

## Research Article

## DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR ESTIMATION OF LACIDIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Shantha Kumar P.T.\*, Nagaraju P.T., U. Siva Kumar.

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagara, karnataka – 571422, India.

\*Corresponding author's E-mail: [nraju04@gmail.com](mailto:nraju04@gmail.com)

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## ABSTRACT

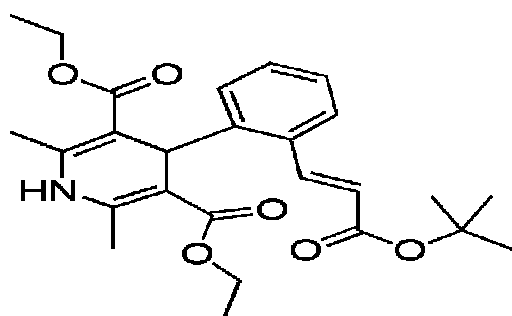
A simple, specific, accurate and precise high performance thin layer chromatography (HPTLC) method has been developed for determination of Lacidipine in bulk and pharmaceutical dosage forms. The method uses aluminium plates coated with silica gel 60 F<sub>254</sub> as stationary phase and Toulene : Ethylacetate (7.5 : 2.5 v / v) as mobile phase. Densitometric evaluation of the separated bands was performed at 239 nm using Camag TLC Scanner-3 with win CAT 1.4.4 software. The  $R_f$  value of Lacidipine was  $0.42 \pm 0.02$ . The validated calibration range was 1000-3500 ng per spot ( $r^2 = 0.9991$ ). The limit of detection was 0.011 ng / spot and Limit of quantitation was 0.034 ng / spot. Results of analysis were validated statistically and by recovery studies. The method was validated according to the ICH guidelines with respect to linearity, accuracy, precision and robustness. Thus the proposed method can be used successfully for routine analysis of Lacidipine from tablet formulations.

**Keywords:** Lacidipine, HPTLC, Validation, Pharmaceutical formulation.

## INTRODUCTION

Lacidipine<sup>1</sup> is a calcium channel blocker drug. Lacidipine is a highly vascular selective newer dihydropyridines suitable for once daily administration. It is claimed to attain higher concentration in vascular smooth muscle membrane; approved only for use as antihypertensive. Chemical name of Lacidipine is (E)-4-[2-[3-(1,1-Dimethylethoxy)-3-oxo-1-propenyl]phenyl]-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylic acid diethyl ester. It is having a molecular formula of C<sub>26</sub>H<sub>33</sub>NO<sub>6</sub> and its molecular weight of 455.55 g/mol. Its molecular structure;

Figure 1: Structure of lacidipine



Literature survey reveals that several analytical methods have been reported for the estimation of Lacidipine by LC-DAD<sup>[2]</sup>, HPLC<sup>[3]</sup> LC-MS<sup>[4,5]</sup> and UV<sup>[6]</sup> methods. Only one HPTLC<sup>[7]</sup> method was developed and applied in the determination of Lacidipine in biological fluids. No validated HPTLC 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 HPTLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition.

## MATERIALS AND METHODS

## Reagent and Pharmaceutical preparations:

An analytically pure sample of Lacidipine was procured as gift sample from Cipla Health Care, Ahmadabad (India). All chemicals including Acetone and glacial Acetic Acid were of A.R.grade from S.D.Fine-chem, Merck, Fischer scientific, and Spectrochem, Mumbai. Tablets of 2 mg were procured from local pharmacy.

## Preparation of Solutions

## Preparation of standard stock solution:

The standard Lacidipine 100 mg was weighed accurately and transferred to volumetric flask 100 ml. It was dissolved for sonication for few minutes and diluted up to the mark with methanol to obtain final concentration of 1000 µg/ml and the resulting solution was used as working standard solution.

## Preparation of sample solution:

For the estimation of Lacidipine in tablets formulations by this method. 20 branded tablets were weighed and triturated to fine powder. Tablet powder equivalent to 10 mg of Lacidipine was weighed and transferred to 100 ml volumetric flask than dissolved with methanol and further diluted with methanol. It was kept for ultrasonication for few min; the solution was then filtered through Whatman filter paper No. 41 and further dilution was made with methanol to get the final stock solution of 100 mcg/ml.

## Chromatography:

Analysis was performed on 10 cm × 10 cm HPTLC silica gel 60 F<sub>254</sub> plates (Merck, Darmstadt, Germany) with concentrating zone. The TLC plates were pre-washed with methanol. Activation was done in an oven at 50°C for 5



min. Sample and standard zones were applied to the layer as bands by means of a Camag (Muttentz Switzerland) Linomat V automated spray-on applicator equipped with a 100- $\mu$ L syringe (Hamilton, Reno, Nevada, USA). The slit dimensions 6 mm  $\times$  0.45 mm and scanning speed of 20 mm/sec was employed. Ascending development of the plate, migration distance 60 mm, was performed at  $25 \pm 2^\circ$  C with Toulene : Ethylacetate (7.5: 2.5 v/v), as mobile phase in a Camag twin trough chamber previously saturated for 30 min. The average development time was 20 min. After development the plate was dried at  $50^\circ$ C in an oven for 5 min. Densitometric scanning at  $\lambda = 239$  nm was then performed with a Camag TLC Scanner-3 equipped with Win CAT 1.4.4 software, using a deuterium light source.

## RESULTS AND DISCUSSION

Literature survey revealed that few HPLC and UV methods have been reported for estimation of Lacidipine which are sophisticated, but costly and time consuming. As one HPTLC method has been reported so far for estimation of Lacidipine, the present study was aimed at development of speedy and cost effective HPTLC technique for determination of Lacidipine as bulk and in dosage forms.

Various blends of solvent systems in varying proportions were tried as mobile phase. However, mobile phase consisting of Toulene: Ethylacetate in the ratio of 7.5:2.5 (v/v) was found to be more suitable with  $R_f$  values of 0.42, with saturation time of 10 minute. The selection of wave length was based on maximum absorbance for optimum sensitivity. The drug showed good linearity in the range of 1000-3500 ng per spot with coefficient of correlation value 0.9991 for peak area. From the recovery studies, the accuracy results were within the range of 99.45 -101.18 % and were found to be highly accurate. The limit of detection was 0.011 ng / spot and Limit of quantitation was 0.034 ng / spot. Robustness of the method checked after deliberate alterations of the analytical parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2).

### Validation of the method:

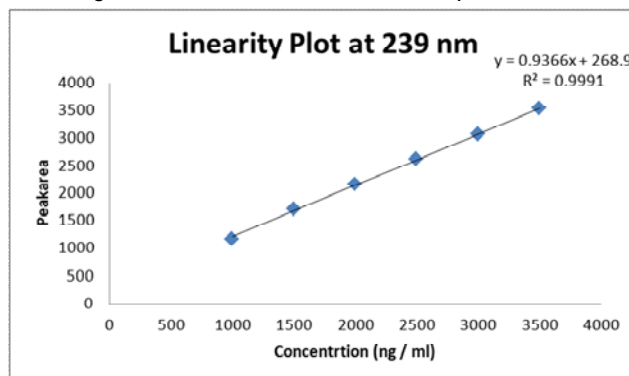
#### Linearity:

Appropriate volume of aliquots from standard Lacidipine stock solutions were prepared and applied on the TLC plate in the range of 1.2-2.4  $\mu$ L to give a series of spots covering the range from 1000 to 3500 ng/spot with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 239 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area and shown in (Fig.2). Calibration parameters are given in table: 1.

**Table 1:** Calibration parameters for Lacidipine

Parameters	Results
Linearity Range	1000-3500 ng/spot
Correlation co-efficient	0.9991
Slope	0.9366
Intercept	268.9

**Figure 2:** Calibration curve of Lacidipine at 239 nm



#### Precision:

The precision expressed as standard deviation or relative standard deviation.

**System precision:** Standard solution of Lacidipine was prepared as per testing procedure and injected into the HPTLC system in six replicates. The values of % relative standard deviation (RSD) for peak area obtained in six replicate injections were reported in Table: 2.

**Table 2:** System Precision results for Lacidipine (n=6)

SI. NO.	$R_f$	Peak area
1	0.42	1799.4
<b>Mean</b>	0.426	1851.5
<b>SD</b>	0.0063	24.81
<b>% RSD</b>	1.47	1.34

**Method precision:** The six different determinations of Lacidipine sample (from tablets) were performed as per testing procedure. % RSD was calculated and which are shown in Table: 3.

**Table 3:** Method Precision results for Lacidipine (n=6)

SI. NO.	Peak area	% Assay
1	1234	100.78
<b>Mean</b>	1220.66	99.81
<b>SD</b>	12.14	0.521
<b>% RSD</b>	0.994	0.529

#### Accuracy:

To check the accuracy of the method, recovery studies were conducted after addition of standard drug solution at three different levels i.e. 50 %, 100 %, and 150 % to

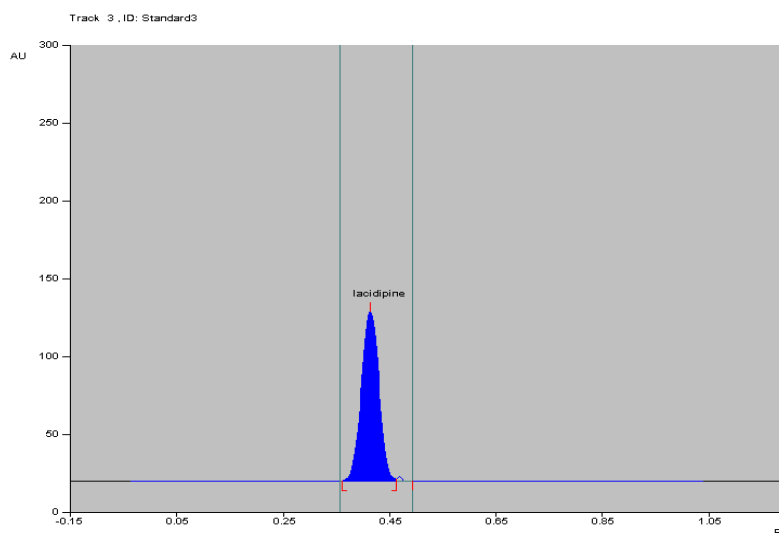
pre-analyzed sample solution. The results are given in table: 4.

#### Specificity:

To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate,

developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peak of standard Lacidipine as shown in (Fig.3). The  $R_f$  value of drug was 0.42.

**Figure 3:** Typical HPTLC chromatogram of standard solution of Lacidipine



**Table 4:** Recovery studies of Lacidipine (n=3)

Sample	Initial Amount (ng/spot)	Amount added	Amount recovered (ng/spot)	Recovery $\pm$ SD (%)	% RSD
Sinopil	1500	1200	1193.45	99.45 $\pm$ 0.34	0.352
	1500	1500	1517.78	101.18 $\pm$ 0.54	0.539
	1500	1800	1794.32	99.68 $\pm$ 0.85	0.871

**Table 5:** Ruggedness studies of Lacidipine (n=6)

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery $\pm$ SD (%)	Amount found (mg)	Recovery $\pm$ SD (%)
Sinopil	2	1.996	99.80 $\pm$ 0.11	1.998	99.92 $\pm$ 0.14

#### Ruggedness:

Ruggedness was carried out under the different condition like different days and different analyst. The ruggedness results were shown in Table: 5.

#### CONCLUSION

The validated HPTLC method proved to be simple, less expensive, fast, accurate, precise and ruggedness and thus can be used for routine analysis of Lacidipine in bulk and pharmaceutical dosage forms.

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**About Corresponding Author: Mr. P.T. Shantha Kumar**

Mr. P.T. Shantha Kumar graduated and post graduated at JNT University, Hyderabad, INDIA. At post graduation level taken specialization in Pharmaceutics, completed project thesis in "Process Validation of Terbinafine Hydrochloride Tablets 250mg". Currently working as Asst. Professor under JNTU Hyderabad.