The Malaysian Journal of ANALYTICAL SCIENCES ISSN 1394-2506

DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF LACIDIPINE IN PURE FORM AND IN PHARMACEUTICAL DOSAGE FORM

(Perkembangan dan Validasi Satu Kaedah KCPT Bagi Penentuan Lacidipine Tulen dan Dalam Dos Farmaseutikal)

Mannur Vinodh*, Mastiholimath Vinayak, Kharya Rahul, Patware Pankaj

Department of Quality Assurance,
KLE University's College of Pharmacy, Belgaum- 590 010, Karnataka, India

**Corresponding author: vinodhmannur@yahoo.com*

Abstract

A simple and reliable high-performance liquid chromatography (HPLC) method was developed and validated for Lacidipine in pure form and pharmaceutical dosage form. The method was developed on Xbridge C-18 column (150 mm x 4.6 mm, 5 µm) with a mobile phase gradient system of ammonium acetate and acetonitrile. The effluent was monitored by PDA detector at 240 nm. Calibration curve was linear over the concentration range of 50 – 250 µg/ml. For Intra–day and inter–day precision % RSD values were found to be 0.83% and 0.41% respectively. Recovery of Lacidipine was found to be in the range of 99.78-101.76%. The limits of detection (LOD) and quantification (LOQ) were 1.0 and 7.3 µg/ml respectively. The developed RP-HPLC method was successfully applied for the quantitative determination of lacidipine in pharmaceutical dosage.

Keywords: Lacidipine, HPLC, Pharmaceutical dosage form, Validation

Abstrak

Suatu kaedah kromatografi cecair berprestasi tinggi (KCPT) yang mudah dan boleh dipercayai telah dibangunkan dan divaildasi bagi penentuan Lacidipine dalam bentuk tulen dan bentuk dos farmaseutikal. Kaedah ini telah dibangunkan manggunakan turus Xbridge C-18 (150 mm x 4.6 mm, 5 µm) dengan sistem campuran fasa bergerak ammonium asetat dan asetonitril. Efluen telah dipantau oleh pengesan PDA pada 240 nm. Keluk penentukuran adalah linear sepanjang julat kepekatan 50 - 250 µg/ml. Kepersisan (% RSD) intra-hari dan antara hari didapati antara nilai 0.83% dan 0.41% masing - masing. Perolehan semula Lacidipine didapati berada dalam julat 99.78 - 101.76 %. Had pengesanan (LOD) dan had kuantifikasi (LOQ) adalah masing - masing pada 1.0 dan 7.3 µg/ml. Kaedah RP-HPLC ini telah berjaya digunakan dalam penentuan kuantitatif Lacidipine bagi dos farmaseutikal.

Kata kunci: Lacidipine, HPLC, bentuk dos farmaseutikal, Pengesahan

Introduction

Lacidipine is a calcium channel blocker, anti hypertension and anti anginal drug. Chemically Lacidipine is 3, 5-diethyl4-{2-[(1***E***)-3-(***tert***-butoxy)-3-oxoprop-1-en-1-yl]phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5dicarboxylate (Figure 1). It has a molecular formula of $C_{26}H_{33}NO_6$ and a molecular weight of 455.54 g/mol [1].

From a physico-chemical point of view, lacidipine is slightly soluble in water, while it is more soluble in some widely used solvents as ethanol, methanol and acetone. It is very sensitive to the action of temperature and light. Lacidipine absorb light in the wavelength at 240nm [2]. Lacidipine has also shown anti-atherosclerotic and antioxidant effects. It has long duration of action because of its high degree of lipophilicity. The active *trans* form is used in therapy. Lacidipine or its metabolite inhibits the angiotensin converting enzyme, other hormone receptors, or ion channels [3].

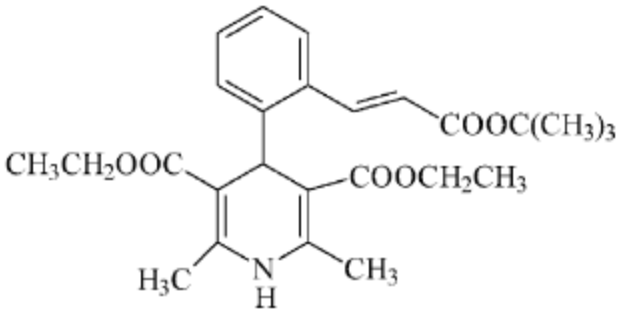

Figure 1. Structure of Lacidipine

Literature survey reveals that several analytical methods have been reported for the estimation of Lacidipine by LC-PDA, [4] High Performance Thin Layer Chromatography, [5] LC-MS, [6] and UV [7] method. Only one HPLC [8] method was developed and applied in the determination of Lacidipine in biological fluids. 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 with gradient sysytem.

From the analytical methods, it is possible to obtain the required information (about quality, purity, and concentration of the drug (analyte) in the dosage form) both qualitatively and quantitatively by the systematic approach. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw materials used and final products obtained meet the required specifications. The continuous and wider usage of same drugs report new toxicities and resistance. Under these conditions standard analytical procedures for some drugs may not available in pharmacopoeias. So, it becomes necessary to develop newer analytical methods [9].

Materials and Methods

Chemicals and Reagents

An analytically pure sample of Lacidipine was procured as gift sample from Cadila Health Care. (Ahmedabad, India). HPLC grade Acetonitrile and Water was procured from Sigma Aldrich and Millipore (India) Ltd., Bangalore. HPLC grade methanol and ammonium acetate was procured from Sigma Aldrich and Merck Ltd., Mumbai, India. Tablet formulations SINOPIL were procured from a local pharmacy with labeled amount of 2 mg per tablet.

Instrumentation

The HPLC system consisted of a Alliance Waters (Waters Corporation, MA, USA) equipped with a Waters e2695 Separations Module in a quaternary gradient mode, a Waters 2998 PDA detector , HPLC Pump Waters 2774 pump and Injector Loop Rheodyne, Model No. 2767, Made in USA 20 µl volume loop . Data acquisition was performed by the Empower 2 software.

Chromatographic Condition

Chromatographic analysis was performed on a Xbridge C-18 column with 150 mm x 4.6 mm i.d. and 5 µm particle size. The mobile phase consisted of acetonitrile : 5mM ammonium acetate (95:5 v/v) and was pumped gradientically at a flow rate of 1.0 ml/min and a column temperature of 30^{o}C. The injection volume was 5 µl. The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system. The effluent was monitored by PDA detector at 240 nm [10].

Preparation of Standard Solutions

The standard stock solution of Lacidipine was prepared with methanol to a concentration of 1000 µg/ml. Standard solutions 200 µg/ml were prepared using stock solution in methanol [11].

Procedure for pharmaceutical preparations

Five Sinopil tablets (2mg lacidipine per tablet) were finely powdered, homogenized and the powder equivalent to 2mg lacidipine was weighed, transferred into a 10 ml volumetric flask and diluted up to the mark with methanol. The mixture was sonicated for at least 30 min to aid dissolution and then filtered through a whatman filter paper no. 41. An appropriate volume of filtrate was diluted further with methanol so that the concentration of Lacidipine in the final solution was within the desired concentration. The sample solution was then analyzed by HPLC as shown in Figure 2 [11].

Results and Discussion

Method development and optimization

The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of Acetonitrile : 5mM Ammonium acetate (95:5 v/v) accomplished at 240 nm by using an Xbridge C-18 column (150 mm x 4.6 mm, 5 µm). The retention time was 8.309 min at a flow-rate of 1.0 ml/min and the injection volume was 5 µl. The total run time for an assay was approximately 15 min (figure 3) [12].

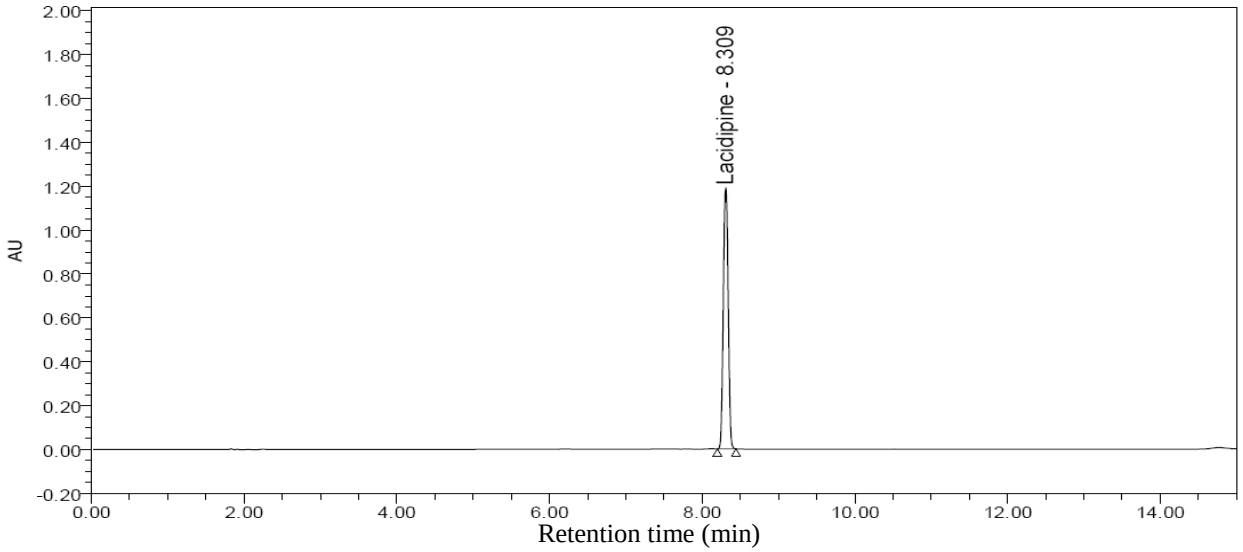

Figure 2**.** Chromatogram of Pure Lacidipine at 240 nm

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 suitable injections were determined. For all sample analyses, the efficiency and %RSD were found ≥ 2000 Theortical plate and ≤ 2% respectively [13]. USP tailing factor and capacity factor was found to be ≤ 1.5 and ≥ 3. : System suitability test for Lacidipine as shown in Table 1.

Table 1. System suitability test for Lacidipine

Name	Area	% Area	K Prime	USP Plate Count	USP Tailing
Lacidipine	5152284	99.89	7.9128	68652.9803	1.1454

Linearity

Calibration curve was constructed for Lacidipine pure drug by plotting the concentration of compound versus peak area response. Standard solutions containing 50, 100, 150, 200 and 250 µg/ml of Lacidipine were injected into the HPLC column (Figure 3). The linearity was calculated by the least square regression method [13]. The regression equations were calculated from the calibration graphs as shown in Table 2.

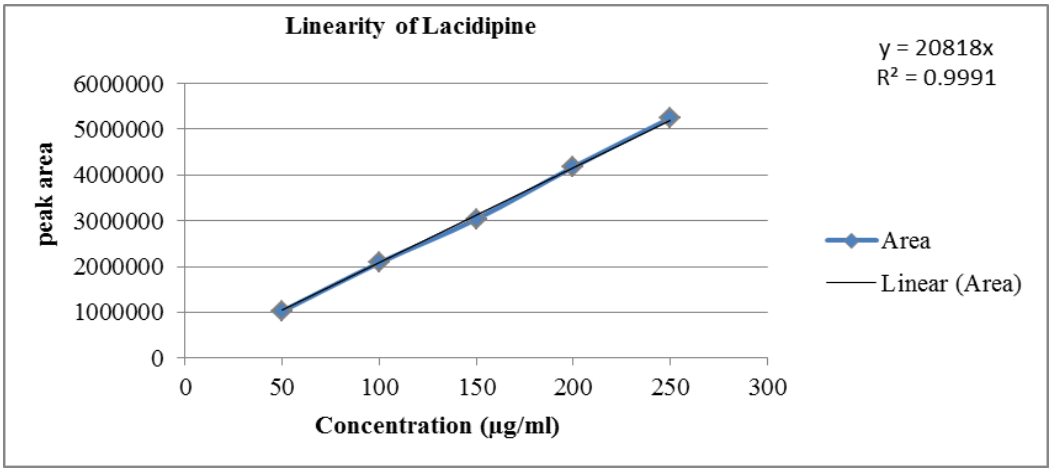

Figure 3. Calibration curve of Lacidipine at 240 nm

Table 2. Linearity result for Lacidipine

Sample No.	Conc. (mcg)	Area
1	50	1028346
2	100	2084209
3	150	3036851
4	200	4177264
5	250	5246360

Accuracy

Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100 and 120% of the standard concentration of Lacidipine (200 µg/ml). The results obtained as shown in Table 3 indicated that recovery was excellent and not less than 100% ± 2 [13].

Table 3. Accuracy results for Lacidipine

Sample No.	Peak Area Count			% Recovery	% Recovery
	Standard Sample	Control Sample	Spiked Sample		
1	6865562	6863948	6967884	99.976	101.490
2	6954103	6879907	7075508	98.933	101.745
3	7003864	6923248	7029465	98.840	100.365
4	7075876	6953203	7089847	98.266	100.197
5	7021791	6902210	7101875	98.297	101.140
6	7082635	6949177	7029678	98.115	99.252
AVG	7033972	6911949	7049043	98.73	100.69
STDEV	115945.1	36444.8	50003.33		
% RSD	1.648359	0.527272	0.709363		

Sensitivity

Limit of detection (LOD) and quantification (LOQ) were estimated from the signal-to-noise ratio. LOD and LOQ values were found to be 1.0 and 7.30 µg/ml respectively [13].

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 [13]. % RSD was calculated and shown in Table 4a and 4b.

Table 4a. Precision results for Lacidipine

PRECISION (INTERDAY)		
Sample No.	**Retention time (min)**	**Area**
1	8.911	8545162
2	8.9	8522625
3	8.885	8502433
4	8.907	8557291
5	8.893	8594801
AVG	8.8992	8544462
STDEV	0.0105	35151.03
% RSD	0.117961	0.41139

Table 4b. Precision results for Lacidipine

PRECISION (INTRADAY)		
Sample No.	**Retention time (min)**	**Area**
1	8.889	3724579
2	8.889	3727733
3	8.902	3739043
4	8.897	3794402
5	8.895	3776488
AVG	8.8944	3752449
STDEV	0.00555	31246.06
% RSD	0.062396	0.832685

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 [13]. Results of reproducibility are shown in Table 5.

Table 5. Ruggedness studies of Lacidipine by RP-HPLC method

	Analyst 1	**Analyst 2**
Sample No.	**Area**	**Area**
1	3825716	5423628
2	3850258	5284504
3	3878128	5365237
AVG	3851367	5357790
SD	26223.6	69860.35
% RSD	0.680891	1.303902

*Average of three determinations.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in wavelength 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 [13]. The results are shown in Table 6.

Table 6. Robustness studies of Lacidipine by RP-HPLC method

Condition	Modification	Mean Area ± SD*	% RSD	Mean t_r ± SD* (min)
Mobile Phase Flow Rate (ml/min)	4.9	6605607 ± 21733.04	0.329	8.928 ± 0.308
	5	5303876 ± 24212.63	0.456	8.299 ± 0.205
	5.1	8435185 ± 36118.45	0.428	8.903 ± 0.171
Wavelength (nm)	238	3520884 ± 25087.63	0.712	8.910 ± 0.008
	240	5297928 ± 25051.38	0.472	8.363 ± 0.608
	242	8547928 ± 54659.3	0.534	8.526 ± 0.325

*Average of three determinations.

Conclusion

A rapid and simple RP-HPLC method for determination of Lacidipine has been developed and validated. The method involved a mobile phase consisting of Acetonitrile: 5mM Ammonium acetate (95:5 v/v) accomplished at 240 nm. The retention time was 8.309 min at a flow-rate of 1.0 ml/min and the injection volume was 5 µl. The total run time for an assay was approximately 15 min. 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 gradient system was more reliable and reproducible for 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.

Acknowledgement

The authors wish to thank D. Raj Aanand and Kartikeyai Diwedi from Jubilant Chemsys R&D Noida (U.P.) for providing the facilities to carry out this research work.

References

1. British National Formulary; 58th Edition (September 2009) British Medical Association and Royal Pharmaceutical Society of Great Britain, London (http://bnf.org/bnf/bnf/current/).
2. Martindale. (1996). The Extra Pharmacopeia, Royal Pharmaceutical Society, London. 31st ed., 898.
3. Tripathi, K. D. (2008). Essentials of medical pharmacology jaypee brothers medical Publishers, 6th ed., 531-532.
4. Baranda, A.B., Berasaluce, O., Jimenez, R.M., Alonso, R.M. (2005). LC-DAD Determination of calcium channel Blockersby Using an Experimental Design Approach. Chromatographia, 61(9-10) : 447-453.
5. Kharat, V.R., Verma, K.K., Dhake, J.D. (2002). Determination of Lacidipine from urine by HPTLC using off-line SPE. J Pharm Biomed Anal, 28(3-4) : 789-793.
6. Marco Garzotti. (2003). Lacidipine, a potential peroxynitrite scavenger investigation of activity by liquid chromatography and mass spectrometry. Rapid Commun Mass Spectrum, 17(4) : 272-278.
7. Filippis, P.De., Bovina, E. E., Fiori, J., Cavrini, V. (2002). Photo degradation studies on Lacidipine in solution: basic experiments with a cis–trans reversible photoequilibrium under UV-A radiation exposure. J Pharm Biomed Anal 27(1-5) : 803-812.
8. Ramesh, G., Vamshi Vishnu, Y., Chinna Reddy, P., Shravan Kumar, Y., Madhusudan Rao, Y. (2009). Development of High performance liquid chromatography method for Lacidipine in rabbit serum. Anal Chim Acta, 632(2) : 278-283.
9. Rashmin. (2008). An introduction to analytical method development for pharmaceutical formulations. 6(4) : 102-115.

10. Yamsani, M., Gannu, R., Yamsani, V., Reddy, P., and Yamsani, S. Available online 17 November (2008). Developed high performance liquid chromatographic (HPLC) method for quantification of Lacidipine in rabbit serum: Application to pharmacokinetic study.
11. Channabasavaraj, K.P., Nagaraju P.T., Shantha Kumar P.T., Srinivas Reddy P. November – December (2010). Reverse phase HPLC method for determination of lacidipine in pharmaceutical preparations. Volume 5, Issue 2, Article-021.
12. Braggio, S., Sartori, S., Angeri, F., Pellegatti, M. (1995). Automation and validation of the high-performance liquid chromatographic-radioimmunoassay method for the Determination of lacidipine in plasma. J of Chromatography B, 669 : 383-389.
13. Tang, J., Zhao, R., Cheng, G., Wenxing, P. (2008). Ultra-performance liquid chromatography-tandem mass spectrometry for the determination of lacidipine in human plasma and its application in a pharmacokinetic study. J of Pharm and Biomed Ana, 47: 923–928.