Development and Validation of Lercanidipine Hydrochloride and Atenolol by Using RP-HPLC and UV Spectroscopy

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Abstract: *Lercanidipine hydrochloride (LCD), a dihydropyridine calcium channel blocker is used in the treatment of hypertension. Atenolol (ATN), a cardio selective β-blocker is used as antihypertensive, antianginal and antiarrhythmic. The proposed method was development and validation of UV-Spectrophotometric and RP-HPLC for determination in bulk and tablet dosage form. In UV Spectroscopic method, LCD showed λ_{max} at 237 nm in 50% v/v acetonitrile. The RP-HPLC analysis was performed by using a mobile phase of methanol: water (95:5 v/v), at a flow rate of 1.0 ml/min. By comparing validation results of both the methods, UV-spectrophotometric method is found to be simple, economical and rapid and RP-HPLC method were found to more precise, accurate, rugged and robust.*

Key words: *Atenolol, Lercanidipine hydrochloride, UV-spectrophotometr, RP-HPLC*

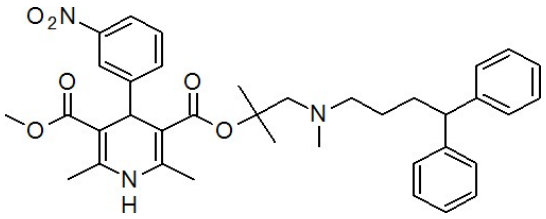
Introduction

LCD, a dihydropyridine calcium channel blocker used in the treatment of hypertension. Lercanidipine hydrochloride is available in the tablet dosage form. ATN is a cardio selective β-blocker used in the management of hypertension, angina pectoris, cardiac arrhythmia, antianginal and myocardial infarction [1,2]. Combination of LCD and ATN is used in the management of hypertension. Very few methods are reported in literature for determination of LCD as well as atenolol in pharmaceutical method is reported formulations. Therefore, it was thought meaningful to develop simple, precise, rapid spectrophotometric, RP- HPLC methods for analysis of lercanidipine hydrochloride in bulk and tablet dosage form [3, 4].

Lercanidipine Hydrochloride

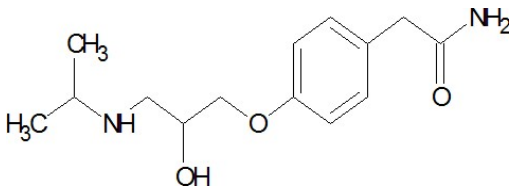
(2-[(3, 3-diphenylpropyl) methylamino]-1, 1-dimethylethyl methyl 1, 4 dihydro-2, 6 dimethyl-4-(m-nitrophenyl)-3, 5 pyridinedicarboxylate hydrochloride)

ATN a cardio selective β-blocker is used as antihypertensive, antianginal and antiarrhythmic. ATN is official in IP, BP and USP. The combination of both these drugs is recently launched in market [7,8]. Several spectrophotometric, chromatographic methods are also reported for determination of ATN alone and in combination with other drugs from pharmaceutical formulations and biological fluids [9].



Atenolol

(4-(2- hydroxy-3- isopropylaminopropoxy)-phenylacetamide)



Methods

The following methods have been developed for determination of LCD alone and in combination with ATN;

Method I: Development of UV-Spectrophotometric Method for Determination of Lercanidipine Hydrochloride in Bulk and Tablets

In 50% *v/v* acetonitrile, LCD showed absorbance maxima at 237 nm. Linearity was observed in the concentration range 4 - 24 µg/ml. The proposed method was applied for pharmaceutical formulation and % label claim was found to be 100.02%. The amount of drug estimated from the pharmaceutical formulation was found to be in good agreement with the label claim. The method was validated for accuracy, precision and ruggedness. Accuracy of the proposed method was assessed by recovery experiments, carried out at three different levels i.e. at 80%, 100%, and 120%. The mean percentage recovery was found to be in range 99.55 - 100.05% Precision of the method was assessed by repeatability, intra-day and inter-day variations. As % R.S.D. values were found to be less than 2, indicate method is accurate and precise. The results did not show any statistical difference between operators suggesting that method developed was rugged. Validation parameters are reported in Table 1.

Method II: Development and Validation of RP-HPLC Method for Determination of Lercanidipine Hydrochloride in Bulk and Tablets.

RP-HPLC method has been developed for estimation of LCD in bulk and tablet formulation. The HPLC analysis was performed on the Phenomenex Gemini C_{18} (250 mm × 4.60 mm), 5µm particle size in isocratic mode, at 25 ^{0}C temperature using a mobile phase consisting of methanol: water (95:5 *v/v*), at a flow rate of 1.0 ml/min. The detection was carried out at 235 nm. The average retention time for LCD was found to be 5.38 min. Linearity was observed in the concentration range 4 - 28 µg/ml (r^2 = 0.9992). The LOD and LOQ for LCD were found to be 0.24 µg/ml and 0.71 µg/ml, respectively.

The method has been successively applied for the determination of LCD in tablets. There was no interference from the excipients commonly present in tablets. The drug content was found to be 99.92 %. Accuracy of the method was checked by recovery experiments at three different levels 80 %, 100 % and 120 %. The mean percentage recovery was found to be within the limits of acceptance criteria with average recovery in the range 99.07 – 100.12%. The % R.S.D. below 2.0 proved accuracy of proposed method.

The results did not show any statistical difference between operators, suggesting that methods developed was rugged. According to USP (621), system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution; results are reported in Table 2; and chromatogram in

Fig No. 1observations were found to be within limit.

Result and Discussion

UV-spectrophotometric and RP-HPLC methods have been developed for determination of LCD in bulk and tablet dosage form. By comparing this method, it can be concluded that UV-spectrophotometric method is simple, economical and rapid as method. But, RP-HPLC method was found to more precise, accurate, rugged and robust. All these developed methods can be used for routine analysis of LCD in pharmaceutical formulation.

Table 1: Validation Parameters

Parameters	**Results**
Linearity and range (µg/ml)	4 – 24
Regression equation (Y = mX +C)	Y= 0.03995X + 0.00415
% Recovery (n = 9)	99.87 ± 0.28
Precision (% R.S.D.)	
Intra-day (n = 3) Inter-day (n = 3) Repeatability (n = 6)	0.31 - 1.45 0.41 - 1.35 0.34
Ruggedness (% R.S.D.)	
Analyst –I (n = 3) Analyst –II (n = 3)	0.52 0.23

Table 2: System Suitability Test Parameters

System Suitability Parameters	**Observation**
Retention Time (t_R)	5.38 min
Capacity Factor (K^1)	
Theoretical Plate (N)	4522
Tailing Factor (As)	1.36

Table 3: Validation Parameters

Parameters	**Observation**
Linearity range (µg/ml)	4 – 28
Regression equation	Y = 48085 X + 31022
LOD (µg)	0.24
LOQ (µg)	0.71
% Recovery (n = 9)	99.52 ± 0.54
Precision (%R.S.D.)	
Intra- day (n = 3)	0.49 – 0.76
Inter-day (n = 3)	0.63 – 1.05
Repeatability (n = 5)	0.84
Ruggedness (%R.S.D.)	
Analyst I (n = 6)	0.54
Analyst II (n = 6)	0.69
Robustness	Robust
Specificity	Specific

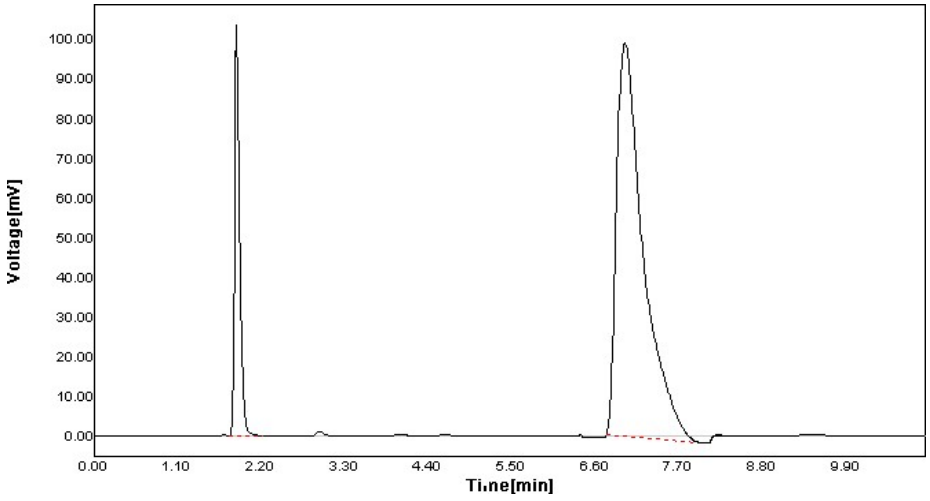


Fig. 1: Chromatogram of Lercanidipine Hydrochloride and atenolol

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