002, India.

it is important that HPLC methods are to be developed for estimating lercanidipine. The aim of this study is to develop a simple, precise, rapid and accurate reversed phase HPLC method for the determination of lercanidipine in bulk drug samples or in pharmaceutical dosage forms.

EXPERIMENTAL

Lercanidipine and ethamsylate were gift samples from M/s Aristo Pharmaceutical Industries Ltd., Bhopal, India. Methanol and water used were of HPLC grade (Qualigens). All other regents 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} 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 an internal standard for HPLC estimation of lercanidipine. 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 diluted to 100 mL with methanol to give 10 μg/mL (stock-II). 1 mL of stock-II was added to standard lercanidipine solutions.

Preparation of stock solution of lercanidipine: About 100 mg of lercanidipine 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 lercanidipine.

Chromatographic conditions: Ethanol and phosphate buffer 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 90:10 (methanol: phosphate buffer pH 8). 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 lercanidipine 0.05-1 mL quantities of solution were transferred to 10 mL volumetric flasks. To this solutions 1 mL of ethamsylate (internal standard) containing 10 μg/mL was added and volume was made up to 10 mL with methanol to get 0.5, 1, 2, 4, 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 lercanidipine in pharmaceutical dosage forms.

Assay of lercanidipine tablets: 20 Tablets (containing 10 mg) were weighed, finely powdered and an accurately weighed sample of tablets equivalent to 100 mg of Lercanidipine 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 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 lercanidipine and 1 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 for a set of drug solutions (10 or 20 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 = (SD/mean) × 100].

Accuracy: The accuracy of HPLC assay method was assessed by adding known amount (20 or 30 μg) of the drug to drug solution of known concentration (20 μg/mL) along with 1 μg internal standard and subjecting the samples to the proposed HPLC method. The known amount of drug solution (20 or 30 μg/mL) was also added to the volumetric flask containing the powder sample of the tablet formulation with known amount of the drug and internal standard. The drug was estimated as per the procedure described above for the estimation of lercanidipine in tablet 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 previous 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 lercanidipine in bulk drug samples and its tablet formulations using most commonly employed RP-C_{18} column with UV detection.

The run time was set at 10 min and retention times for lercanidipine and internal standard (ethamsylate) were 5.5 min and 2.1 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 lercanidipine 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.

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

Concentration of lercanidipine (μg/mL)	Mean (± SD) peak area ratio (n =5)	CV (%)
0.5	0.454	1.80
1.0	0.892	1.21
2.0	1.818	1.32
4.0	3.513	1.82
8.0	7.271	1.89
10	9.121	1.78
20	17.923	2.94
40	35.352	1.76
80	72.705	0.68
100	90.882	1.32

Regression equation (from 0.5 to 100 μg/mL):
Y = -0.115 + 0.908 X (r = 0.9999)

The peak areas of both the drug and internal standard were reproducible as indicated by low coefficient of variation (3 %). When the concentration of lercanidipine and its respective peak area ratios were subjected to regression analysis by least squares method, a good linear relation ship (r = 0.9999) was observed between the concentration of lercanidipine and the respective peak areas in the range 0.5-100 μg/mL. The regression of lercanidipine concentration over its peak area ratio was found to be Y = -0.115 + 0.908 X (Where Y = ratio of peak area of drug to that of internal standard, X= concentration of lercanidipine). This regression equation was used to estimate the amount of lercanidipine either in tablet formulation or in validation study (precision and accuracy) than 2 % (Table-2). Thus results show that the proposed HPLC method is highly reproducible. When a known amount of drug solution (20 or 30 μg) was added to a known amount of drug solution (20 μg),there was a high recovery (98.7 ± 1.20 %) of lercanidipine (Table-3) indicating that the proposed method is highly accurate. The HPLC method, developed in the present study has also been used to quantify lercanidipine in tablet dosage forms (containing 10 mg of drug) as per procedures described above. The average drug content was found to be 97.6 % of the labeled amount (Table-4). No interfering peaks were found in the chromatogram indicating that excipients used in the tablet

TABLE-2
PRECISION OF THE PROPOSED HPLC METHOD

Lercanidipine concentration (μg/mL)	Concentration of lercanidipine (μg/mL) found on			
	Inter-day		Intra-day	
	Mean (n = 5)	CV (%)	Mean (n = 5)	CV (%)
10	10.09	1.89	10.14	2.50
20	20.12	1.25	20.09	1.80

TABLE-3
RECOVERY OF LERCANIDIPINE

Amount of drug added (μg)	Mean (± SD) amount (μg) recovered (n = 5)	Mean (± SD) % of recovery (n =5)
20	20.03 ± 0.06	100.01 ± 0.30
30	29.61 ± 0.08	98.7 ± 1.20

TABLE-4
MEAN (± SD) AMOUNT OF LERCANIDIPINE IN TABLET DOSAGE FORMS BY PROPOSED HPLC METHOD

Brand of the tablet	Labeled amount (mg)	Observed amount (mg)	Purity (%)
AAA	10	9.76 ± 1.04	97.6 ± 0.99
BBB	10	9.51 ± 0.89	95.1 ± 0.69

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 tablet dosage form and subjected to the estimation of the drug by proposed method. There was high recovery of lercanidipine (98.32 ± 1.20 %) indicating that the proposed procedure for the determination of lercanidipine in the tablet dosage forms is highly accurate.

REFERENCES

1. Remington: The Science and Practice of Pharmacy, edn. 19, p. 965 (1995).
2. The Merck Index, Merck & Co. Inc., New York, edn. 13, p. 937 (2001).
3. V.A.P. Jabor, E.B. Coetho, D.R. lfa, P.S. Bonato, N.A.G. dos Santos and V.L. Lanchote, *J. Chromatogr. B*, **796**, 429 (2003).
4. A. Alvarez-Lueje, S. Pujol, L.J. Nunez-Vergara and J.A. Squella, *J. AOAC Int.*, **85**, 1247 (2002).
5. M. Garzotti and M. Hamdan, *J. Chromatogr. B*, **770**, 53 (2002).
6. N. Erk, *Pharmazie*, **58**, 801 (2003).
7. A.A. Luege, S. Pujol, J.A. Squella and L.J. Nunez-Vergara, *J. Pharm. Biomed. Anal.*, **31**, 1 (2003).

(*Received*: 30 November 2006; *Accepted*: 14 September 2007) AJC-5855