SPECTROPHOTOMETRIC QUANTITATIVE ESTIMATION OF ATENOLOL AND LOSARTAN POTASSIUM IN BULK DRUGS AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, economical and reproducible UV Spectrophotometric method has been developed for the simultaneous estimation of Atenolol and Losartan Potassium in bulk and in marketed combined tablet dosage form. The stock solutions were prepared in pH 1.2 buffer followed by further required dilutions with pH 1.2 buffer. Vierordt’s simultaneous equation method was developed and is validated statistically as per ICH guidelines. The absorbance maxima of Atenolol and Losartan Potassium were found to be 223.6nm & 205nm respectively. Linearity was observed by linear regression equation method for both drugs in different concentration range. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The % R.S.D. were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods. Statistical analysis proves that the proposed method can be effectively applied for the simultaneous estimation of these two drugs in bulk & combined dosage forms.

Keywords: Atenolol, Losartan Potassium, Vierordt’s simultaneous equation method, etc.

INTRODUCTION

Atenolol chemically, 2-[4-{(2RS)-2-hydroxy-3-[(1-methylethyl)-amino]-propoxy]-phenyl] acetamide (fig. 1) is a selective β1 receptor antagonist, a drug belonging to the group of beta blockers, a class of drugs used primarily in cardiovascular diseases such as hypertension. Atenolol competitively blocks beta-adrenergic receptors in the heart and juxtaglomerular
apparatus. They lead to decreased heart rate decreasing the work load by the heart. They do not produce coronary vasodilatation but lead to a shift and redistribution of coronary circulation to the ischemic areas. It decreases the release of renin from the kidney, thus lowering the blood pressure.\[1-3\] Losartan potassium (2-butyl-4-chloro-1-{(2′-1H-tetrazol-5-yl) biphenyl-4-yl} methyl)-1Himidazol-5-yl) methanol is used in the treatment of hypertension. It is a strong non-peptide antihypertensive agent, which exerts it action by specific blockade of angiotensin II receptors.\[3,6\]

In the literature survey, some methods are reported in combination for estimation of Atenolol and Losartan Potassium by HPLC\[7,8\], HPTLC\[9\] and some methods reported in combination for the estimation in pharmaceutical dosage form in methanol by UV Spectrophotometry.\[10\] Extensive literature survey reveals that no Spectrophotometric method is available for simultaneous determination of Atenolol and Losartan in combination in HCl buffer of pH 1.2. Aim of present work was to develop simple, precise, accurate and economical Spectrophotometric methods for simultaneous determination of binary drug formulation in biological fluids at three different pH. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines.\[11\]

**Figure 1 Structural formula of Atenolol. Figure 2 Structural Formula of Losartan Potassium**

**MATERIALS AND METHODS**

**Instruments**

Quantitative estimation was performed on Shimadzu UV 1700 double beam UV-Visible spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra was recorded on a medium scan speed, setting slit width to be 1 nm and sampling interval to be auto. pH meter (Labindia) was used.
Chemicals and Reagents
AT and LP obtained from USV Ltd, Baddi. A commercial sample AT and LP tablets were procured from local market and used within their shelf-life period. Potassium Chloride and Hydrochloric acid was procured from Loba Chemie Pvt. Ltd., Mumbai and S.D. Fine Chemical Limited, India respectively. To prepare HCl Buffer of pH 1.2 50ml of 0.2M KCl was taken in 200ml volumetric flask. 85ml of 0.2M HCl was added to the volumetric flask. This solution was diluted to 200ml with distilled water.

Vierordt’s simultaneous equation method\textsuperscript{[12-14]}
This method of analysis is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. The quantification analyses of AT (X) and LP (Y) in a binary mixture were performed with the following equations:

\[
C_x = \frac{(A_2a_{y1} - A_1a_{y2})}{(a_{x2}a_{y1} - a_{x1}a_{y2})} \quad \text{(Eqn. 1)}
\]

\[
C_y = \frac{(A_1a_{x2} - A_2a_{x1})}{(a_{x2}a_{y1} - a_{x1}a_{y2})} \quad \text{(Eqn. 2)}
\]

Where -

- \(C_x\) and \(C_y\) are the concentration of X and Y, respectively in the diluted sample.
- \(a_{x1}\) and \(a_{x2}\) are absorptivities of X at \(\lambda_1\) and \(\lambda_2\).
- \(a_{y1}\) and \(a_{y2}\) are absorptivities of Y at \(\lambda_1\) and \(\lambda_2\).

Preparation of Standard Stock solution
Standard stock solution of 100 µg/ml of AT and LP were prepared by dissolving accurately weighed quantity (10mg) of each drug in 100 ml HCl buffer of pH 1.2. The aliquots of standard stock solution of drugs were diluted separately with pH 1.2 buffer to obtain working standard solutions with final concentration of 10 µg/ml of each drug and each working standard solution was scanned between 200-380 nm in Shimadzu UV visible spectrophotometer. Overlain absorption spectrum of both drugs was recorded and is depicted in Figure 3. The spectra exhibit major absorbance maxima at 223.6 nm and 205 nm for AT and LP, respectively which revealed that the peaks are well satisfying the criteria for obtaining maximum precision based on AT and LP, respectively.

For the preparation of calibration curve of AT and LP at selected two wavelengths; the standard solution (100 µg/ml) was further diluted with HCl buffer of pH 1.2 to obtain different concentration range for AT and LP and the absorbance was measured at 223.6nm and 205nm for both drugs and calculated the absorptivity.
Analysis of Tablet Formulations
For analysis of commercial formulations of tablets, 20 tablets were weighed, powdered and accurately weighed and finely powdered. The amount of powder equivalent to 50 mg of AT and 50 mg of LP were weighed and transferred into 100 ml volumetric flask. Methanol (10 mL) was added to it and sonicated for 10 minutes and volume was made up to mark with pH 1.2 buffer. The solution was filtered through Whatman filter paper No. 41. From the above solution suitable aliquot was diluted with pH 1.2 buffer to get concentration 10 µg/mL of each drug. The absorbance of sample solutions were measured at both selected wavelengths.

Figure 03 Overlain Absorption Spectra of Atenolol and Losartan in pH 1.2 buffer.

Validation of proposed method\(^{(11)}\)
The method was validated according to ICH guidelines for validation of analytical procedure in order to determine sensitivity, linearity, precision, and accuracy.

• **Linearity:** The linearity of measurement was evaluated by analyzing different concentration of standard solution of both drugs at the \(\lambda_{\text{max}}\) of AT i.e. 223.6 nm and at \(\lambda_{\text{max}}\) of LP i.e. 205 nm. The response was plotted against concentration of the analyte. Linearity of the calibration curve was demonstrated by applying least square regression analysis to the plot obtained.

• **Accuracy:** To ascertain accuracy of the proposed method, different levels of drug concentrations (LQC, MQC and HQC) were prepared from independent stock solution and analyzed. Accuracy was assessed as the mean percentage Bias.

• **Precision:** Precision was studied to find out intra-day and inter-day variations in the proposed method. Different levels of prepared drug concentrations were run in triplicate in
the same day (intra-day variation) and for three consecutive days (inter-day variation). % Relative standard deviation (% RSD) were calculated which should be less than 2%.

- **Limit of detection (LOD) and Limit of quantitation (LOQ):** LOD was determined using the relation 3.3 σ/s where ‘σ’ is the standard deviation of the response and ‘s’ is the slope of the calibration curve. The standard deviation of the response can be obtained either by measuring the standard deviation of the blank response or by calculating the residual standard deviation of the regression line or by calculating the standard deviation of the y-intercept of the regression line, i.e. the standard error of the estimate. Similarly, LOQ was determined using the relation 10 σ/s.

**RESULT AND DISCUSSIONS**

Simultaneous equation method was proposed as a suitable method for the analysis of drugs AT and LP in dosage forms. A series of standard solutions were prepared for AT and LP and absorbance’s of solutions were recorded at 223.6nm and 205nm to plot a calibration curve of absorbance versus concentration. The calibration curves were found to be linear in concentration range under study (Table-2). Regression equation and Absorptivity values of AB and TEL were determined at selected wavelengths are presented in Table- I.

**Table 1 Result of Analytical method Development in 1.2 pH buffer**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atenolol at 233.6nm</th>
<th>Losartan at 223.6nm</th>
<th>Atenolol at 205nm</th>
<th>Losartan at 205nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.0341</td>
<td>0.0617</td>
<td>0.035</td>
<td>0.0955</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.001</td>
<td>0.0014</td>
<td>0.001</td>
<td>0.0018</td>
</tr>
<tr>
<td>Correlation coefficient(r²)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

The concentration of two drugs in mixture was calculated by using following equations.

\[ C_x = \frac{(A_1a_{x1} - A_2a_{y1})}{(a_{x1}a_{y2} - a_{x2}a_{y1})} \]  …… (Eqn. 1)

\[ C_y = \frac{(A_2a_{x1} - A_1a_{x2})}{(a_{x1}a_{y2} - a_{x2}a_{y1})} \]  …… (Eqn. 2)

Where –

\[ C_x \text{ and } C_y \text{ are the concentration of X and Y, respectively in the diluted sample.} \]

The absorbance of the diluted samples at \( \lambda_1 \) (Atenolol \( \lambda_{\text{max}} \)) are \( A_1 \) (\( A_1 = a_{x1}bc_x + a_{y1}bc_y \)).

The absorbance of the diluted samples at \( \lambda_2 \) (Losartan \( \lambda_{\text{max}} \)) are \( A_2 \) (\( A_2 = a_{x2}bc_x + a_{y2}bc_y \)).
\[ a_{x1} \text{ and } a_{y1} \text{ are the absorptivities of Atenolol and Losartan Potassium respectively at } \lambda_1 \text{ i.e. 223.6 nm. } a_{x2} \text{ and } a_{y2} \text{ are the absorptivities of Atenolol and Losartan Potassium respectively at } \lambda_2 \text{ i.e. 205 nm.}

To prove the validity and applicability of the proposed method, studies were carried out as per ICH Guidelines and their results are stated in Table 3. Satisfactory results were obtained with % Bias and %RSD value less than 2%; thus conforming the accuracy and precision of proposed method. LOD and LOQ values for AB and TEL are stated in Table 3.

### Table 2 Validation of proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atenolol at 233.6nm</th>
<th>Losartan at 223.6nm</th>
<th>Atenolol at 205nm</th>
<th>Losartan at 205nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s Law Limit (µg/ml)</td>
<td>6-35</td>
<td>3-18</td>
<td>5-30</td>
<td>2-12</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.0418</td>
<td>0.17</td>
<td>0.046</td>
<td>0.1</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.1263</td>
<td>0.51</td>
<td>0.138</td>
<td>0.33</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>0.117-0.246</td>
<td>0.094-0.172</td>
<td>0.094-0.172</td>
<td>0.07-0.12</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.177-0.527</td>
<td>0.246-0.41</td>
<td>0.246-0.41</td>
<td>0.17-0.37</td>
</tr>
<tr>
<td>Accuracy (% Bias)</td>
<td>0.032-0.84</td>
<td>0.029-0.037</td>
<td>0.03-0.08</td>
<td>0.01-0.03</td>
</tr>
</tbody>
</table>

The percentage of purity of AT and LP in tablet dosage form is shown in Table 2. The precision of the spectrophotometer system was determined using the %RSD of the absorbance for six replicate injections of the drug. The %RSD was less than 2 indicating precision of the method. Precision data were present in Table 3.

### Table 3 Determination of AB and TEL in combined tablet dosage form

<table>
<thead>
<tr>
<th>Brand</th>
<th>Tablet content</th>
<th>Label content (mg)</th>
<th>Amount found (mg)</th>
<th>%Amount*</th>
<th>± SD*</th>
<th>RSD (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>AT</td>
<td>50</td>
<td>49.69</td>
<td>99.38</td>
<td>± 0.065</td>
<td>0.155</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>50</td>
<td>49.81</td>
<td>99.62</td>
<td>± 0.048</td>
<td>0.174</td>
</tr>
<tr>
<td>II</td>
<td>AT</td>
<td>50</td>
<td>50.09</td>
<td>100.18</td>
<td>± 0.071</td>
<td>0.183</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>50</td>
<td>50.04</td>
<td>100.08</td>
<td>± 0.053</td>
<td>0.195</td>
</tr>
</tbody>
</table>

*Mean of six readings
CONCLUSION
A convenient and rapid UV method has been developed for simultaneous estimation of Atenolol and Losartan Potassium in available dosage form. The assay provides a linear response across a wide range of concentrations. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. Intra-day and inter-day % R.S.D. values were found to be less than 2 % as required by ICH guidelines, which indicates the validity of methods; hence, this method can be easily and conveniently adopted for routine analysis of Atenolol and Losartan Potassium in pure form and its dosage forms.

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REFERENCES


