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

Mahabal Rashmi H, Deshchougule Sayali G, Suryawanshi Vishnu M, Chabukswar Anuruddha R, Choudhari Vishnu P*, Kuchekar Bhanudas S
Pharmaceutical Analysis and Quality Assurance Department, MAEER’s Maharashtra Institute of Pharmacy, MIT Campus, Paud Road, Kothrud, Pune, 411038, MS, India.
Received on : 05.05.2010 Revised : 14.09.10 Accepted : 17.09.10

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 ìg/ml for ATE and 5-60 ìg/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 \(\alpha\)1 receptor antagonist, a drug belonging to the group of \(\beta\)-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\(^6\) 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.\(^6\)-\(^15\) 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). Spectroscopy grade methanol purchased from Merck, Mumbai 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.

*Correspondence : viraj1404@rediffmail.com
Preparation of Standard Stock Solution

Standard stock solutions of pure drugs containing 1000 µg/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 µg/ml of ATE + 30 µg/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 µg/ml at 353.93 nm with a correlation coefficient of 0.999. LER exhibits linearity over a concentration range of 5-60 µg/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.

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 = \( \frac{\text{Abs}_{270.33} \text{(ATE + LER)} - \left( \frac{\text{abs}_{270.33} \text{(ATE)}}{\text{abs}_{353.93} \text{(LER)}} \right) \times \text{abs}_{353.93} \text{(LER)}}{1 - 2.19 \times \text{abs}_{353.93} \text{(LER)}} \)

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 (\( \bar{\bar{\lambda}} - \bar{\bar{\lambda}_2} \)) and 280-285 nm (\( \bar{\bar{\lambda}_3} - \bar{\bar{\lambda}_4} \)) 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).

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

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

\[
C_{ATE} = A_2 x a_{y1} - A_1 x a_{y2} / a_{X2} x a_{Y1} - a_{X1} x a_{Y2} \ .......... (3)
\]

\[
C_{LER} = A_1 a_{X2} - A_2 a_{X1} / a_{X2} a_{Y1} - a_{X1} a_{Y2} \ .......... (4)
\]

Where, 
\( a_{X1} \) (6721) and \( a_{X2} \) (16447) are absorptivities of ATE at (\( \bar{\bar{\lambda}_1} - \bar{\bar{\lambda}_2} \)) and (\( \bar{\bar{\lambda}_3} - \bar{\bar{\lambda}_4} \)) respectively. \( a_{Y1} \) (48975) and \( a_{Y2} \) (29479) are absorptivities of LER at (\( \bar{\bar{\lambda}_1} - \bar{\bar{\lambda}_2} \)) and (\( \bar{\bar{\lambda}_3} - \bar{\bar{\lambda}_4} \)) respectively.

A1 and A2 are Absorbances of mixed standard at (\( \bar{\bar{\lambda}_1} - \bar{\bar{\lambda}_2} \)) and (\( \bar{\bar{\lambda}_3} - \bar{\bar{\lambda}_4} \)) 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
Spectrophotometric method - Atenolol & Lercanidipine
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
Repeatability 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 εmax 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atenolol</th>
<th>Lercanidipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (nm)</td>
<td>200.3</td>
<td>200.4</td>
</tr>
<tr>
<td>εmax (µg/ml)</td>
<td>2500</td>
<td>2500</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>99.9-101</td>
<td>99.5-100.7</td>
</tr>
<tr>
<td>Correlation Coef.</td>
<td>0.9968</td>
<td>0.9968</td>
</tr>
<tr>
<td>Precision (%)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>εmax (µg/ml)</td>
<td>99.5-101</td>
<td>99.5-100.7</td>
</tr>
</tbody>
</table>

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.

ACKNOWLEDGEMENT
The authors are thankful to Zest Pharma, Indore;
Glenmark Pharmaceuticals, Sinnar, Nasik for providing
gift samples. The authors are thankful to Management
of MAEER’s Maharashtra Institute of Pharmacy, Pune
for providing necessary facility for the work.

REFERENCES
1. Indian Pharmacopoeia, Ghaziabad: The Indian
Pharmacopoeia Commission 2007, p747-748.
2. The Merck Index 14th edn. Whitehouse Station.
spectrophotometric estimation of atenolol and
amlodipine besylate by multi-wavelength method.
The Indian Pharmacist. 2007; 6(5): 5962.
4. Kasture A V, Ramteke Madhuri. Simultaneous UV-
Spectrophotometric methods for the estimation
of atenolol and nifedipine in solid dosage forms.
67(6): 752.
5. Kasture A V, Ramteke Madhuri. Simultaneous UV-
spectrophotometric method for the estimation
of atenolol and amlodipine besylate in combined
dosage form. Indian Journal of Pharmaceutical
Sciences. 2006; 68(3):394.
N, Suresh B, Sankar SR. Simultaneous
estimation of atenolol and amlodipine in
formulations by reversed Phase-HPLC. Indian
59(4):171.
7. Durga Rao D, Satyanarayana N V, Sait
S S, Ramakoti Reddy Y and Mukkanti K.
Simultaneous determination of Losartan
potassium, Atenolol and hydrochlorothiazide in
pharmaceutical preparation by stability indicating-
UPLC. Chromatographia, 2009; 70:647.
8. Argekar A P, Sawant J G. Simultaneous
determination of Atenolol and Nitrendipine in
pharmaceutical dosage form by HPTLC. Journal
of Liquid Chromatography and Related
9. Charde S, Kumar L, Saha R. Development and
validation of high performance liquid
chromatographic method for estimation of
Lercanidipine in Rabbit serum, Analytical letters.


