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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for the Estimation of Lacidipine in Bulk and Dosage Form by RP-HPLC

Dandu Girija[1*], Akkimi Padma[1], A.Thirupathireddy[2], B. Venkateswara reddy[3]

[1,]Assistant professor, Department of Pharmaceutical Analysis, Sankar Reddy Institute of Pharmaceutical sciences, Salakalveedu(v), Bestavaripeta(M), Prakasam(Dist) Andha pradesh pin -523370.
[2]Associate Professor, Department of Pharmaceutics, Sankar Reddy Institute of Pharmaceutical sciences, Salakalveedu(v), Bestavaripeta(M), Prakasam(Dist) Andha pradesh pin – 523370.
[3] Professor, Department of Pharmaceutics, Sankar Reddy Institute of Pharmaceutical sciences, Salakalveedu(v), Bestavaripeta(M), Prakasam(Dist) Andha pradesh pin – 523370.

A B S T R A C T

The aim of present research work method development and validation for the quantitative estimation of Lacidipine in pure and Pharmaceutical dosage forms by RP-HPLC. Chromatographic separation was carried out on an Intersil C-18 column using a mobile phase consisting of Phosphate Buffer pH-3:Methanol (60:40 v/v). The mobile phase was pumped at a rate of 1.0 ml/min and the UV detection wavelength was measured at 284 nm. The linearity was found to be in the range of 50-120 µg/ml and retention time was 4.7 min. Mean percentage recovery of Lacidipine was found to be 99.89%. The developed method was validated stastically according to ICH guidelines(Q2R1). So the developed method was linear, precise, accurate and robust, the proposed method wassuccessfully applied for the quantitative determination of Lacidipine in pharmaceutical dosage forms.

Key words: Lacidipine, RP-HPLC, Method validation, Intersil C-18 column, Retention time, Mobilephase

A R T I C L E I N F O

Corresponding Author
Dandu Girija
Assistant professor,
Sankar Reddy Institute of Pharmaceutical sciences,
Bestavaripeta(M), Prakasam(Dist) Andhra Pradesh.
MS-ID: AJCPR3780

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C O N T E N T S

1. Introduction

Lacidipine is a calcium channel blocker drug. Lacidipine is a highly vascular selective newer dihydro pyridines suitable for once daily administration. It is claimed to attain higher concentration in vascular smooth muscle membrane; approved only for use as anti-hypertensive. Calcium channel blockers can be safely given to patients with obstructive lung disease and peripheral vascular disease in whom β-blockers are contraindicated. The problem of rebound worsening of angina on withdrawal after chronic use is less with calcium channel blockers than with β-blockers. Lacidipine is used effectively in Angina pectoris, Hypertension, Cardiac arrhythmias and Cardiomyopathy.

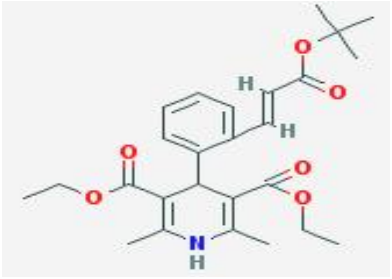

Fig 1: Structure of Lacidipine

Literature survey reveals that few methods have been reported for the estimation of lacidipine in bulk and dosage form by using HPLC method. Then need to develop new RP-HPLC method for the estimation of estimation of lacidipine in bulk and its formulations.

2. Materials and Methods

Materials :

API of Lacidipine was procured from gift sample of aurobindo pharma, Hyderabad. HPLC grade water, methanol was purchased from merck laboratories, Mumbai. Potassium hydrogen orthophosphate and ortho phosphoric acid were obtained from sd fine.chemicals, Mumbai, india.

Instrument used:

The liquid chromatographic system consists of Agilent 1100 chemostation HPLC with UV-VIS detector, binary pump and septum injector valve with 20 µl fixed loop. The analytes were monitored at 284 nm. Chromatographic analysis was performed on Inertsil C18 ODS column having 250 mm× 4.6 mm i.d. and 5µm particle size.

Chromatographic conditions:

Flow rate**:**	1.0 ml/min
Column **:**	Intersil C-18 (250 x 4.6 mm, 5 µm)
Detector wavelength**:**	284 nm
Column temperature**:**	Ambient
Injection volume**:**	20 µl
Run time**:**	8 min

Preparation of mobile phase:

7.0 grams of potassium dihydrogen phosphate was weighed and dissolved in 1000 ml volumetric flask with HPLC water. pH of this solution was adjusted to 3.0 with orthophosphoric acid. 600 ml (60 %) of above buffer solution and 400 ml of methanol (40 %) wasmixed. Thissolution was filtered through 0.45 µ membrane filter under vacuum and degassed in ultrasonic water bath for 5 minutes.

Preparation of working stock solution of Lacidipine:

About 10 mg of Lacidipine was weighed and transferred into 10 ml volumetric flask and diluted up to the mark with water to get the concentration of 1000 µg/ml. From this, 1 ml was pipetted out into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 100 µg/ml. above the stock solution further respected dilutions were prepared and analysed.

Analysis of formulation:

Twenty tablets were weighed and finely powdered. Tablet powder equivalent to 10mg of Lacidipine was weighed accurately and transferred into 10 ml volumetric flask, diluted up to the mark with water to get the concentration of 1000 µg/ml. From this solution, 4 ml was pipetted out and transferred into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 40µg/ml. The final solution was injected into chromatographic system for three times.

Validation of Analytical Method

Method validation was done from the according to ICH guidelines Q2 R1. Method validation parameters like specificity, linearity, accuracy, precision, robustness and system suitabiltiy.

Accuracy:

Accuracy was performed in triplicate for various concentrations of lacidipine equivalent to 50, 100 and 150% of standard amount was injected into HPLC system as per the test procedure.

Preparation of 50% solution (With respect to target assay concentration):

10 mg of Lacidipine was weighed and transferred into 10 ml volumetric flask and diluted up to the mark with water to get the concentration of 1000 µg/ml. From this, 1 ml was pipetted out into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 100 µg/ml. (stock solution)Further 0.5 ml of lacidipine solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 50 µg/ml.

Preparation of 100% solution (With respect to target assay concentration):

1 ml of lacidipine solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 100 µg/ml.

For preparation of 150% solution (With respect to target assay concentration):

1.5 ml of lacidipine solution was pipetted out from the above stock solution into a 10 ml volumetric flask and diluted up to the mark with the mobile phase to get a concentration of 150 µg/ml.

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard solution was made and the response factor of drug peak and % RSD were calculated. In the inter-day variation studies, six repeated injections of

standard solution were made for six consecutive days and response factor of drugs peak and % RSD were calculated. From the data obtained, the developed method was found to be precise.

Linearity:

Different concentrations (50-120 µg/ml.) of the pure drug were injected into the chromatographic system. Calibration curve of Lacidipine was constructed by plotting peak area vs. applied concentration of Lacidipine. The obtained results have shown an excellent correlation between peak area and concentration of pure drug within the concentration range. The correlation coefficient for the average area versus concentration of analyte was calculated.

Limit of Detection and Limit of Quantification:

The sensitivity of the proposed method for measurement of Lacidipine was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ).The LOD and LOQ were calculated by using the slope and SD of response (intercept).The mean slope value and SD of response were obtained after plotting six calibration curves.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of robustness, a number of method parameters such as pH, flow rate, column temperature, injection volume, detection wavelength, or mobile phase composition, are varied within a realistic range, and the quantitative influence of the variables is determined.The sample was analyzed separately by slightly changes in the analytical method asGiven below: Changing the flow rate of mobile phase to 1.0 ± 0.2 ml / min(0.8,1.2).The retention time values are measured. By changing ratio of the mobile phase i.e. Buffer (pH -3): Methanol.

3. Results and discussion

Specificity: Specificity of the method was ascertained by comparing the chromatogram obtained from formulation and standard drug.No interference from blank or placebo is observed at the retention time of the drug. The retention time of the standard drug and the drug from formulation was same, so the method was specific. Specificity data was shown in table no 1. Chromatogram was shown in fig 2.

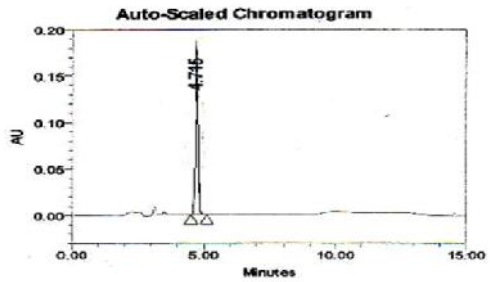

Fig 2: Chromatogram of Lacidipine at 284 nm

Precision: (System precision/ System suitability):

The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

Method Precision:

Sample solution of same concentration is prepared for six different times and injected once in to the system. Results were shown in table 3.

Acceptance criteria: The %RSD for the area and Rt of the six standard injections results should not be more than 2%.The %RSD of Rt and area values of lacidipine for system precision, method precision intra-day precision and inter-day precision was found to be 0.5&1.4 in the acceptance limit of less than 2%.

Linearity:

The linearity of the method was determined at 50-100%. The results are shown in table 4.

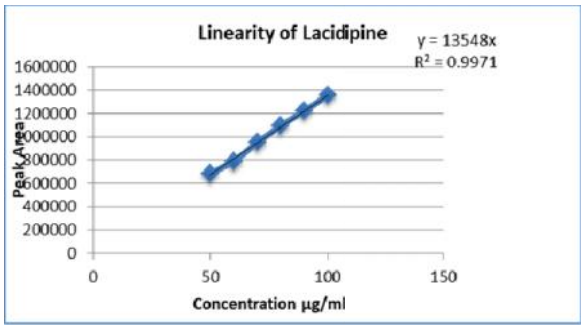

Fig 3: Calibration curve of Lacidipine at 284 nm

Acceptance criteria: The relationship between the concentrations (%) of Lacidipine should be linear in the specified range and correlation should not be less than 0.999. The correlation coefficient for linear curve obtained between concentrations Vs area for standard preparation of Lacidipine is 0.997 respectively.

Accuracy:

The accuracy of the method was inferred by establishing the precision and linearity studies of standard drug.

Acceptance criteria: The % recovery for each level should be between 98.0 to 102.0%. The % recovery of lacidipine is with in acceptance criteria 99.68%-99.76% respectively.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

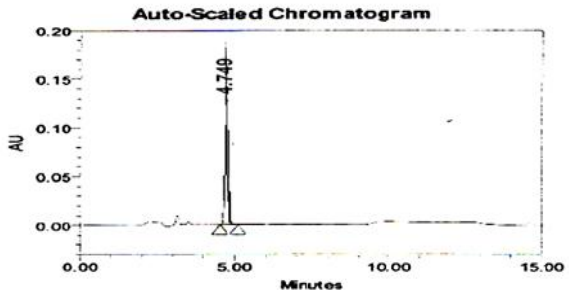

Fig 4: Chromatogram at 0.8 ml / min

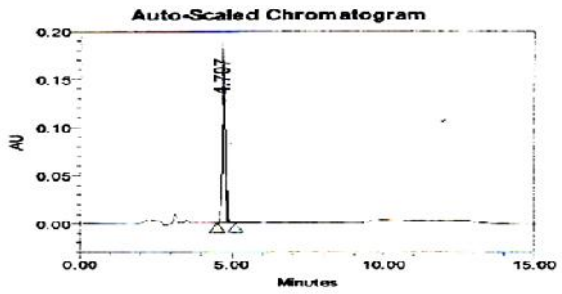

Fig 5: Chromatogram at 1.2 ml / min

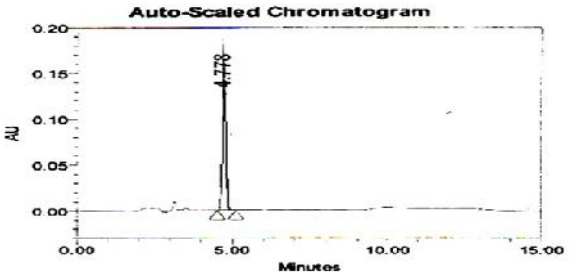

Fig 6: Chromatogram at 50:50% Mobile Phase

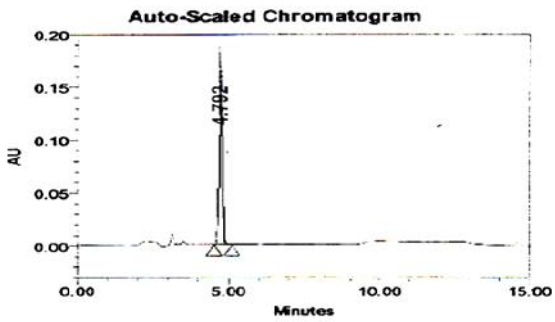

Fig 7: Chromatogram at 70:30% Mobile Phase

4. Conclusion

The present research work concluded that the developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately robust and cost effective can be used for determination of lacidipine in pharmaceutical formulation. The method was validated as per ICH guidelines.

Table 1: Specificity results for Lacidipine

S.No	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	R_t:4.715 min	λ_{max} : 284 nm

Table 2: System precision results of Lacidipine by RP-HPLC method

Property	Values	Required limits
Retention time (Rt)	4.71 ± 0.10	RSD ≤ 1%
Theoretical plates (N)	2884.74	$N > 2000$
Tailing factor (T)	1.30	$T \leq 2$

Table 3: Precision results for Lacidipine by RP-HPLC

Sr. No.	Concentration (µg/ml)	Intraday precision (Area)	Interday precision (Area)
1	100	1376511	1377057
2	100	1381573	1395823
3	100	1379278	1335498
4	100	1365340	1362906
5	100	1370017	1371072
6	100	1366426	1373002
Mean		1373191	1369226
Std.Dev		6867.454	19818.73
%RSD.		0.500109	1.44744

Table 4: Linearity results for Lacidipine

Conc. (µg / ml)	Peak Area
50	685562
60	787267
70	950209
80	1098229
90	1219339
100	1353326
Correlation coefficient	0.997

Table 5: Accuracy results for Lacidipine

Sample No.	Spike Level (%)	Amountadded (µg / ml)	Amountfound (µg/ ml)	% Recovery	Mean % Recovery
1	50	80	80.05243	99.87	99.76
	50	80	80.04786	99.63	
	50	80	80.0565	99.79	
2	100	100	99.89514	99.90	99.90
	100	100	99.90428	99.90	
	100	100	99.887	99.90	
3	150	120	120.0524	99.68	99.68
	150	120	120.0479	99.72	
	150	120	120.0565	99.65	

Table 6: Results for Robustness (Change in flow rate)

SI. No	Change in flow rate	R.T	Peak area	USP plate count	USP Tailing
1	0.8 ml / min	4.749	1311648	2879.69	1.20
2	1.2 ml / min	4.707	1311122	2799.97	1.20

Table 7: Results for Robustness (Change in Mobile phase composition)

SI. No	Change in mobile phase	R.T	Peak area	USP plate count	USP Tailing
1	50:50	4.778	1353086	2897.84	1.20
2	70: 30	4.792	1347456	2864.73	1.20

Table 8: Summary and Validation Parameters for RP-HPLC

S. No	Parameter	Result	Acceptance Criteria
1	Retention time	4.74 min	k'> 2
2	Tailing factor	Less than 2	A_s< 2
3	Theoretical plate	More than 2000	N> 2000
4	Linearity range µg/ml	50-100 µg/ml	-
5	Slope	13548	-
6	Intercept	65.83	-
7	Correlation coefficient	0.997	>0.999
8	Intraday precision	0.5	NMT 2%
9	Interday precision	1.4	NMT 2%
10	%Recovery	99.68-99.76%	98%-102%
11	Limit of detection	1.87	-
12	Limit of quantification	5.68	-

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