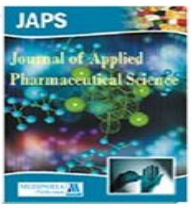
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received: 11-07-2011
Revised on: 14-07-2011
Accepted: 19-07-2011

Spectrophotometric determination of lacidipine in bulk and tablet dosage form

C. Moorthi, Aju. M, Elsadig, Prezzy and G. Sumithira

ABSTRACT

New, simple, accurate, sensitive, economical, and reproducible UV-spectrophotometric method was developed and validated for the estimation of Lacidipine in bulk and tablet dosage form. Lacidipine shows maximum absorbance at 615.7 nm and linearity (Beer's Lamberts law) was found to be in the range of 10-70 µg/ml. The apparent molar absorptivity and Sandell's sensitivity coefficient were found to be 2.84 x 10^3 L mol^{-1} cm^{-1} and 0.16043 indicating the high sensitivity of the proposed methods. The slope, intercept and correlation coefficient were also calculated. Interference of common excipients with the proposed method was also studied and found there was no interference of common excipients with the proposed method. The method was validated by determining its sensitivity, accuracy and precision which proves the suitability of the developed method for the routine estimation of Lacidipine in pharmaceutical formulations.

Key words: Lacidipine, UV Spectrophotometry, Para Dimethyl Amino Benzaldehyde, Schiff's base.

C. Moorthi, Aju. M, Elsadig, Prezzy and G. Sumithira
Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India.

***For Correspondence**:
C. Moorthi,
Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India
Tel.: + 91-9787440311

INTRODUCTION

Lacidipine is a once-daily, orally-administered, lipophilic dihydropyridine calcium antagonist with an intrinsically slow onset of activity approved only for use in mild to moderate hypertension, and is widely used in therapy since early 90s. Lacidipine works by relaxing and opening up the blood vessels. This allows blood to circulate more freely around the body, lowering blood pressure and allowing the heart to work more efficiently. It has a long duration of action and a high degree of vascular selectivity (McCormack et al., 2003). In addition to calcium channel-modulated vasodilation, lacidipine displays antioxidant activity greater than that of other dihydropyridine calcium antagonists (Marco Garzotti et al., 2003). Chemical name of Lacidipine is (E)-4-[2-[3-(1,1-Dimethyl ethoxy)-3-oxo-1-propenyl] phenyl]-1,4- dihydro-2,6-dimethyl-3,5-pyridine dicarboxylic acid diethyl ester (Figure 1). It has a molecular formula of $C_{26}H_{33}NO_6$ and a molecular weight of 455.543 g/mol (Nagaraju et al., 2011). Literature survey reveals that several analytical methods have been reported for the estimation of Lacidipine by LC-DAD (Baranda et al., 2005), UPLC-TMS (Tang et al., 2008), HPTLC (Kharat et al., 2002), HPLC (Ramesh et al., 2009), LC-MS, UV (Nagaraju et al., 2011) and Electrochemical (Juan et al., 1999). However, the current study aimed to develop a new UV spectrophotometric method for estimation of Lacidipine in pharmaceutical formulations with good accuracy, simplicity, sensitive, precision, reproducible and economy.

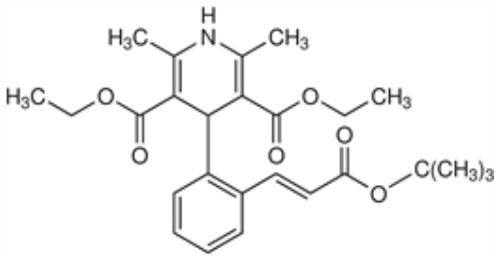


Fig. 1: Structure of Lacidipine [Source*:* Martindale: The Complete Drug Reference]

MATERIALS AND METHODS

Materials

Pure analytical grade sample of Lacidipine was procured as gift sample from GSK Pharmaceuticals Ltd, Mumbai. Two different brands of lacidipine were procured from local pharmacy. Analytical grade methanol, Para Dimethyl Amino Benzaldehyde and Concentrated Hydrochloric acid were procured from E-Merck.

Instrumentation

Shimadzu UV - 1800 UV/VIS spectrophotometer with 1 cm matched quartz cell was used for spectral measurements. Seiko (Japan) DSC model 220c was used to determine the impurity profiling of Lacidipine and IR spectra were recorded using Perkin-Elmer Paragon 1000 FT-IR spectrophotometer.

Methodology

Functional group confirmation of Lacidipine

FT-IR spectrophotometer was utilized to confirm the presence of basic nucleus and functional group of Lacidipine.

Impurity profiling of Lacidipine

DSC study of the Lacidipine was performed at a scanning rate of 10 °C/min. 10 mg of Lacidipine was heated on sealed aluminium pans from 30 °C to 400 °C to confirm its purity.

Principle of method

Secondary amine group which is present in 1, 4-dihydropyridine molecule of Lacidipine reacts with Para Dimethyl Amino Benzaldehyde to form a Schiff's base which produces blue color.

Preparation of standard stock solution

10 mg of pure Lacidipine was dissolved in 10 ml of methanol to get a concentration of 1000 µg/ml (Stock solution I). 1 ml of stock solution I was further diluted to 10 ml with methanol to get final concentration 100 µg/ml (Stock solution II).

Selection of Wavelength

1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid were added to 5 ml of stock solution II in 50 ml standard volumetric flask which was then heated for 4 minutes in a boiling water bath to develop blue color which is then cooled at room temperature and final solution was made up to the mark using methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) in the visible region between 550 - 670 nm.

Effect of concentration and volume of reagents

The effect of change in concentration and volume of Para Dimethyl Amino Benzaldehyde and hydrochloric acid were studied at 550 to 625 nm for a fixed concentration of lacidipine (50 µg/ml).

Color stability study

Concentration of 10 to 40 µg/ml of Lacidipine was prepared from the stock solution I. To each concentration, 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid were added and heated in a boiling water bath for 4 minutes to develop color. Final solution was cooled and volume was made up to the mark using methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) at 617.5 nm for 90 mins at the interval of 15 minutes.

Beer's Lamberts Law plot

Concentration of 10 to 70 µg/ml of Lacidipine was prepared from the stock solution I. To each concentration, 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid were added and heated in a boiling water bath for 4 minutes to develop color. Final solution was cooled and volume was made up to the mark using methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) at 617.5 nm.

Assay of the branded Lacidipine tablets

Two different brands of lacidipine tablets were taken for analysis. Twenty tablets were weighed to calculate the average weight, powdered and 10 mg of powder was transferred to 10 ml volumetric flask where it is dissolved using methanol by shaking the solution for 30 minutes. Final solution is filtered using Whatman filter paper. 1 ml of filtrate was mixed with 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid and heated in a boiling water bath for 4 minutes to develop blue color and the final solution was cooled and volume was made up to the mark using methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) at 617.5 nm.

Interference of excipients with the proposed method

The interference study of the additives used for the formulation of the tablets can be done by mixing 100 mg each of talc, starch, magnesium striate and lactose with 10 mg of pure lacidipine individually in 10 ml of volumetric flasks to that methanol was added to dissolve the drug by shaking the solution for 30 minutes. Final solution is filtered using Whatman filter paper. 1 ml of filtrate was mixed with 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid and heated in a boiling water bath for 4 minutes to develop blue color. The final solution was cooled at room temperature and volume was made up to the mark with methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) at 617.5 nm.

Validation of the developed methods

Recovery studies

To ensure the accuracy and reproducibility of the results obtained. The recovery experiments were performed for two different brands of the tablets. The tablets powder equivalent to 10 mg was transferred to 10 ml volumetric flask. A known volume of solution (1 mg/ml) of pure sample of Lacidipine was added to flask and methanol was added to dissolve the drug by shaking the solution for 30 minutes. Final solution is filtered using Whatman filter paper. Rejecting the first few ml of filtrate, 1 ml of filtrate was mixed with 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml of concentrated hydrochloric acid and heated in a boiling water bath for 4 minutes to develop blue color and the final solution was cooled and volume was made up to the mark with methanol. The absorbance spectrum of resulting solution was measured against respective blank solution (without drug) at 617.5 nm. This experiment was repeated three times for each brand of Lacidipine tablet by adding known volume of standard solution of Lacidipine.

Precision

The precision is a measure of the ability of the method to generate reproducible results. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as Relative Standard Deviation (RSD) percentage. For this, 10 and 70 µg/ml of the solution was measured three times in a day and the same was repeated in next three days and then the percentage RSD was calculated.

Accuracy

Accuracy of the proposed method was found out by comparing the results obtained by the proposed method with the result obtained by reported methods. The reported method was UV spectrophotometric method (λ_{max} at 522.5 nm). 10 mg of lacidicipine was dissolved in methanol and volume was adjusted to 10 ml with methanol to get 1 mg/ml. To 0.5 ml of working standard drug solution in a 10 ml volumetric flask, 1 ml of 1% w/v of vanillin in methanol and 1 ml of 85 % v/v phosphoric acid were added then the solution was heated on a water bath for 25 minutes, cooled, made up to 10 ml with phosphoric acid and kept for 5 min, then the absorbance of these solutions were measured at 522.5 nm against reagent blank. Similarly 0.5 ml of working standard was treated using proposed method and absorbance was measured and compared with the reported method.

RESULT AND DISCUSSION

Functional group confirmation of Lacidipine

Basic nucleus and functional group of Lacidipine has been confirmed and listed in table 1.

Impurity profiling of Lacidipine

DSC study showed the melting point in the range of 174 - 175 °C which confirms the purity of Lacidipine.

Table 1: Basic nucleus and functional group of Lacidipine.

Frequency cm^{-1}	Indication
720 & 740	-$(CH_2)_n$- Skeletal vibration
810	-characteristic for academic ring
840	-NH- Bending vibration
1000	-CO- Stretching band
1070 & 1170	-CO- Stretching vibration
1290	-characteristic for ester group
1350	-[-CO- Stretching vibration for ester group
1660	-C=O- Stretching vibration
3280	-NH- Stretching vibration

Selection of Wavelength

The absorption spectral analysis showed that the maximum absorption at 615.7 nm with minimum effective concentration of 0.1% w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml concentrated hydrochloric acid. Summary of absorption spectral analysis is shown in figure 2.

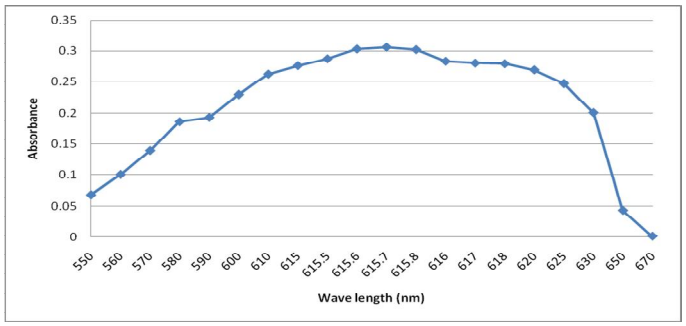


Fig. 2: Absorption spectral data for Lacidipine (50 µg/ml) with 0.1 % w/v Para Dimethyl Amino Benzaldehyde and 0.2 ml hydrochloric acid.

Effect of concentration and volume of reagents

Low concentration of Para Dimethyl Amino Benzaldehyde did not affect the calibration curve but gives low absorbance. While more concentration of Para Dimethyl Amino Benzaldehyde didn't showed significant difference in absorption spectrum and calibration curve. Hence 1 ml of 0.1% w/v Para Dimethyl Amino Benzaldehyde seems to be satisfactory. However, concentrated hydrochloric acid was added directly as the lower concentration produced low color intensity. Summary of change in concentration and volume of Para Dimethyl Amino Benzaldehyde are given in table 2 and table 3.

Table 2: Effect of concentration of Para Dimethyl Amino Benzaldehyde.

Wave length (nm)	Para Dimethyl Amino Benzaldehyde concentration		
	0.05% w/v	0.1% w/v	0.2% w/v
	Absorbance		
550	0.32	0.068	0.070
570	0.087	0.139	0.142
580	0.128	0.186	0.186
600	0.191	0.230	0.232
610	0.206	0.262	0.265
615.7	0.283	0.306	0.308
620	0.211	0.269	0.270
625	0.193	0.247	0.250

Table 3: Effect of volume of Para Dimethyl Amino Benzaldehyde.

Wave length (nm)	Para Dimethyl Amino Benzaldehyde volume		
	0.5 ml	1 ml	2 ml
	Absorbance		
550	0.042	0.068	0.070
570	0.097	0.139	0.145
580	0.123	0.186	0.193
600	0.180	0.230	0.238
610	0.197	0.262	0.262
615.7	0.231	0.306	0.313
620	0.192	0.269	0.275
625	0.160	0.247	0.251

Color stability study

Developed blue chromogen has found to be stable for 75 mins. The Summary of the color stability is shown in figure 3.

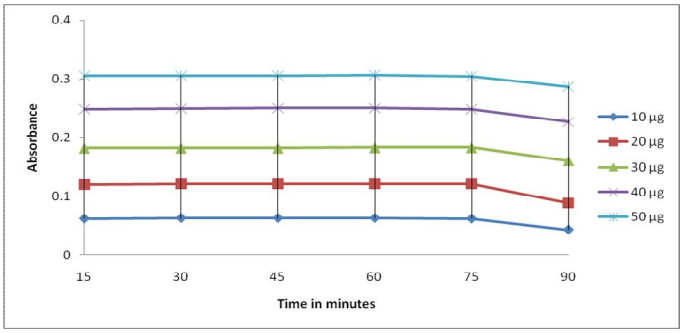


Fig. 3 Stability of color produced by drug with Paradimethyl Amino Benzaldehyde.

Beer's Lamberts Law plot

The proposed method obeyed Beer's Lamberts law in the range of 0-70 µg/ml of Lacidipine which has been shown in figure 4. The optimum concentration and quantity of Para Dimethyl Amino Benzaldehyde is 0.1 w/v and 1 ml respectively and 0.2 ml of concentrated hydrochloric acid.

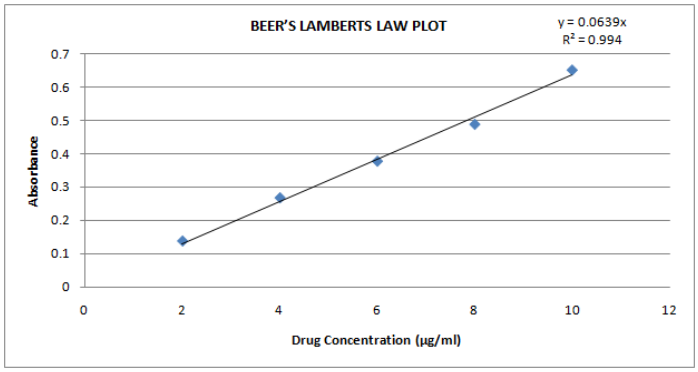


Fig. 4: Beer's Lamberts Law plot for the proposed method

Assay of the branded Lacidipine tablets

The applicability of the proposed method for the assay of Lacidipine in tablet dosage form was examined by analysing the branded Lacidipine tablets and the results were tabulated in table 4. The results obtained were good agreement with the label claims. The results were reproducible with low % RSD values.

Interference of excipients with the proposed method

The results of the estimation of Lacidipine in the synthetic mixtures containing Lacidipine and excipients like talc, lactose and magnesium stearate are shown in table 5 and it is indicated that the excipients are not interfering in the estimation of the drug using the proposed method.

Validation of the developed methods

The validation parameters of the proposed method are listed in table 6.

Recovery studies

Recovery studies were close to 100% that indicates the accuracy and precision of the proposed methods. Results of Recovery studies are listed in table 7.

Precision

The precision (measurements of intra-day and inter-day) results (Table 8) showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Accuracy

Drug content obtained by the proposed method was comparable with the reported method (Table 9). Hence the proposed method is highly accurate.

CONCLUSION

The proposed method was found to be simple, sensitive, accurate and reproducible and can be used for routine quality control analysis of Lacidipine in bulk and in pharmaceutical formulations.

REFERENCES

Baranda AB., Berasaluce O., Jimenez RM., Alonso RM. LC-DAD Determination of calcium channel Blockers by Using an Experimental Design Approach. Chromatographia. 2005; 61: 447-453.

Juan A. Squella, Anny E. Iribarren, Juan C. Sturm, and Luis J. Núñez-Vergara. Electrochemical Determination of Lacidipine. Journal of AOAC International. 1999; 82: 1077.

Table 4: Data for assay of branded tablets using 0.1 % w/v Para Dimethyl Amino Benzaldehyde and Hydrochloric Acid.

Brands	Label claim (in mg)	Weight of standard powder taken (in mg)	Average weight of tablet taken (in mg)	Weight of tablet powder taken (in mg)	Absorbance		Content of drug (in mg)	Average Content (in mg) ± S.D
					Standard	Test		
B1	4	10.2	310.2	728.8	0.374	0.346	4.010	3.9885 ± 0.009172
				778.0		0.370	4.006	
				753.4		0.354	3.974	
				730.5		0.346	3.980	
				750.3		0.353	3.971	
B2	2	10.2	305.2	1500.6	0.374	0.375	2.058	2.0540 ± 0.004510
				1525.0		0.381	2.059	
				1520.3		0.380	2.054	
				1501.8		0.375	2.058	
				1518.2		0.374	2.038	

Table 5: Interference of excipient with the proposed method.

Excipients	Absorbance at 615.7 nm at 60 µg/ml concentration of Lacidipine
Lactose 100 mg	0.375
Starch 100 mg	0.377
Talc 100 mg	0.376
Magnesium stearate 100 mg	0.377

Table 6: Validation parameters.

Characters	Values
λ_{max}	615.7 nm
Beer's Lamberts law range	0 – 70 µg/ml
Slope	0.006189
Intercept	-0.00316
Regression equation	Y = 0.006189 C- 0.00316
Correlation coefficient	1.007482
Molar extinction coefficient ($L\ mol^{-1}cm^{-1}$)	2.84×10^3
Sandell's sensitivity	0.16043

Table 7: Summary of Recovery Studies Data.

Brands	Average weight of tablet taken (in mg)	Weight of standard powder taken (in mg)	Standard absorbance	Weight of tablet powder taken (in mg)	Amount of drug added (in mg)	Content of drug (in mg)	% recovery	Average % recovery ± S.D
B1	310.2	10.2	0.374	842.0	1	5.011	102.26	100.42
				790.5	1	4.977	98.85	±
				788.2	1	4.990	100.15	1.2169
B2	305.2	10.2	0.374	1558.0	1	3.0505	99.65	100.7 ±
				1526.1	1	3.1183	100.95	0.6718
				1520.1	1	3.0690	101.50	

Table 8: Precision of Proposed method.

Concentration (µg/ml)	Intra-day (% RSD)	Inter-day (% RSD)
10	0.72	1.02
70	0.68	0.97

Table 9: Comparison of proposed method with reported method.

Brand	Drug content determined by reported method (mg)	Drug content determined by proposed method (mg)
B1	4.0580	3.9885
B2	1.9706	2.0540

Kharat VR., Verma KK., Dhake JD. Determination of Lacidipine from urine by HPTLC using off-line SPE. J Pharm Biomed Anal. 2002; 28: 789-793.

Marco Garzotti. Lacidipine, a potential peroxynitrite scavenger investigation of activity by liquid chromatography and mass spectrometry. Rapid Commun Mass Spectrum. 2003; 7: 272-278.

McCormack PL., Wagstaff AJ. Lacidipine: a review of its use in the management of hypertension. Drugs. 2003; 63: 2327-2356.

Nagaraju P.T., Channabasavaraj K.P., Shantha Kumar P.T. Development and Validation of Spectrophotometric Method for estimation of Lacidipine in Tablet Dosage Form. International Journal of PharmTech Research. 2011; 3: 18-23.

Ramesh G., Vamshi Vishnu Y., Chinna Reddy P., Shravan Kumar Y., Madhusudan Rao Y. Development of High performance liquid chromatography method for Lacidipine in rabbit serum. Anal Chim Acta. 2009; 632: 278-283.

Tang J., Zhu R., Zhao R., Cheng G., Peng W. Ultra-performance liquid chromatography-tandem mass spectrometry for the determination of lacidipine in human plasma and its application in a pharmacokinetic study. J Pharm Biomed Anal. 2008; 47: 923-928.