DEVELOPMENT AND VALIDATION OF SIMULTANEOUS EQUATION METHOD FOR DETERMINATION OF METOPROLOL AND AMLODIPINE IN COMBINED DOSAGE FORM

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ABSTRACT
A simple, rapid, precise, accurate and economical spectrophotometric method requiring no prior separation has been developed for the simultaneous estimation of amlodipine and metoprolol in tablet dosage form. Amlodipine and Metoprolol exhibit absorption maxima at 237.5 and 223 nm respectively. The development of simultaneous equation method obeyed Beer’s Law in the concentration range of 5-25 µg/ml for both the drugs. The proposed method is recommended for routine analysis of amlodipine and metoprolol as it is rapid, precise, accurate and reproducible. The results of the tablet analysis were validated with respect to recovery (accuracy), linearity, limit of detection and limit of quantification according to ICH guidelines and found to be satisfactory.

Keywords: Simultaneous Equation Method, Amlodipine, Metoprolol, ICH guidelines.

INTRODUCTION
Amlodipine (AML), chemically \((RS)\)-3-ethyl-5-methyl 2-(2-aminoethoxy) methyl)-4-[2-chlorophenyl]-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate (Fig.1), is a long-acting calcium channel blocker used in the management of hypertension by inhibiting the inward movement of calcium by binding to L-Type calcium channels in the heart and in smooth muscle of the coronary and peripheral vasculature relaxing the smooth muscle and dilating arterioles thereby decreasing peripheral resistance and hence improve blood pressure and in angina pectoris by improving blood flow to the myocardium\(^{1,2}\) whereas Metoprolol (MET), chemically \((RS)-1\text{-}[(\text{Isopropylamino})\text{-}3\text{-}[(2\text{-}methoxy ethyl})\text{-}phenoxy\text{]}\text{propan}-2\text{-}ol\) (Fig. 2) is a selective \(\beta_1\) receptor blocker used in the treatment of several diseases of cardiovascular system especially hypertension. It is a beta adrenergic blocking agent, which reduces chest pain and lowers high blood pressure.\(^{3}\)

Literature survey revealed the estimation of AML and MET with other drugs using UV\(^{4-14}\), HPLC\(^{15-22}\) and HPTLC\(^{23}\). So far this combination has only been analysed using HPLC\(^{24}\)and no spectrophotometric method has been reported. So the present study is focussed on a successful attempt to estimate AML and MET using UV spectroscopy.

MATERIALS AND METHODS

Standard bulk drug samples of amlodipine and metoprolol were provided as gift samples. Tablets of combined dosage form were procured from the local market. All other reagents used were of analytical grade.

A double-beam Shimadzu UV-visible spectrophotometer, 1800 with a pair of 1 cm matched quartz cells were used to measure the absorbance of the solutions.

Preparation of Standard Solutions

10 mg of MET was accurately weighed and transferred to a 10 ml volumetric flask containing small amount of methanol. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to get a solution of 1000 µg/ml and then further diluted to get 10 µg/ml. Solution of AML was also prepared in a similar way to get a concentration of 10 µg/ml.

Determination of Absorption maxima

By scanning standard solutions in the UV-VIS spectrophotometer in the wavelength range of 200-400 nm, an overlain spectrum was recorded (Fig.3). Using the overlain spectra of AML and MET in methanol, the wavelength maxima of both drugs, i.e., 237.5 nm \((\lambda_1)\) and 223.0 nm \((\lambda_2)\), were selected as two sampling wavelengths for this method. The prepared stock solutions were diluted to get solutions of concentrations of 5-25 µg/ml. The absorbance of these solutions were measured at the selected wavelengths and absorptivities were determined. The concentrations of the drugs were obtained by using following equations:

\[
C_x = \frac{A_x \cdot a_x - A_y \cdot a_y}{a_y - a_x} \quad \text{Eq. 1} \\
C_y = \frac{A_y \cdot a_y - A_x \cdot a_x}{a_x - a_y} \quad \text{Eq. 2}
\]

Where, \(A_x\) and \(A_y\) are absorbance of mixture at 237.5 nm and 223 nm respectively, \(a_x\) and \(a_y\) are absorptivities of AML at \(\lambda_1\) and \(\lambda_2\) respectively and \(a_y\) and \(a_x\) are absorptivities of MET at \(\lambda_1\) and \(\lambda_2\) respectively. \(C_x\) and \(C_y\) are the concentrations of AML and MET respectively. The results of analysis are given in Table-1.

Application of the developed method on tablet dosage form

Twenty tablets were weighed, crushed and an accurately weighed sample equivalent to 20 mg of MET and 2 mg of AML was transferred to a 10 ml measuring flask and then by standard addition method 18 mg of pure AML was added in order to bring MET and AML in ratio of 1:1. The drug powder was dissolved in methanol with sonication, filtered through Whatman filter paper and then volume was made up to 10 ml with methanol to get stock solution of 1000 µg/ml of each drug and then further diluted to get 10 µg/ml. All determinations were carried out three times at 237.5 and 223 nm and then the concentration of both the drugs was calculated using Equation 1 and 2 and the results are given in Table 1.
Fig. 3: Overlaid spectrum of AML and MET showing $\lambda_{\text{max}}$ of AML at 237.5 nm and $\lambda_{\text{max}}$ of MET at 223 nm

Table 1: Result of Analysis in Marketed Formulation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim</th>
<th>Amount found (mg)</th>
<th>% drug found ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>5 mg</td>
<td>5.003</td>
<td>100.06±0.740</td>
</tr>
<tr>
<td>MET</td>
<td>50 mg</td>
<td>50.15</td>
<td>100.3±1.267</td>
</tr>
</tbody>
</table>

Values expressed mean ± SD (n=3)

Statistical Validation

The described method has been validated for the assay of both the major components of bulk drug using following parameters according to ICH guidelines.25-26

Limit of detection and limit of quantification

Limit of detection (LOD) is the minimum concentration of the analyte that can be detected by the instrument. Limit of quantification (LOQ) is the concentration of analyte that can be quantified. These parameters were calculated for the proposed method based on the standard deviation (SD) of the y-intercept and the slope (S) of the calibration curves. LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) and the values obtained are given in Table 2.

Linearity

Linearity was studied by preparing solutions at different concentration levels. Calibration curve was plotted using standard solutions of 5µg/ml to 25µg/ml and regression analysis was carried out. Regression coefficients are reported in Table 2 and linearity graph is shown in Fig.4.

Precision

Precision of the method was studied by intra- and inter-day variations in the test method of AML and MET. Intra-day precision was run in triplicate on the same day and inter-day precision for three consecutive days. Precision and accuracy data is shown in Table 3 and 4.

Fig. 4: Linearity graphs of AML and MET plotted between absorbance on X-axis and concentration (µg/ml) on Y-axis
and MET in pharmaceutical dosage form. Linearity of both the drugs was analysed on selected wavelengths (237.5 nm and 223 nm). The method discussed in the present work provides a convenient, simple, rapid, accurate, precise and economical way to estimate AML. Simultaneous equation method using derivative UV-Spectrophotometry was used to estimate the concentrations of AML and MET in pharmaceutical dosage form. Linearity of both the drugs was assessed on selected wavelengths (237.5 nm and 223 nm). The method discussed in the present work provides a convenient, simple, rapid, accurate, precise and economical way to estimate AML.

RESULTS & DISCUSSION

Recovery

It is a measure of closeness between actual value and the analytical value and is calculated by applying test procedure for a number of times at three different levels viz. 80%, 100% and 120%. Samples of concentration 10µg/ml were prepared and analyzed using the proposed method. Percentage recovery was calculated using the equation for the method and the results are given in Table 5. Values expressed mean ± SD (n=3)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
<th>Simultaneous Equation Method</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>10</td>
<td>100.23 ± 0.057</td>
<td>0.057</td>
</tr>
<tr>
<td>MET</td>
<td>10</td>
<td>99.90 ± 0.520</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 5: Recovery Study

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount added (µg/ml)</th>
<th>Amount recovered (µg/ml)</th>
<th>%Recovery ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>80% (9µg/ml)</td>
<td>9.036</td>
<td>100.5±0.081</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>100% (10µg/ml)</td>
<td>10.07</td>
<td>100.7±0.082</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>120% (11µg/ml)</td>
<td>11.154</td>
<td>101.4±0.326</td>
<td>0.321</td>
</tr>
<tr>
<td></td>
<td>80% (9µg/ml)</td>
<td>9.108</td>
<td>101.2±0.244</td>
<td>0.241</td>
</tr>
<tr>
<td>MET</td>
<td>100% (10µg/ml)</td>
<td>10.03</td>
<td>100.3±0.025</td>
<td>0.204</td>
</tr>
<tr>
<td></td>
<td>120% (11µg/ml)</td>
<td>10.813</td>
<td>99.3±0.244</td>
<td>0.248</td>
</tr>
</tbody>
</table>

Values expressed mean ± SD (n=3)

Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantification (LOQ). Precision of the proposed method was determined by inter- and intra-day precision methods and the results range from 99.98-100.23% in case of inter-day precision and 98.26-100.06% in intra-day precision. % RSD calculated was less than equal to 2 which indicate the accuracy and reproducibility of the method. Results are shown in Table 3 and 4.

Drug content in the tablet was directly calculated from the given equations and the results ranges from 100.06-100.3% as shown in Table 1. The proposed method was validated according to the ICH guidelines. Standard deviation (SD) and % relative standard deviation (%RSD) is calculated in Table 2 & 3. Low values of standard deviation show the accuracy, repeatability and reproducibility of the method. The accuracy of the method was proved by performing recovery studies on the commercial formulation at 80, 100 and 120% level. Recovery ranges from 98.3-101.4% (Table 5). The results of recovery study indicate that these drugs could be quantified simultaneously and that there is no interference of the excipients present in the formulation.

From statistical data it is clear that the method is repeatable and specific for the analysis of two drugs in combination and since none of the methods is reported for simultaneous estimation of metoprolol and amlodipine from combined dosage form, the developed method can be used for routine analysis of two components without prior separation.

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REFERENCES


