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# SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ATENOLOL AND LERCANIDIPINE HYDROCHLORIDE IN COMBINED DOSAGE FORM

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**ABSTRACT**

Two simple, rapid, accurate and economic spectrophotometric methods have been developed for the determination of Atenolol (ATE) & Lercanidipine Hydrochloride (LER) in combined dosage form. The first method is Area under curve (AUC) and second is Absorption Corrected method. The wavelength ranges 260-264 & 280-285 were selected to determine Atenolol (ATE) & Lercanidipine (LER), respectively in combined formulation by AUC method. The second method was absorption corrected method in which both the drugs exhibited strong absorbance at 270.33 nm. However, LER exhibited strong absorbance at 353.93, at which ATE showed zero absorbance. Hence, 353.93 nm was selected as one wavelength for determination of LER without interference from ATE. Quantitative estimation of ATE was carried out by subtracting the absorption due to LER at 270.33 nm using experimentally calculated absorption factor. Beer's law was obeyed in the concentration range of 25-300 ì g/ml for ATE and 5-60 ì g/ml for LER for both the methods. The results of analysis have been validated statistically and recovery studies confirmed the accuracy and reproducibility of the proposed methods which were carried out by following ICH guidelines.

**Keywords:** *Lercanidipine Hydrochloride; Atenolol; Area under curve; Absorption corrected.*

## INTRODUCTION

Atenolol, (RS)-4-(2-Hydroxy-3-isopropylamino-propoxy)phenylacetamide is an analogue of acetamide[1]**.** Atenolol is a selective â1 receptor antagonist, a drug belonging to the group of â-blockers, a class of drugs used primarily in cardiovascular diseases. Lercanidipine Hydrochloride is 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-[(3,3-diphenyl-propyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride[2]. Lercanidipine is a calcium channel blocker of the dihydropyridine class & is used in the treatment of hypertension.

Literature survey reveals that various methods have been reported for estimation of ATE such as UV spectrophotometry[3-5], reverse phase HPLC, UPLC, HPTLC[6-8] individually and in combination dosage form with other drugs. For LER various analytical methods have been reported for its individual estimation and in combined dosage form which includes HPLC, electrophoresis, LC-MS, extractive spectrophotometry, visible spectrophotometry, HPLC with electrochemical detection.[9-15] Two methods have been reported for simultaneous analysis of ATE and LER in its combination which include TLC- Densitometry and second order derivative spectrophotometry[16-17]. Here an attempt has been made to develop simple, rapid and accurate area under curve and absorption corrected spectroscopic methods[18-19] for simultaneous estimation of ATE and LER from its formulation. The proposed methods are optimized and validated as per International Conference on Harmonization (ICH) guidelines.

## Materials and Methods

### Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells was used for spectrophotometric method. All weighing were done on electronic balance (Model Shimadzu AUW-220D). Spectroscopic grade methanol was used throughout the study. Ultrasonicator (Model 5.5 150H) was used for sample solution preparation.

### Reagents and chemicals

Pure drug sample of ATE and LER were kindly supplied as gift sample by Zest Pharma, Indore and Glenmark Pharmaceuticals, Sinnar, Nasik, respectively. These samples were used without further purification. Tablet formulation manufactured by Sun Pharmaceutical Industries (Lotensyl, Batch No. AD 92286) was purchased from local market containing ATE (50 mg) and LER (10 mg) per tablet. Spectroscopy grade methanol purchased from Merck, Mumbai was used throughout the study.

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**Preparation of Standard Stock Solution**

Standard stock solutions of pure drugs containing 1000 ìg/ml of ATE and LER were prepared by dissolving separately, 50 mg of each drug in 50 ml methanol.

**Preparation of Sample Stock Solution**

For formulation analysis, twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of ATE + 10 mg of LER was weighed and dissolved in 40 ml of methanol with the aid of ultrasonicator for 10 min and solution was filtered through Whatman paper No. 41 into a 50 ml volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask, volume was made up to mark. The solution was suitably diluted with methanol to get of 150 ìg/ml of ATE + 30 ìg/ml of LER and proposed methods were followed for analysis.

**PROCEDURE**

**Method A: Absorption Corrected Method**

Standard solutions of ATE and LER were scanned in spectrum mode from 200 nm to 400 nm at 0.2 band width and 200 nm/min scan rate. The overlain spectrum (Fig.1) of the two drugs indicated that both the drugs exhibited strong absorbance at about 270.33 nm. However LER exhibits strong absorbance at 353.93 nm at which ATE shows zero absorbance. Hence 353.93 nm was selected for the determination of LER without interference of ATE. The linearity range of ATE is 25-300 ìg /ml at 353.93 nm with a correlation coefficient of 0.999. LER exhibits linearity over a concentration range of 5-60 ìg/ml both at 270.33 nm and at 353.93 nm. The correlation coefficient for LER was found to be 0.999 at both the wavelengths. Absorbance of both the drugs recorded were found to be practically additive at 270.33 nm

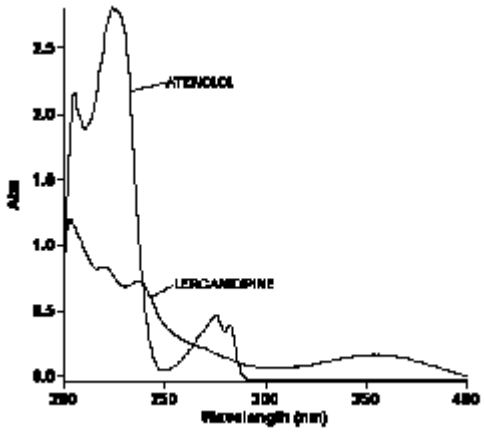


***Fig. 1:*** *Overlay spectrum of ATE and LER in methanol, ATE (100µg/ml) and LER (20µg ml$^{-1}$)*

**Determination of Absorption Factor at Selected Wavelengths**

ATE and LER solution in methanol of known concentrations were scanned against blank on spectrophotometer. The value of absorption factor was found to be 2.19. Quantitative estimation of ATE and LER was carried out using following equation:

Corrected Absorbance of ATE at 270.33 nm = $Abs_{270.33}$ (ATE + LER) – [($abs_{270.33}$ (ATE)/ $abs_{353..93}$ (LER)] × $abs_{353.93}$ (LER) or

Corrected Absorbance of ATE at 270.33nm = $abs_{270..33}$ (ATE + LER) – 2.19 × $abs_{353.93}$LER)

Where abs= Absorption value at given wavelength.

**Method B: Area under curve**

For the simultaneous determination using the Area under curve (AUC) method, suitable dilutions of the standard stock solutions (1000 µg/ml) of ATE and LER were prepared separately in methanol. The solutions of drugs were scanned in the range of 200-400 nm. For Area under curve method, sampling wavelength ranges selected for estimation of ATE and LER were 260-264 nm (ë1-ë2) and 280-285 nm (ë3-ë4) and area were integrated between these selected wavelength ranges for both drugs, which showed linear response with increasing concentration hence the same wavelength range were used for estimation of tablet formulation. By using integrated areas two simultaneous equations (eq. 1 & 2) were constructed and solved to determine concentrations of analytes. Concentration of analytes in mixed standard and the sample solution were calculated using equation (3) and (4).

$A_1 = 6721\ C_{ATE} + 48975\ C_{LER}$..... (1) 260-264nm (1)

$A_2 = 16447\ C_{ATE} + 29479 C_{LER}$..... (2) 280-285 nm (2)

$C_{ATE} = A_2 \times a_{Y1} - A_1 \times a_{y2} / a_{X2} \times a_{Y1} - a_{X1} \times a_{Y2}$ ..... (3)

$C_{LER} = A_1 ax_2 - A_2 ax_1 / ax_2 ay_1 - ax_1 ay_2$..... (4)

Where,

$a_{X1}$ (6721) and $a_{X2}$ (16447) are absorptivities of ATE at (ë1-ë2) and (ë3-ë4) respectively. $a_{Y1}$ (48975) and $a_{y2}$ (29479) are absorptivities of LER at (ë1-ë2) and (ë3-ë4) respectively.

A1 and A2 are Absorbances of mixed standard at (ë1-ë2) and (ë3-ë4) respectively. $C_{ATE}$ and $C_{LER}$ is the conc. of ATE & LER, respectively in g/100 ml.

**Recovery Studies**

The accuracy of the proposed methods were checked by recovery study, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (50 %, 100 % and 150 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for

spiking of the drugs standard solution was 75 ìg/ml of ATE and 15 ìg/ml of LER for both the methods.

**Precision of the Method**

Reparability of the methods were studied by repeating the methods six times. To study intra-day precision, method was repeated 3 times in a day. Similarly the method was repeated on five different days to determine inter-day precision.

## RESULTS AND DISCUSSION

Practically no interference from tablet excipients was observed in these methods. As their ëmax differ more than 20 nm, absorption corrected method was tried for their simultaneous estimation in formulation. Quantitative estimation of ATE was carried out by subtracting interference of LER using experimentally calculated absorption factor. Both the methods are accurate, simple, rapid, precise, reliable, sensitive and economical.

These methods were validated as per ICH guidelines. The values of % RSD and correlation coefficient were satisfactory. The results of the formulation analysis are presented in Table 1. Result of the recovery study indicates that there is no interference due to excipients present in the formulation. The % Recovery (% RSD) of ATE by Method A and Method B was found to be in the range of 98.9-101(0.33-1.47) and 99.5-100.7(0.35-1.52), respectively and that of LER by Method A and Method B was found to be in the range of 99.2-100.6(1.1-1.56) and 98.4-100.2(1.21-1.6), respectively. Result of precision study summarized in Table 1 indicates that methods are precise.

***Table 1:*** *Optical characteristics of the proposed methods and results of formulation*

| Parameter | | ATENOLOL | | LERCANIDIPINE | |
|---|---|---|---|---|---|
| | | Method A | Method B | Method A | Method B |
| λ (nm) | | 270.33 | Area Integrated between 280-264 nm | 353.9487 | Area Integrated between 280-285 nm |
| Beer's law limit (µg/ml) | | 25-300 | 25-300 | 5-60 | 5-60 |
| Regression Equation(y) = mx + c | Slope (m) | 0.004206 | -- | 0.008859 | -- |
| | Intercept(c) | -0.004055 | -- | -0.023541 | -- |
| Correlation coefficient | | 0.99988 | -- | 0.99989 | -- |
| Precision (%R.S.D.) | Repeatability (n=5) | 0.56 | 0.66 | 0.76 | 0.72 |
| | Intra-day (3X3 times) | 0.57 | 0.76 | 0.55 | 0.56 |
| | Inter-day(3X5 days) | 0.59 | 0.87 | 0.65 | 0.83 |
| | Analyst | 0.54 | 0.72 | 0.70 | 0.75 |
| Formulation Analysis (% Assay, %RSD) n=6 | | 99.98, 0.84 | 99.91, 1.01 | 100.89, 1.1 | 101.12, 1.24 |

*RSD is relative standard deviation*

## CONCLUSION

The proposed methods are simple, precise, accurate and rapid for the determination of ATE and LER in combined tablet dosage forms. Analysis of authentic samples containing ATE and LER showed no interference from the common additives and excipients. Hence, recommended procedure is well suited for the assay and evaluation of drugs in pharmaceutical preparations. Thus these methods can be easily and conveniently adopted for routine quality control analysis.

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