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Original Research Article

Validated UV and RP-HPLC Method Development for the Estimation of Anti-Hypertensive Drug in Marketed Formulation

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ABSTRACT

Lacidipine (LAC) is a calcium channel blocker used in treatment of hypertension. In present study a simple, sensitive and accurate isocratic reverse phase high performance liquid chromatography (RP-HPLC) and UV spectrophotometric method was developed for determination of lacidipine in tablets formulation. Different analytical performance parameters such as linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantification (LOQ) were determined according to International Conference on Harmonization ICH Q2B guidelines. The RP-HPLC method was developed by the isocratic technique on a reversed-phase Thermo C18 (250 × 4.6 mm, 5μm) column with mobile phase consisting of methanol: water (80:20v/v) at flow rate of 1.0 ml/min. The retention time for LAC was 3.778±0.3min. The UV spectrophotometric determinations were performed at 240 nm using methanol as a solvent. The linearity range for LAC was 5-25 μg/ml for both HPLC and UV method. The linearity of the calibration curves for each analyte in the desired concentration range was good (r2 >0.999) by both the HPLC and UV methods. The method showed good reproducibility and recovery with percent relative standard deviation less than 2%. Moreover, the accuracy and precision obtained with HPLC co-related well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence successfully applied for determining the assay and in vitro dissolution of a marketed formulation.

Key words: HPLC, UV Spectrophotometry, Lacidipine, Pharmaceutical formulation, Method validation, Quantitative analysis.

INTRODUCTION

Lacidipine (LAC) is a calcium channel blocker, anti hypertension and anti anginal drug. Chemically LAC 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 (British National Formulary, 2009). From a physico-chemical point of view, LAC 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. LAC absorbs light in the wavelength at 240nm (Martindale, 1996). LAC 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. LAC or its metabolite inhibits the angiotensin converting enzyme, other hormone receptors, or ion channels (Tripathi, 2008). Literature survey reveals that several analytical methods have been reported for the estimation of LAC by LC-PDA, (Baranda et al., 2005) High Performance Thin Layer Chromatography, (Kharat et al., 2002) LC-MS, [Garzotti, 2003] and UV (Filippis et al., 2002) method. Only one HPLC (Ramesh et al., 2009) method was developed and applied in the determination of LAC in biological fluids. The aim of this study was to develop a RP-HPLC and UV method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition. 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 (Rashmin, 2008). To assess the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines. (ICH, 2005; ICH, 1995)

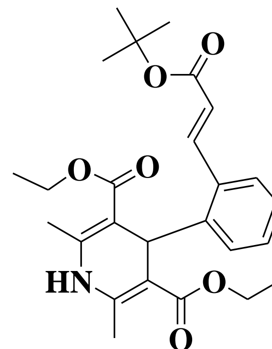

Figure1: Chemical structure of lacidipine

EXPERIMENTAL

Reagents and chemicals

An analytically pure sample of Lacidipine was procured as gift sample from Cadila Health Care. (Ahmedabad, India). HPLC grade methanol and water was procured from Sigma Aldrich and Millipore (India) Ltd., Bangalore. Tablet formulations LACIDIP were procured from a local pharmacy with labeled amount of 4 mg per tablet of Incepta Pharmaceuticals Limited.

Instrument

In UV-spectrophotometric method, Labindia model- 3000 + series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

UV spectrophotometric method

Determination of wavelength of maximum absorbance (λ_{max}) of Lacidipine

Wavelength of maximum absorption was determined by scanning 15µg/ml solution of LAC using UV spectrophotometer from 200 to 400 nm. This showed maximum absorbance at 240.0 nm (Figure 2).

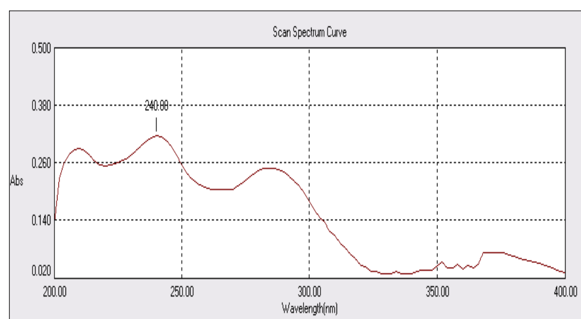

Figure 2 Selection of λ_{max} of lacidipine

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving 100 mg of drug in 50 ml of methanol and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with methanol to get a concentration of 1000 µg/ml (Stock-A) for drug.

Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of LAC and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with methanol that gave concentration of 100 µg/ml (Stock-B).

Preparation of working standard solution

0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with methanol. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for LAC.

Preparation of the calibration curves of the drug

The calibration curve was prepared by scanning test samples ranging from 5-25µg/ ml at 240 nm for LAC. The calibration curve was tested by validating it with inter-day and intra-day measurements. Mean of n =5 determinations was plotted as the standard curve (Figure3).

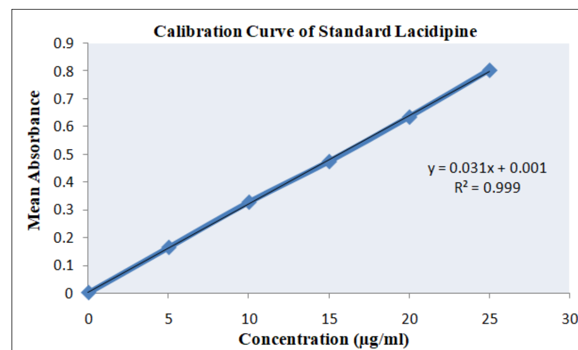

Figure 3 Calibration curve of standard lacidipine

RP-HPLC method

Chromatographic condition

The isocratic mobile phase consisted of methanol: water in the ratio of (80:20 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 240 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

10mg of LAC was weighed accurately and transferred to separate 10 ml volumetric flask, and the volume was adjusted to the mark with mobile phase to give a stock solution of 1000 μg/ml.

Working standard solution

From stock solutions of LAC 1 ml was taken and diluted up to 10 ml with mobile phase. From this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25 μg/ ml concentration.

Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 5-25 μg/ml for LAC solutions manually in triplicate to the HPLC system at detection wavelength of 240 nm. Mean of n =5 determinations was plotted as the standard curve (Figure 4). The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both inter day and intra-day measurements.

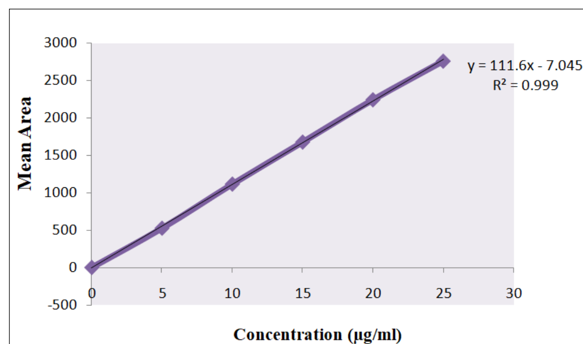

Figure 4 calibration graph of lacidipine

System Suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 μg/ml of LAC were injected separately and chromatogram was recorded. The result of system suitability parameter is reported in Table1.

Table 1 Results of system suitability parameters

Parameters	Haloperidol
AUC	1116.158
No. of Theoretical Plates	2564.667
Tailing Factor	1.420
Retention time	3.778

Validation Parameters

Linearity

Linearity was studied by analyzing five standard solutions (n=5) in the range of 5-25 μg /m1 of LAC in both UV spectrophotometric and HPLC method. Calibration curves with concentration verses absorbance or peak area was plotted for each method and the obtained data were subjected to regression analysis using the least squares method. Linearity of LAC was established by response ratios of drug. Response ratio of drug was calculated by dividing the absorbance or peak area with respective concentration (Table 2).

Table 2 Response ratios of lacidipine

Concentration (µg/ml)	HPLC Method		UV Method	
Haloperidol	AUC	RR	ABS	RR
5	526.043	105.21	0.163	0.033
10	1116.158	111.62	0.327	0.033
15	1678.723	111.92	0.473	0.032
20	2245.401	112.28	0.634	0.032
25	2762.283	110.50	0.803	0.033

Accuracy

The validity and reliability of the proposed methods was assessed by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies were carried out by adding known amount of standard solution of LAC to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods. In UV Spectrophotometric method, the value of mean recoveries was found to be in the range of 97.88% to 99.73% for LAC. The value of SD and %RSD less than 2 indicate the accuracy of the method. In RP-HPLC method, the value of mean recoveries was found in the range of 100.00% to 100.66 % for LAC. Total amount of drug found and percentage recovery was calculated. Results of recovery studies are reported in Table 3.

Table 3 Results of recovery study

Recovery Level%	% Mean±SD*	
	U.V Method	**RP-HPLC Method**
80%	99.58±0.877	100.66
100%	99.73±0.702	100.66
120%	97.88±0.834	100.00

* Value of three replicate and three concentrations

Precision

Precision was determined by repeatability and intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD is less than 2 indicate the precision of method. Result of precision shown in Table 4.

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase, flow rate and temperature were made to check the method's capacity to remain unaffected. Results of robustness are reported in Table 5.

LOD and LOQ

LOD and LOQ of described method were observed as 0.25µg/ml and 0.68µg/ml for LAC in UV spectrophotometric method and 0.570 µg/ml and 0.500 µg/ml for LAC in RP-HPLC method, based on the SD of response and slope, which meet the requirement of new method.

Analysis of marketed formulation

20 tablets (LACIDIP 4 mg) were weighed and ground to a fine powder. An equivalent amount

to 4 mg of LAC was taken in 10 ml volumetric flask. This was dissolve in 5 ml of diluents by sonication for about 10 minutes. The volume was made up to the mark by diluents as per the UV spectrophotometry method and RP-HPLC method. The solutions were filtered (whatman filter paper no.41). The filtrate was used to prepare samples of different concentration. The statistical evaluation of tablet analysis by both methods is reported in Table 6.

Table 4 Results of precision

Parameters	UV Method		RP-HPLC Method	
Precision (Mean± SD)*	LAC	%RSD	LAC	%RSD
Repeatability	99.50±0.145	0.235	98.75±0.645	0.698
Intra-day Precision	99.57±0.109	0.109	99.13±0.546	0.551
Inter-day Precision	95.56±0.3139	0.328	97.50±0.500	0.512
Analyst to Analyst (1)	98.75±0.125	0.223	99.60±0.110	0.158
Analyst to Analyst (2)	99.50±0.132	0.254	99.80±0.225	0.159

*Average of 5 replicate and 5 concentrations.

Table 5 Result of robustness of formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5 ºC	+ 5 ºC
Lacidipine	0.34	0.67	0.58
Flow rate		(-10%)	(+10%)
Lacidipine	0.49	0.79	0.99
Mobile phase ratio		- 2 %	+ 2 %
Lacidipine	0.34	0.85	0.90

Table 6 Results and statistical parameters for tablet analysis

S. No	Drug	Label claim	Amount found	% Label claimed	SD*	%RSD*
UV Method	LAC	4	3.98	99.50	0.145	0.235
RP-HPLC	LAC	4	3.99	99.80	0.225	0.159

*Average of five determination

Result and discussion

RP-HPLC and UV-Spectrophotometric methods were developed for LAC which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The chromatographic conditions were optimized in order to provide a good performance of the assay. The retention times (Rt) of LAC was 3.778±0.3min. The chromatograms have been shown in Figure 5. A five-point calibration curve was constructed with working standards and was found linear (r2 = 0.999) for each of the analytes over their calibration ranges. The slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed HPLC method was accurate, precise, reproducible and very sensitive.

For UV Method: Y = 0.03 x +0.001 (r2= 0.999)

For RP-HPLC: Y = 111.6 x -7.045 (r2= 0.999)

All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines. Table 3 lists the percent recovery (content uniformity) of LAC in the commercial formulations by HPLC and UV methods. The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 4). Moreover, the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest. Hence, the methods were suitably employed for assaying LAC in commercial marketed formulation (Table 6).

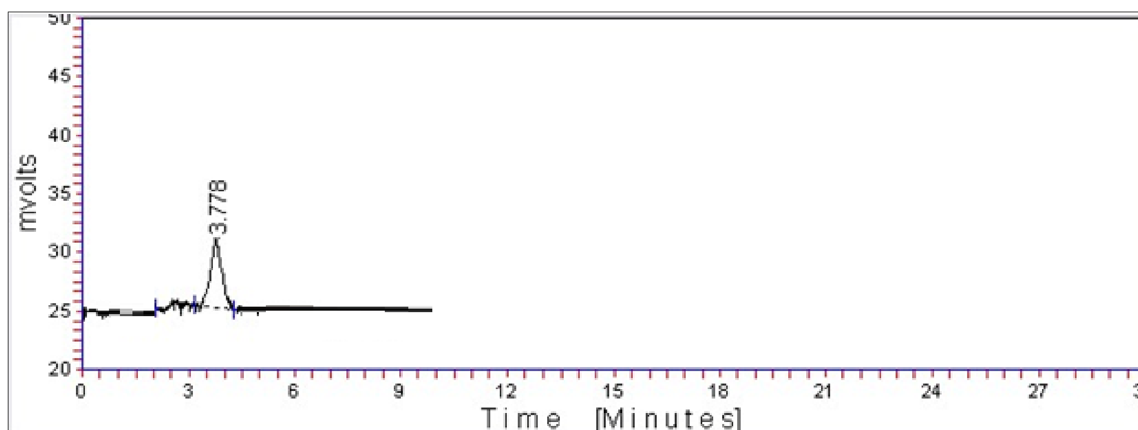

Figure 5 Chromatogram of lacidipine

Conclusion

The HPLC method and the UV spectrophotometric method for the determination of LAC in pharmaceutical formulations were found to be simple, rapid, precise, accurate and sensitive. Moreover, the UV method offers a cost effective and time saving alternative to HPLC method of analysis for LAC from formulations.

The HPLC method enables faster quantification of LAC within run time of eight minutes without interference of excipients. In summary, the proposed methods can be used for routine quality control of pharmaceutical formulation containing LAC.

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