Research Article

REVERSE PHASE HPLC METHOD FOR DETERMINATION OF LACIDIPINE IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple and reliable high-performance liquid chromatography (HPLC) method was developed and validated for Lacidipine in pharmaceutical preparations. The method was developed on Thermo Hypersil RP C-18 column (100 mm x 4.6 mm, 3.5 µm) using a mobile phase of Acetonitrile: water (65 : 35, v/v). The effluent was monitored by PDA detector at 239 nm. The total run time was 7 min with a flow rate of 1.0 ml/min. Calibration curve was linear over the concentration range of 10 – 50 µg/ml. For Intra–day and inter–day precision % RSD values were found to be 0.481% and 0.487% respectively. Recovery of Lacidipine was found to be in the range of 99.26-100.97%. The limits of detection (LOD) and quantification (LOQ) were 0.03 and 0.1 µg/ml, respectively. The developed RP-HPLC method was successfully applied for the quantitative determination of lacidipine in pharmaceutical dosage forms.

Keywords: Lacidipine, HPLC, Pharmaceutical preparation, Validation.

INTRODUCTION

Lacidipine[1] is a calcium channel blocker drug. Chemically Lacidipine is (E)-4-[2-[3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl]phenyl]-1,4-dihydro-2,6-dimethyl-3,5pyridine dicarboxylic acid diethyl ester (Figure 1). It has a molecular formula of $C_{26}H_{33}NO_6$ and a molecular weight of 455.55 g/mol.

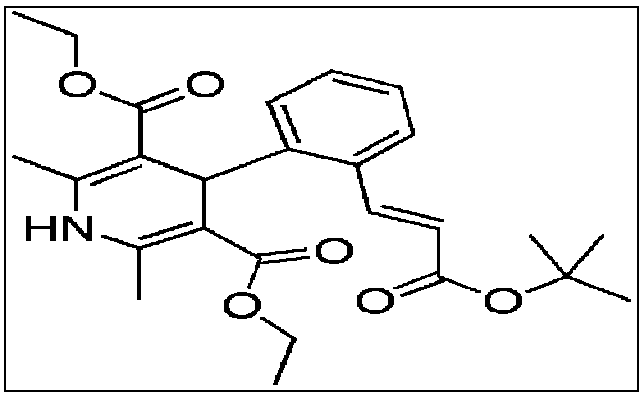

Figure 1: Structure of lacidipine

Literature survey reveals that several analytical methods have been reported for the estimation of Lacidipine by LC-DAD[2], High Performance Thin Layer Chromatography[3] LC-MS[4,5] and UV[6] method. Only one HPLC[7] method was developed and applied in the determination of Lacidipine in biological fluids. No validated HPLC methods for quantitative determination of Lacidipine in bulk drug samples and formulations were reported till date. The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition.

MATERIALS AND METHODS

Chemicals and Reagents

An analytically pure sample of Lacidipine was procured as gift sample from Cipla Health Care. (Ahmedabad, India). HPLC grade Acetonitrile and Water was procured from E. Merck (Ahmedabad). Tablet formulation SINOPIL were procured from a local pharmacy with labeled amount 2 mg per tablet.

Instrumentation

The HPLC system consisted of a Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a Waters 2487 PDA detector. Data acquisition was performed by the EM-power 2 software.

Chromatographic Condition

Chromatographic analysis was performed on a Thermo Hypersil reversed phase C-18 column with 100 cm x 4.6 mm i.d. and 3.5 µm particle size. The mobile phase consisted of Acetonitrile : Water (65 : 35 v/v) and was set at a flow rate of 1.0 ml/min. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system. The effluent was monitored by PDA detector at 239 nm.

Preparation of Solutions

Preparation of Standard Solutions

The stock standard solution of Lacidipine was prepared with methanol to a concentration of 50 µg/ml. Five standard solutions ranging from 10 to 50 µg/ml (10, 20, 30, 40 50 mcg/ml) were prepared in methanol by a serial dilution. Three quality control (QC) samples at the

concentrations of 50%, 100% and 150% were prepared from the stock standard solution.

Procedure for pharmaceutical preparations

The average tablet mass calculated from mass of tablets of Sinopil (2mg Lacidipine tablet, which was composed of Lacidipine and some excipients). They were then finely ground, homogenized and portion of the powder was weighed accurately, transferred into a 10 ml volumetric flask and diluted up to mark with methanol. The mixture was sonicated for at least 30 min to aid dissolution and then filtered through a whatman no 41 paper. An appropriate volume of filtrate was diluted further with methanol so that the concentration of Lacidipine in the final solution was within the working range. The sample solution was then analyzed by HPLC (figure 2).

RESULTS AND DISCUSSION

Method development and optimization

The development of the RP-HPLC method for determination of drugs has received considerable attention in recent years because of its importance in routine quality control analysis. A RP–HPLC method was proposed as a suitable method for the estimation of Lacidipine in pharmaceutical dosage form. A good separation was achieved using an Thermo Hypersil RP C-18 column (100 mm x 4.6 mm, 3.5 µm). The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of Acetonitrile : Water (65:35 v/v) accomplished at 239 nm. The retention time was 5.13 min at a flow-rate of 1.0 ml/min and the injection volume was 20 µl. The total run time for an assay was approximately 7 min.

Validation of the method

System suitability

A system suitability test of the chromatographic system was performed. Five replicate injections for a system suitability test were injected into the chromatographic system. Relative standard deviation and column efficiency for the five suitability injections were determined. For all sample analyses, the efficiency and %RSD were found ≥ 2000 and ≤ 1.84% respectively.

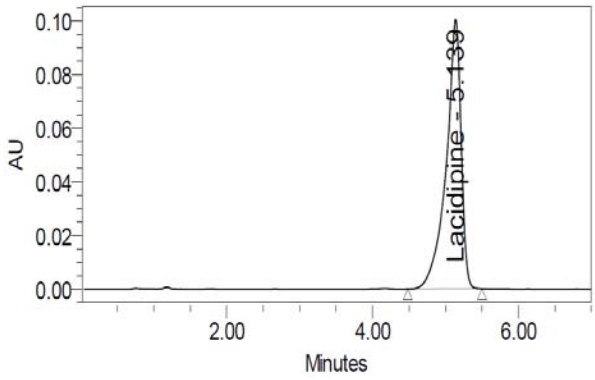

Figure 2: Chromatogram of Lacidipine at 239 nm

Linearity

Calibration curve was constructed for Lacidipine standard by plotting the concentration of compound versus peak area response. Standard solutions containing 10, 20, 30, 40 and 50 µg/ml of Lacidipine were injected into the HPLC column (Figure 3). The linearity was calculated by the least square regression method. The regression equations were calculated from the calibration graphs (Table: 1).

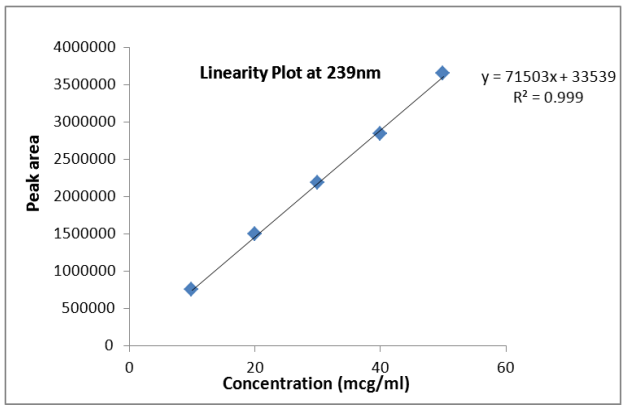

Figure 3: Calibration curve of Lacidipine at 239 nm

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 50, 100, and 150% of the standard concentration of Lacidipine (20 µg/ml). The results obtained (Table: 2) indicate that recovery was excellent, not less than 100% ± 2.

Table 2: Accuracy results for Lacidipine

Sample	Percentage recovery	Mean
50%	100.97	100.06
50%	99.96	
50%	99.26	
100%	100.58	100.22
100%	100.23	
100%	99.86	
150%	100.33	100.03
150%	99.90	
150%	99.88	

Sensitivity

Limit of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ values were found to be 0.10 and 0.25 µg/ml, respectively.

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated presented in Table 3.

Table 1: Linearity results for Lacidipine

Conc (µg / ml)	10	20	30	40	50
Peak Area	744111	1489810	2176527	2837012	3645648
Correlation Coefficient	0.999				

Table 3: Precision results for Lacidipine

Sr. No.	Concentration (µg / mL)	Intraday precision (Area)	Interday precision (Area)
1	100	1648340	1643826
2	100	1646723	1642686
3	100	1653535	1650328
4	100	1655408	1659038
5	100	1635163	1638412
6	100	1634726	1645696
Mean		1647834	1646858
Std.Dev		7936.0	8036.2
%RSD.		0.4816	0.4879

Table 4: Ruggedness studies of Lacidipine by RP-HPLC method

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD* (%)	Amount found (mg)	Recovery ± SD* (%)
SINOPIL	2	1.98	98.50 ± 0.77	2.03	101.50 ± 0.49

*Average of six determinations.

Table 5: Robustness studies of Lacidipine by RP-HPLC method

Condition	Modification	Mean area ± SD*	RSD (%)	Mean t_R ± SD* (min)
Mobile phase composition Acetonitrile : Water (v / v)	55 : 45	1482407 ± 1165.436	0.021	7.440 ± 0.015
	65 : 35	1427141 ± 1575.686	0.019	5.139 ± 0.031
	75 : 25	1511420 ± 2900.825	0.018	3.837 ± 0.014
Mobile phase flow rate (ml / min)	0.8	1865079 ± 1033.194	0.0043	6.368 ± 0.095
	1.0	1446157 ± 1114.536	0.0046	5.139 ± 0.044
	1.2	1246644 ± 4498.892	0.0056	4.318 ± 0.016

*Average of three determinations.

Reproducibility (Ruggedness)

In addition to intra and inter day precision reproducibility study was also carried out and it was checked by determining precision on the same instrument, but by a different analyst. Results of reproducibility are shown in Table: 4.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in composition of mobile phase and flow rate. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The results are shown in Table: 5.

CONCLUSION

A rapid and simple RP-HPLC method for determination of Lacidipine has been developed and validated. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method, including linearity, accuracy, sensitivity, precision, ruggedness and robustness. The chromatographic run time of 7 min allows the analysis of a large number of samples in a short period of time. Therefore, the method is suitable for analysis of large samples during routine analysis of formulations and raw materials.

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Mr. P.T. Nagaraju graduated at JNTUniversity, Hyderabad, INDIA and post graduated from RGUHS, Karnataka, INDIA. At post graduation level taken specialization in Pharmaceutical Analysis, completed master thesis in “DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHODS FOR THE ESTIMATION OF LACIDIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM”. Gained recognition from Pharmacy Council of India.