SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF ATENOLOL AND LERCANIDIPINE HYDROCHLORIDE IN COMBINED DOSAGE FORM BY RATIO DERIVATIVE AND DUAL WAVELENGTH METHOD

Vishnu P. Choudhari*, Vishnu M. Suryawanshi, Rashmi H. Mahabal, Sayali G. Deshchougule, Kishor P. Bhalerao, Bhanudas S. Kuchekar
Pharmaceutical Analysis and Quality Assurance Department, MAEER's Maharashtra Institute of Pharmacy, MIT Campus, Paud Road, Kothrud, Pune, 411038, MS, India.
***Email:** viraj1404@rediffmail.com

ABSTRACT

A simple, economical, precise and accurate method for simultaneous determination of Atenolol (ATE) and Lercanidipine Hydrochloride (LER) in combined dosage form has been developed. The first method is based on Ratio Derivative (Method A) and second method is based on Dual Wavelength (Method B). The amplitudes 266.98 nm and 386.97 nm wavelength were selected to determine ATE and LER respectively, by method A. In method B, two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. At wavelengths 234.01 nm and 238.66 nm Lercanidipine have equal absorbance therefore these two wavelengths were used to determine ATE and on similar basis 253.33 nm and 286.07 nm were selected to determine LER in combined formulation. The drugs obey Beer's law in the concentration range of 25-125 µg mL^{-1} for ATE and 5-25 µg mL^{-1} for LER by the method A and 50-90 µg mL^{-1} for ATE and 10-18 µg mL^{-1} for LER by the method B. The % assay of LER and ATE was found to be in the range 99.98 – 101.12 % by the proposed methods. Recovery was found in the range of 98.4 - 101.0 % for both analytes by both 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: Atenolol (ATE); Dual Wavelength; Lercanidipine Hydrochloride (LER); Ratio Derivative

INTRODUCTION

Atenolol 1-3, (*RS*)-2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl} acetamide is an analogue of acetamide (Fig.1). Atenolol (Tenormin) is a selective β1 receptor antagonist, a drug belonging to the group of beta blockers (sometimes written β-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-diphenylpropyl)methylamino]-1,1-dimethylethyl methyl ester hydrochloride. 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[4-6], reverse phase HPLC, UPLC, HPTLC[7-9] individually and in combination dosage form with other drugs. For LER various analytical methods have been reported or its individual estimation and in combined dosage form which includes HPLC, electrophoresis, LC-MS, extractive Spectrophotometry, visible Spectrophotometry, HPLC with electrochemical detection.[10-16] Two methods have been reported for simultaneous analysis of ATE and LER in its combination which include TLC- Densitometry and second order derivative Spectrophotometry[17-18]. Here an attempt has been made to develop simple, rapid and accurate Dual Wavelength and Ratio Derivative spectroscopic methods[19-20] 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 a gift sample by Zest Pharma, Indore and Glenmark Pharmaceuticals, Sinner, 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.

Preparation of Standard Stock Solutions and calibration Curve

Standard stock solutions each containing 1000 µg mL^{-1} of ATE and LER were prepared separately in the methanol. The working standard solutions of these drugs were obtained by dilution of the respective stock solution in methanol. For verification of Beer's law a series of

dilutions in the concentration range of 25-125 µg mL^{-1} for ATE and 5-25 µg mL^{-1} for LER were prepared in mixture for method A. Solutions in the concentration rang of 50-90 µg mL^{-1} for ATE **(series A)** and 10-18 µg mL^{-1} for LER (**series B)** were prepared and mixture of both the drugs **(series C)** in same concentration range was prepared for method B.

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 and 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 and volume was made up to mark. The solution was suitably diluted with methanol to get of 75 µg/ml of ATE and 15 µg/ml of LER. The proposed methods were followed for analysis

Procedure

Method A: RATIO DERIVATIVE METHOD:

The method involves dividing the spectrum of mixture by the standardized spectra of each of the analyte and deriving the ratio to obtain spectrum that is dependent of concentration of analyte used as a divisor. Using appropriate dilutions of standard stock solution, the two solutions were scanned separately. The ratio spectra of different ATE standards at increasing concentrations were obtained by dividing each with the stored spectrum of the standard solution of LER (15 µg mL^{-1}) and the first derivative of these spectra traced, illustrated in Fig 1 . Wavelength 266.98 nm was selected for the quantification of ATE in ATE + LER mixture. The ratio and ratio derivative spectra of the solutions of LER at different concentrations were obtained by dividing each with the stored standard spectrum of the ATE (75 µg mL^{-1}) (Fig. 2). Wavelength 386.97 nm was selected for the quantification of LER in LER + ATE mixture. Measured analytical signals at these wavelengths were proportional to the concentrations of the drugs. Calibration curves were prepared from the measured signals at the selected wavelength and concentration of the standard solutions. The amount of ATE (C_{ATE}) and LER (C_{LER}) in tablets and capsules was calculated by using equations 1 and 2, respectively.

C_{ATE} = [Derivative amplitude at 266.98 – (-0.0079)] / (0.014404) ... (1)

C_{LER} = [Derivative amplitude at 386.97 – (0.35)] / (1.2016) ... (2)

Method B:. DUAL WAVELENGTH METHOD:

The spectrum of ATE shows identical absorbance at 253.33 nm (λ_3) and 286.07 nm (λ_4) therefore these two wavelengths were selected for the analysis of LER. All the solutions of **series A** were scanned to ensure that the difference between λ_3 and λ_4 is zero. Similarly, the LER solutions were scanned to determine the two wavelengths, where absorbance is same. These two wavelengths were found to be 234.01 nm (λ_1) and 238.66 nm (λ_2). All the solutions of **series B** were scanned to ensure that difference between (λ_1) and (λ_2) is zero. All the solutions of **series c** were scanned to verify the results.

Recovery studies

The accuracy of the proposed methods was 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 µg/ml of ATE and 15 µg/ml of LER for both the methods.

Precision of the Method

Reparability of the methods was 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.

Table 1: Optical characteristics of the proposed methods and results of formulation analysis & precision study

Parameter		Atenolol		Lercanidipine	
		Method A	Method B	Method A	Method B
λ (nm)		266.98	Difference in absorbance between 234.01 nm and238.66nm	386.97	Difference in absorbance between 253.33 nm and286.07nm
Beer's law limit (µg mL^{-1})		25-125	50-90	5-25	10-18
Regression Equation (y = mx + c)	Slope (m)	0.01444	0.00951	1.2016	0.01147
	Intercept (c)	-0.0079	0.0232	0.35	0.0066
Correlation coefficient		0.99985	0.99983	0.99971	0.99979
Precision (%R.S.D.)	Repeatability (n=5)	0.57	0.68	0.77	0.73
	Intra-day	0.58	0.75	0.56	0.94
	Inter-day	0.59	0.87	0.65	0.83
	Analyst	0.55	0.73	0.71	0.76
Formulation Analysis (% Assay, %RSD) n=6		99.98, 0.84	99.91, 1.01	100.89,1.1	101.12,1.24

Table 2: Result of Recovery studies

Formulation	Recovery Level (%)	Amount spiked (µg mL^{-1})		Recovery (%), % RSD n=3			
				Method A		Method B	
		ATE	LER	ATE	LER	ATE	LER
Tablet	50	37.5	7.5	98.9, 0.56	100.6, 1.10	100.2, 0.68	98.4, 1.21
	100	75	15	101.0, 1.47	99.7, 1.56	100.7, 1.52	99.30, 1.60
	150	112.5	22.5	101.0, 0.33	99.2, 1.23	99.50, 0.35	100.2, 1.24

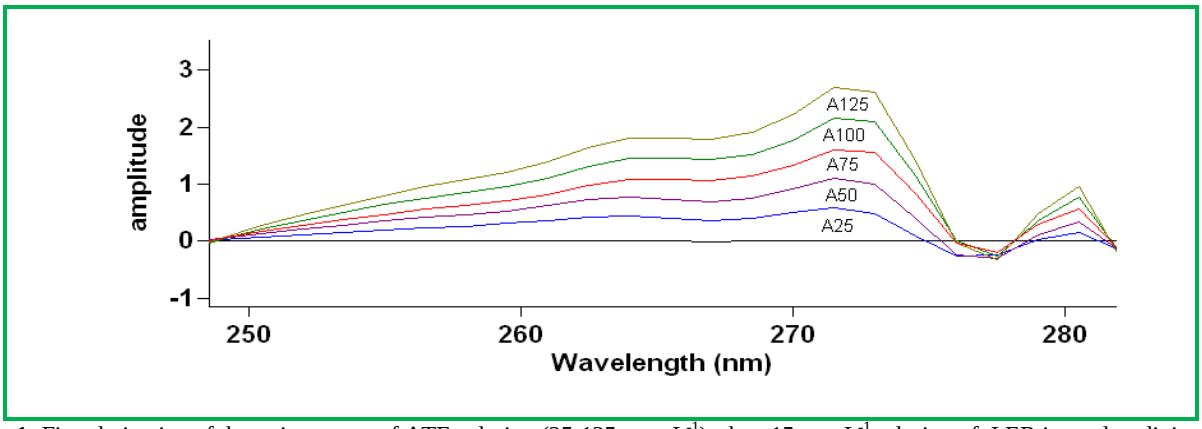

Figure 1: First derivative of the ratio spectra of ATE solution (25-125 µg mL^{-1}) when 15µg mL^{-1} solution of LER is used as divisor.

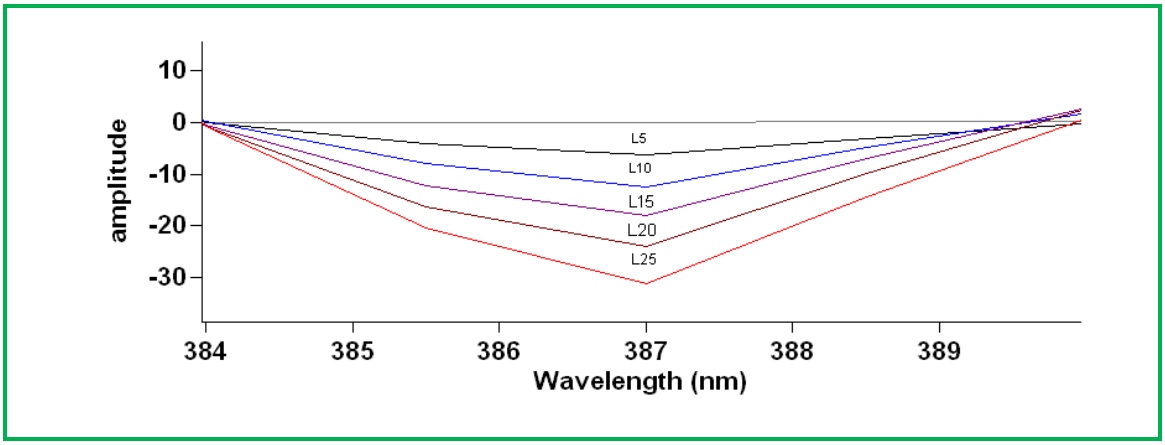

Figure 2: First derivative of the ratio spectra of LER solution (5 – 25 µg mL^{-1}) when 75µg mL^{-1} solution of ATE is used as divisor.

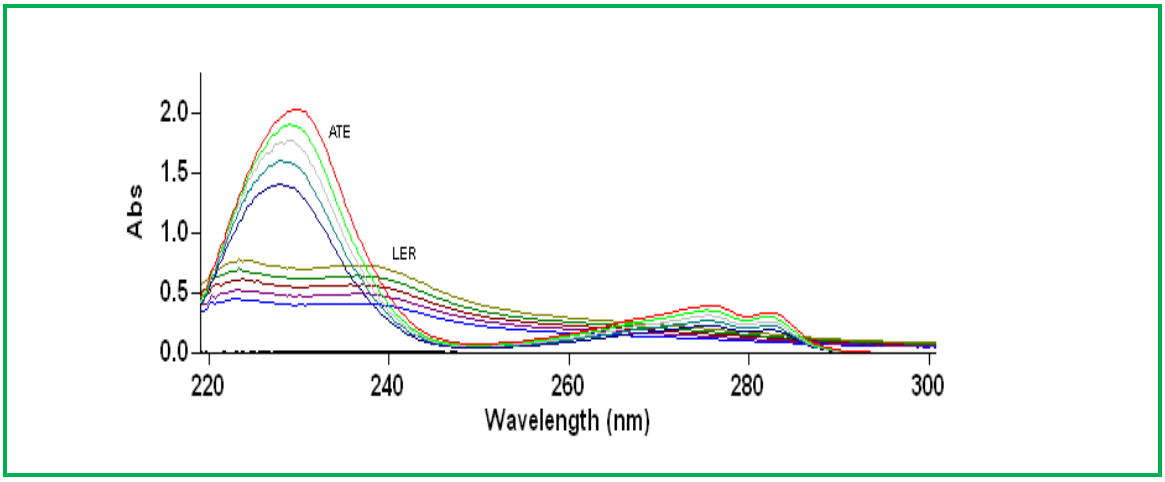

Figure 3: Overlain spectra of Atenolol and Lercanidipine in methanol

RESULTS AND DISCUSSION

ATE and LER the proposed methods for simultaneous estimation of in combined dosage form were found to be accurate, simple and rapid. The developed methods can be used for routine analysis of two drugs in combined dosage forms. Practically no interference from tablet excipients was observed in these methods. Both the methods are accurate, simple, rapid, precise, reliable, sensitive, reproducible and economical.

These methods were validated as per ICH guidelines. The values of % RSD and correlation of coefficient were satisfactory and result of the recovery study indicates that there is no interference due to excipients present in the formulation. Result of formulation analysis and precision study are summarized in table 1 indicated that method is precise. % Recovery (%RSD) for 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) (Table 2).

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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