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Development and Validation of Spectrophotometric Method for estimation of Lacidipine in Tablet Dosage Form

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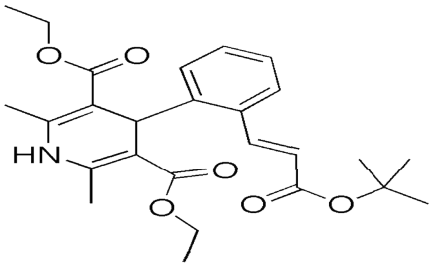
Abstract: Two simple, precise and economical UV methods have been developed for the estimation of Lacidipine in bulk and pharmaceutical formulations. Lacidipine has the absorbance maxima at 240nm (Method A), and in the first order derivative spectra, Showed zero crossing at 240nm, with a sharp peak at 230nm when n=1 (Method B).Drug followed the Beer's Lamberts range of 5–30 µg/ml for the Method A&B.The limits of detection were found to be 0.802 µg/ml and 2.94 µg/ml for Method Aand Method B respectively. The limit of quantification for Method A and Method B were found to be 2.430µg/ml and 8.98 µg/ml respectively. Results of analysis were validated statistically and by recovery studies and were found to be satisfactory.
Key words: Lacidipine, UV Spectrophotometry, Derivative Spectroscopy.

1.INTRODUCTION:

Lacidipine [1] 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 has a molecular formula of $C_{26}H_{33}NO_6$ and a molecular weight of 455.55 g/mol.



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] and HPLC[4], LC-MS[5,6] and UV[7] modified. Apart from above no other spectroscopic methods such as UV/Vis, difference spectrophotometric method, RP-HPLC by using internal standard etc., were reported for this compound.

Hence an attempt has been made to develop new UV method for its estimation in bulk and pharmaceutical formulations with good accuracy, simplicity, precision and economy.

2. EXPERIMENTAL

2.1 Instruments and reagents

A Shimadzu UV - 1800 UV/VIS spectrophotometer was used with 1 cm matched quartz cell.

All the chemicals used were of analytical grade. Methanol A.R. grade was procured from Loba Chem. Ltd., Mumbai. An analytically pure sample of Lacidipine was obtained from Cipla Health Care,

Ahmadabad as a gift sample. Tablet of 2mg were procured from local pharmacy.

2.2 Preparation of standard stock solution

Standard stock solution was prepared by dissolving accurately weighed 100 mg of Lacidipine in Methanol and the volume was made up to 100 ml with Methanol in 100 ml volumetric flask (Stock solution-I, 1000 µg/ml). 10 ml of stock solution-I was diluted to 100 ml with Methanol (Stock solution-II, 100 µg/ml). 1 ml of stock solution-II was taken in 10 ml standard flask diluted to 10 ml with Methanol to get the concentration 10 µg/ml. The absorbance of resulting solution was measured against respective blank solution in the UV region of 200-400 nm, which shows maximum absorbance at 241.1 nm.

2.3 Zero order spectroscopic method

Use above stock solution-II to prepare range of standard solution from 5, 10, 15, 20, 25 and 30. The solutions were scanned in the range from 400-200nm (method A), and the peaks were observed at 240 nm and 280 nm. The wavelength selected for the analysis of the drug was 240nm **(figure 1)**. The drug followed the Beer's-Lamberts law in the range of 5-30 µ g/ml. Using calibration curve the concentration of the sample solution can be determined.

2.4First order derivative spectroscopic method

The first order derivative spectra at n=1 (method B)[8], showed a sharp peak at 230**(figure 2)**. The absorbance difference at n=1 (dA/d) is calculated by the inbuilt software of the instrument which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 5-30 µg/ml and scanned in the first order derivative spectra. The calibration curve of dA/d against concentration of the drug showed linearity.

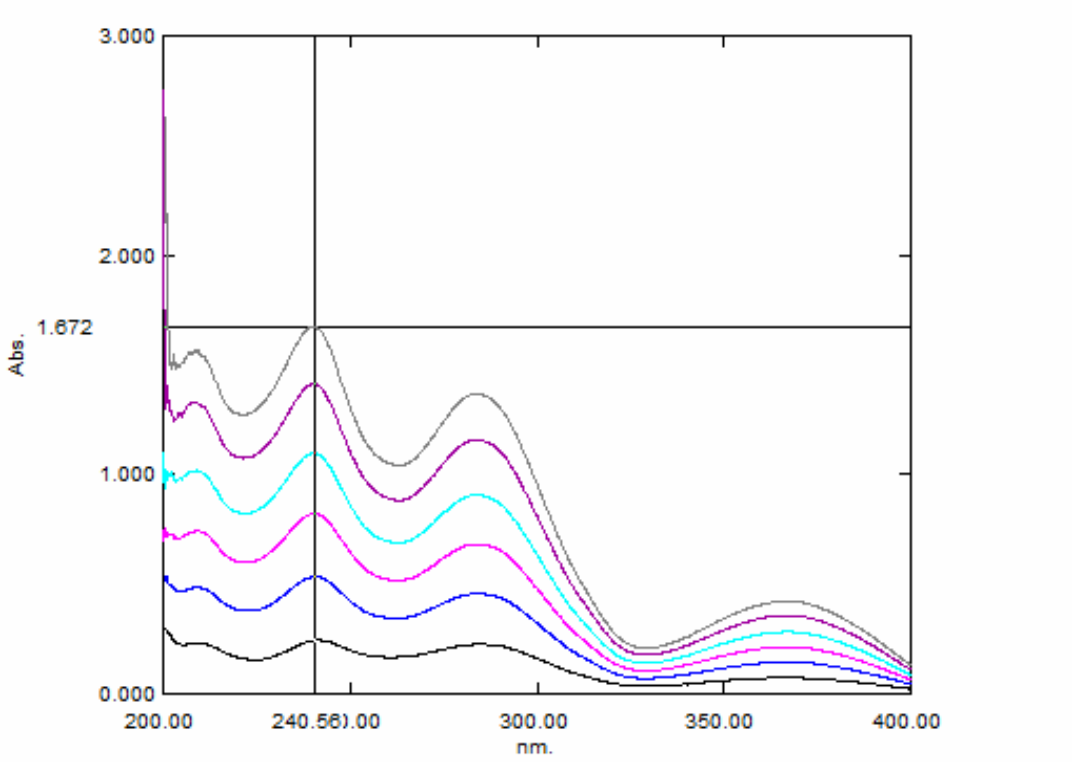


Figure 1: Zero orderderivative spectra of Lacidipine

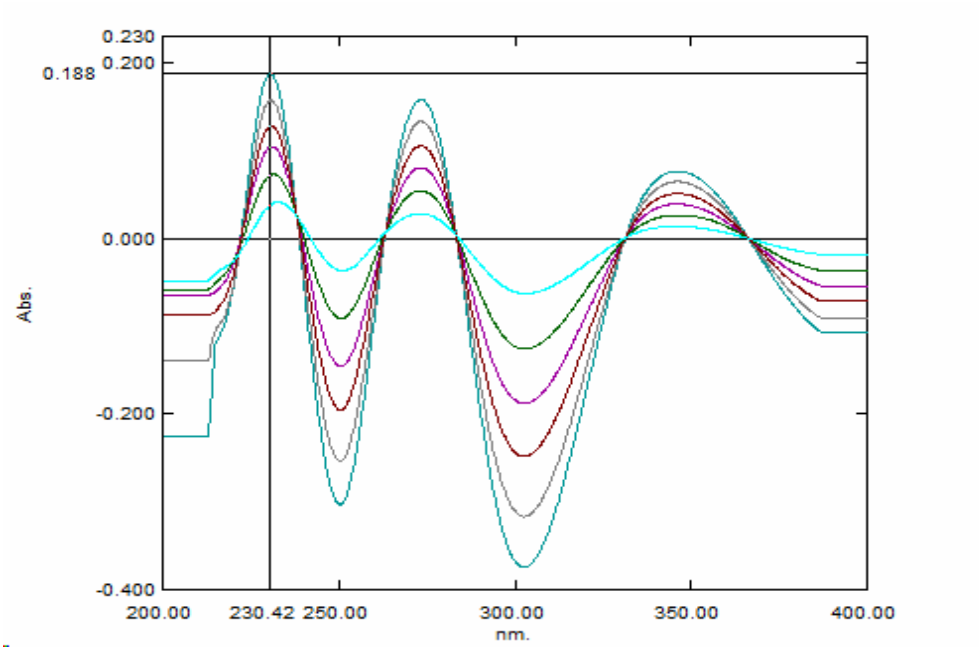


Figure 2: First order derivative spectra of Lacidipine with n=1

2.5 Analysis of the Tablet formulation

20 tablets of Lacidipine were weighed, powdered in glass mortar and the powder equivalent to 10 mg of Lacidipine was weighed accurately and transferred into a 100 ml standard volumetric flask. The contents were dissolved in Methanol and sonicated for thirty minutes. This solution was filtered through 0.45 micron Whatmann filter paper. 1 ml of the filtrate was diluted to 10 ml with Methanol to get the solution of 100 µg/ml. An aliquot of 1 ml of test solution was diluted to 10 ml with Methanol in 10 ml standard volumetric flask to produce the concentration 10 µg/ml.

2.6 Validation of the method

All these methods were validated according to ICH guidelines[9, 10] by carrying out analysis of six replicate sample of tablet. Recovery studies were carried out at three different levels i.e. 50%, 100%, and 150% by adding the pure drug to previously analysed tablet powder sample. From the amount of drug found, percentage recovery was calculated.Precision method was studied as intra-day and inter-day variations. The Ruggedness of marketed formulation was carried out.

Table: 1 Results of calibration curve

Sr. No.	Conc. (µg/ml)	Method A	Method B
		Absorbance at 240 nm	Absorbance at 230 nm
1	5	0.244	0.041
2	10	0.534	0.073
3	15	0.822	0.101
4	20	1.098	0.128
5	25	1.413	0.157
6	30	1.676	0.187

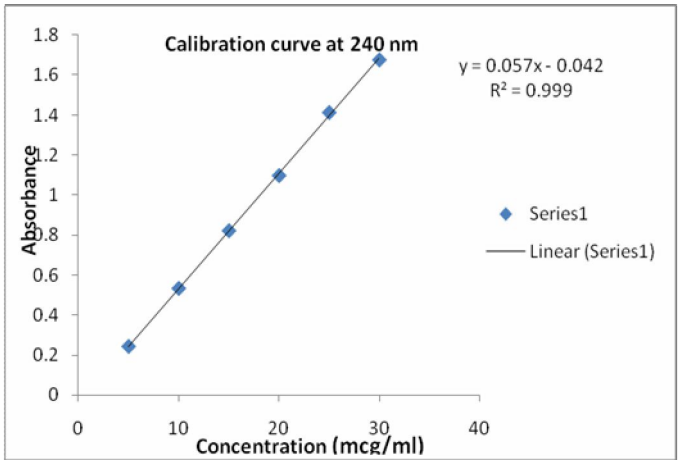


Fig: 3Linearity curve forLacidipine at 240 nm by Zero order derivative spectroscopy

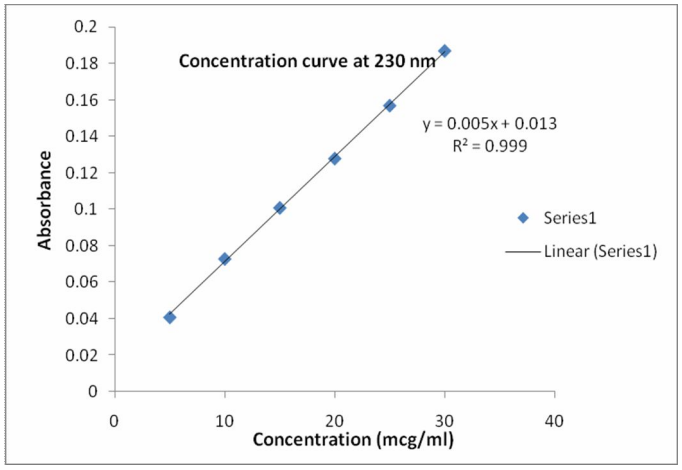


Fig: 4Calibration curve for Lacidipine at 230 nm by first order derivative spectroscopy

Table: 2Optimum conditions, Optical characteristics and Statistical data of the Regression equation in UV method

Parameters	Method A	Method B
λ_{max} (nm)	240	230
Beer's law limits (µg/ml)	5-30	5-30
Molar extinction coefficient (L mol^{-1} cm^{-1})	$0.0549X10^4$	$0.0064 X 10^4$
Sandell's sensitivity (µg/cm^2-0.001 absorbance units)	0.0182	0.156
Regression equation (Y*)	Y= 0.0576 C - 0.0428	Y=0.0058C + 0.0136
Slope (b)	0.0576	0.0058
Intercept (a)	-0.0428	0.0136
Correlation coefficient(r^2)	0.9997	0.9995
% RSD**	0.217	0.69
Limit of detection (µg/ml)	0.802	2.94
Limit of quantitation (µg/ml)	2.430	8.98

*Y= bC + a where C is the concentration of Lacidipine in mcg/ ml and Y is the absorbance at the respective $\lambda_{max.,}$ **Average of six determinations.

Table: 3 Determination of Accuracy results for Lacidipine by zero order derivative spectroscopy

Brand name	Concentration of sample (µg/ml)	Amount of pure drug added(µg/ml)	Amount Recovered (µg/ml)	% Recovery± SD**
Sinopil	20	15	34.9	99.74 ± 0.591
	20	20	39.94	99.85 ± 0.832
	20	25	45.11	100.24 ± 0.613

**Average of six determinations.

Table: 4 Determination of Accuracy results for Lacidipine by First order derivative spectroscopy

Brand name	Concentration of sample (µg/ml)	Amount of pure drug added(µg/ml)	Amount Recovered (µg/ml)	% Recovery± SD**
Sinopil	20	15	15.20	101.09 ± 0.70
	20	20	20.03	100.15 ± 0.06
	20	25	25.06	99.65 ± 0.36

**Average of six determinations.

Table: 5 Determination of Precision results for Lacidipine at 240 nm by Zero order derivative spectroscopy

Conc mcg / ml	Inter-day Absorbance Mean ± SD**	% CV	Intra-day Absorbance Mean ± SD**	% CV
5	0.247 ± 0.0035	1.41	0.241 ± 0.00316	1.31
10	0.532 ± 0.00189	0.35	0.533 ± 0.00208	0.39
15	0.822 ± 0.00216	0.26	0.823 ± 0.00217	0.26
20	1.095 ± 0.00238	0.21	1.095 ± 0.00275	0.25
25	1.411 ± 0.00182	0.12	1.415 ± 0.00182	0.12
30	1.673 ± 0.00221	0.13	1.675 ± 0.00221	0.13

**Average of six determinations.

Table: 6 Determination of Precision for Lacidipine at 230 nm by firstorder derivative spectroscopy

Conc mcg / ml	Inter-day Absorbance Mean ± SD**	% CV	Intra-day Absorbance Mean ± SD**	% CV
5	0.042 ± 0.00100	2.38	0.041 ± 0.00141	3.43
10	0.073 ± 0.00057	0.78	0.075 ± 0.00282	3.76
15	0.102 ± 0.00152	0.14	0.101 ± 0.00353	3.49
20	0.128 ± 0.00081	0.63	0.128 ± 0.00081	0.63
25	0.155 ± 0.00152	0.09	0.157 ± 0.00424	2.70
30	0.186 ± 0.00057	0.30	0.187 ± 0.00070	0.37

**Average of six determinations.

Table: 7 Ruggedness results for Lacidipine at 240 nm by Method A and at 230 nm by Method B

Brand name		Label claim (mg)	Analyst I		Analyst II	
			Amount found (mg)	Recovery ± SD**(%)	Amount found (mg)	Recovery ± SD**(%)
Sinopil	**Method A**	2	1.996	99.80 ± 0.11	1.998	99.92 ± 0.14
	Method B	2	1.988	99.40±0.26	1.9982	99.91 ± 0.33

** Average of six determinations.

3. RESULTS AND DISCUSSION

All the methods A and B for the estimation of Lacidipine in tablet dosage were found to be simple, accurate and reproducible. Beer-lambert's law wasobeyed in the concentration range of 5-30 µg/ml in all these methods. The accuracy of the method was assessed by recovery studies at three different levels i.e. 50%, 100%, 150%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The results for percentage recovery obtained from the amount of drug are given in table 3&4. The %RSD value was less than 2 indicative of accuracy of the method.Results for precision study are reported in Table 5&6. The results of analysis of marketed formulation are shown in Table 7. The values obtained were found to be within the limit. Hence these methods can be useful in routine analysis of Lacidipine in bulk drug and formulations.

4. CONCLUSION

The developed 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.

5. ACKNOWLEDGEMENT

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