SIMULTANEOUS ESTIMATION OF ATENOLOL AND AMLODIPINE BESYLATE IN TABLETS FORMULATIONS BY VIERODT’S METHOD USING U.V. SPECTROPHOTOMETRY

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ABSTRACT
A new UV- Spectrophotometric method has been developed for the simultaneous estimation of atenolol and amlodipine besylate in tablet dosage forms using 0.1N hydrochloric acid (pH 1.2). The method is based on simultaneous equation or Vierordt’s method. The \( \lambda_{\text{max}} \) values for atenolol and amlodipine besylate were found to be 224.6 nm and 239.6 nm respectively. The system obey Beer’s law in the range of 4-28 \( \mu \)g/ml and 4-32 \( \mu \)g/ml with correlation coefficient of 0.9991 and 0.9932 for atenolol and amlodipine besylate respectively. Intraday and Interday precision were found to be 0.08577-1.4682, 0.1080-1.71138, 0.2525-1.6080 and 0.2599-1.3906 respectively. The developed method can be successfully employed for the assay of atenolol and amlodipine besylate in different formulations.

KEY WORDS: Atenolol, Amlodipine besylate, UV-Spectrophotometry, Vierordt’s method

INTRODUCTION
Atenolol (ATE) chemically 2-[4-{(2RS)-2-hydroxy-3-{[(1-methylethyl)amino]propoxyyl} phenyl} acetamide is a \( \beta \)-adrenoreceptor blocking agent primarily used for hypertension, angina pectoris & myocardial infarction. It mainly acts by inhibition of rennin release and angiotension – II (AT-II) and aldosterone production1. Amlodipine besylate (AM) chemically 3-Ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate benzene sulphonate is a long acting calcium channel blocker used for hypertension and angina pectoris2. Amlodipine besylate block the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle and dilating arterioles thereby decreasing peripheral resistance. Hence improving blood pressure; in angina it improves blood flow to the myocardium1.

In, the present study 0.1N hydrochloric acid (pH 1.2) is used as solvent for simultaneous estimation of both the drugs by simultaneous equation or Vierordt’s method using UV- Spectrophotometry. The present method is relying on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC & HPTLC4,5.

METHOD AND MATERIAL
Instrument
Shimadzu-1700 Double beam UV-VIS Spectrophotometer with spectral band width of 1.8nm, wavelength accuracy of \( \pm 2 \) nm and matched quartz cells of 10mm optical path length was used for all spectral and absorbance measurements.

Materials
Atenolol and Amlodipine besylate were obtained as a gift samples from Alembic Pharma, Mumbai, India. All chemicals and buffers were of analytical grade.

Preparation of stock solution of Atenolol and Amlodipine besylate
A standard stock solution of ATE and AM were prepared by dissolving 100mg in 100ml 0.1N hydrochloric acid (pH 1.2) stock solution ‘1’ and further 1ml is pipette out and diluted to 100ml with 0.1N hydrochloric acid (pH 1.2) stock solution ‘2’. Different dilutions were prepared to get working concentrations of 4-28 \( \mu \)g/ml and 4-32 \( \mu \)g/ml for ATE and AM respectively. The aliquots of pure ATE and AM were transferred into a calibrated flasks and total volume was adjusted up to mark with 0.1N hydrochloric acid (pH 1.2).

The absorbance values of the resulting solution were then measured at 224.6 nm and 239.6 nm respectively and calibration curves were plotted between absorbance v/s concentrations.

Accuracy
As a part of determining accuracy of the proposed method, different levels of drug concentrations (LQC, MQC and HQC) were prepared from the independent stock solution.

Precision
Intraday and interday variations were taken to determine intermediate precisions of the proposed method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intraday variations. The same drug concentrations were prepared on three different days to study interday variations. The coefficient of variations (%) of the predicted concentrations from the regression equations was taken as precision.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
LOD was determined using the relation 3.3 \( \sigma/s \) where ‘\( \sigma \)’ is the standard deviation of the response and s is the slope of the calibration curve.

Similarly, LOQ was determined using the relation 10 \( \sigma/s \).
Bench top stability study
In this method stock solution stability was determined by preparing LQC, MQC and HQC at different intervals of time.9

Assay of Formulations
Twenty tablets each of two brands were weighed and ground in to a fine powder. Powder equivalent to 25mg and 5mg of ATE and AM was transferred into 100 ml volumetric flasks and dissolved in 25 ml of 0.1N hydrochloric acid (pH1.2).10,11 The solution was sonicated for 20 minutes and was filtered through Whatmann No.40 filter paper. The residue was washed with hydrochloric acid buffer and washings were added to filtrate. The volume was made up to the mark with 0.1N hydrochloric acid buffer so as to get a concentration of 250.0 µg/ml of atenolol and 500 µg/ml. From this solution, (1.5ml) was pipette out into 10 ml volumetric flask and diluted up to the mark with 0.1N hydrochloric acid buffer (pH 1.2) so as to get a concentration of 80.0 µg/ml and 8.0 µg/ml for amlodipine besylate. The absorbance values of these solutions were measured at 224.6 nm and 239.6 nm respectively.12,13

RESULT AND DISCUSSION
Analytical data
A linear correlation was found between absorbance values at λmax and concentrations of ATE and AM. The optical characteristics such as Beer’s law limits, molar absorptivity values were given in Table1. Regression analysis of Beer’s law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values are reported in Table 1. The graph shows negligible intercept as described by the regression equation Y = a + bX where Y is the absorbance and X concentration in µg/ml, the limit of detection and quantification calculated according to ICH guidelines and reveals a very high sensitivity of the methods.

Method Validation
Accuracy and precision
To evaluate the accuracy and precision of the methods, pure drug solutions at three different levels (within working limits) were analyzed, each determination being repeated three times. The relative standard deviation (%) were in range of 0.08527-1.4682 for atenolol (intraday), 0.1080-1.7118 for atenolol (intraday), 0.2525-1.6080 for amlodipine besylate (intraday) and 0.2599-1.3906 for amlodipine besylate (interday) respectively. These values were significant and can be used for routine analysis.

Application to analysis of commercial samples
In order to check the validity of the proposed method, ATE and AM were determined in some commercial formulations. Table 2 presents the results of the determination from which it is clear that there is close agreement between the results obtained by the proposed methods and the labeled claim. The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powders were spiked with pure ATE and AM standard solutions at three different levels and the concentrations of the sum total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug solution was quantitative (98.14-99.08% and 99.06-99.52% respectively) and reveals that co-formulated substances did not interfere in the determination.

CONCLUSION
The proposed method for determination of amlodipine besylate and amlodipine besylate has been developed and validated. This method was found to be applicable over a range of 4-28 µg/ml for ATE and 4-32 µg/ml for AM and molar absorptivity of 402.50 L mol⁻¹ cm⁻¹ for ATE and 297.00 L mol⁻¹ cm⁻¹ for AM. The methods rely on the use of simple and cheap chemicals and techniques, but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC, HPTLC. Thus this method can be used as an alternative for rapid and routine determination of bulk samples and tablets.

Mathematical Calculations

Amounts of atenolol and Amlodipine besylate were determined by solving the simultaneous equations. Two simultaneous equations were formed using absorptivity coefficient values:

\[ \begin{align*}
A_1 &= 402.50 \times C_1 + 38.84 C_2 \\
A_2 &= 297.00 \times C_1 + 32.22 C_2
\end{align*} \]

Where \( C_1 \) and \( C_2 \) are the concentrations of Atenolol and Amlodipine besylate respectively in gm/liter in the sample solution, \( A_1 \) and \( A_2 \) are the absorbance values of the mixture at the 224.6 nm and 239.6 nm respectively.

ACKNOWLEDGMENT
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REFERENCES
Table 1: Result of Analytical method Development

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atenolol at 224.6nm</th>
<th>Amlodipine Besylate at 239.6 nm</th>
<th>Atenolol at 239.6nm</th>
<th>Amlodipine Besylate at 224.6nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law Limit (µg/ml)</td>
<td>4-28</td>
<td>4-32</td>
<td>50-350</td>
<td>50-350</td>
</tr>
<tr>
<td>Absorptivity (L mol⁻¹ cm⁻¹)</td>
<td>402.50</td>
<td>297.00</td>
<td>38.84</td>
<td>32.22</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.040</td>
<td>0.041</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>intercept</td>
<td>0.003</td>
<td>0.015</td>
<td>0.058</td>
<td>0.017</td>
</tr>
<tr>
<td>Correlation coefficient(r²)</td>
<td>0.999</td>
<td>0.999</td>
<td>0.996</td>
<td>0.999</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.1065</td>
<td>0.2777</td>
<td>3.6580</td>
<td>0.8976</td>
</tr>
<tr>
<td>LOQ(µg/ml)</td>
<td>0.3227</td>
<td>0.8417</td>
<td>11.084</td>
<td>2.7210</td>
</tr>
<tr>
<td>Precision(%RSD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday</td>
<td>0.0857-1.4682</td>
<td>0.2525-1.6080</td>
<td>0.0022-0.2293</td>
<td>0.0221-0.1921</td>
</tr>
<tr>
<td>Intraday</td>
<td>0.1080-1.7118</td>
<td>0.2599-1.3906</td>
<td>0.0021-0.2712</td>
<td>0.0054-0.2342</td>
</tr>
<tr>
<td>Accuracy %Bias</td>
<td>0.0258-0.5372</td>
<td>0.0263-0.3520</td>
<td>0.0423-0.1038</td>
<td>0.0166-0.0617</td>
</tr>
</tbody>
</table>

Table 2: Summary of estimation of ATE and AM in different brands

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Brand</th>
<th>Labeled amount (mg)</th>
<th>Amount found⁺ (mg)</th>
<th>% of Labeled amount*</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Amloras-AT</td>
<td>25 (ATE)</td>
<td>24.90±0.027</td>
<td>99.60±0.581</td>
<td>0.590</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (AM)</td>
<td>4.98±0.169</td>
<td>99.80±0.341</td>
<td>0.336</td>
</tr>
<tr>
<td>2.</td>
<td>Amlopress-AT</td>
<td>25 (ATE)</td>
<td>24.87±0.033</td>
<td>99.58±0.563</td>
<td>0.541</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 (AM)</td>
<td>4.96±0.176</td>
<td>99.71±0.473</td>
<td>0.308</td>
</tr>
</tbody>
</table>

⁺: data represents mean ± SD; n=3

Figure 1: Over lain spectra of synthetic mixture of Atenolol and Amlodipine besylate between 210-310 nm

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