Simultaneous UV Spectrophotometric Methods for Estimation of Amlodipine Besilate and Olmesartan Medoxomil in Tablet Dosage Form

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ABSTRACT

Two methods for simultaneous estimation of Amlodipine besylate and Olmesartan medoxomil in combined tablet dosage form have been developed. The first UV spectrophotometric method was a determination using the simultaneous equation method at 239 nm and 255 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 245 nm (isobestic point) and at 255 nm the maximum absorption of olmesartan medoxomil. The linearity ranges for Amlodipine besylate and olmesartan medoxomil were 2.5 – 30 µg/ml and 4 – 32 µg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 99.76± 0.5861 and 100.39± 0.4168 for simultaneous equation method and 99.91± 0.4216 and 100.16± 0.1446 for Q analysis (absorption ratio) method for Amlodipine besylate and olmesartan medoxomil respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Key Words: Amlodipine besylate, Olmesartan medoxomil, Q–analysis spectrophotometric method.

INTRODUCTION

Amlodipine besylate(AMB) chemically is [ 3-ethyl-5- methyl (4RS)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-methyl-1-hydropyridine-3, dicarboxylate benzene sulfonate. It is long acting calcium channel blocker used as an antihypertensive agent. The chemical structure of AMB is shown in fig. 1. Olmesartan medoxomil (OLME), chemically (5-methyl-2-oxo-1,3-dioxolen-4-
methyl-4-(1-hydroxyl-1-methyl)-2-propyl-1-{4-[2-(tetrazol-5-yl)-phenyl]-phenyl} methyl imidazole-5-carboxylate is a prodrug used as antihypertensive which blocks the vasoconstrictor effect of angiotensin II by selectively blocking the binding of angiotensin II to AT1 receptor in vascular smooth muscle.

UV spectrophotometric area under curve and derivative spectrophotometry methods for estimation of AMB and OLME for tablet dosage form has been reported. Hence attempt has been made to develop and validate in accordance with ICH guidelines, a simple precise accurate and economical spectrophotometric method for quantitative analysis of AMB and OLME in combined tablet.

EXPERIMENTAL SECTION

Pharmacetically pure samples of AMB and OLME were obtained as gifts from Biochem Pharmaceuticals Industries Ltd Mumbai and Glenmark Pharmaceuticals Ltd Mumbai respectively. Methanol AR grade (Loba Ltd) and distilled water (1:4) was used as solvent in the study. Double beam UV/Vis spectrophotometer Shimadzu model 1800 with a pair of 10mm matched quartz cells was used to measure absorbance of the resulting solution.

Preparation of standard stock solution:
Accurately 10 mg each of AMB and OLME was weighed separately and transferred to two different 100ml volumetric flask. Each drug was dissolved by 10 min sonication in 20 ml methanol and then volume was made up to the mark with distilled water. The standard stock solutions (100µg/ml) were further diluted separately to obtain working standard of concentration 10µg/ml of AMB and OLME each.

Study of spectra and selection of wavelengths:
Each working standard solution was scanned between the range 200-400 nm in 1 cm cell against blank. Maximum absorbing wavelength of AMB and OLME were selected from spectral data and isobestic wavelength selected from overlain spectra of zero order. The λ max for AMB, OLME and isobestic point was 239nm, 255nm and 245nm respectively.
Method I:
In quantitative estimation of two components by simultaneous equation method, absorbances were measured at the maximum absorption wavelengths of two drugs. From the spectra of AMB and OLME absorbances were measured at selected wavelengths i.e. 239nm (λ₁) and 255nm (λ₂) the maximum absorption of AMB and OLME respectively. The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbance and absorptivity coefficient in the following sets of equations.

\[
C_x = \frac{A_1 \, a_y - A_2 \, a_y}{a_x - a_x} \quad \text{Eq. (i)}
\]

\[
C_y = \frac{A_1 \, a_x - A_2 \, a_x}{a_y - a_y} \quad \text{Eq. (ii)}
\]

Where, A₁ and A₂ are absorbances of mixture at 208 nm and 237.5 nm respectively, a₁ and a₂ are absorptivities of AMB at λ₁ and λ₂ respectively and a₁ and a₂ are absorptivities of OLME at λ₁ and λ₂ respectively. Cₓ and Cᵧ are concentrations of AMB and OLME respectively.

Method II:
In Q analysis method the absorbances were measured at the isobestic point and maximum absorption wavelength of OLME. From overlain spectra of AMB and OLME (fig) absorbances...
were measured at the selected wavelengths i.e. 245nm (isobestic point) and at 255nm, the maximum absorption of OLME. The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbance and absorptivity coefficients in the following sets of equations.

For OLME
\[
C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{a_1}{A}
\]

For AMB
\[
C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{a_2}{A}
\]

Where,
- \( Q_0 \) = Absorbance of sample at 237.5 nm
- \( Q_1 \) = Absorbance of sample at 242.5 nm
- \( Q_2 \) = Absorbance of sample at 255 nm
- \( a_1 \) = Absorptivity of LP at 237.5 nm
- \( a_2 \) = Absorptivity of LP at 242.5 nm
- \( A \) = Absorptivity of AB at 237.5 nm
- \( A \) = Absorptivity of AB at 242.5 nm

\( A \) = Absorbance of sample at isoabsorptive point, \( a_1 \) and \( a_2 \) = Absorptivities of LP and AB respectively at isoabsorptive point.

Procedure for analysis of tablet formulation:
Twenty tablets were accurately weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 20 mg OLME was dissolved in 20 ml methanol and sonicated for 20 min and volume was made up to 100ml. The solution was filtered through Whatman filter paper No 41 and aliquot portion of filtrate was diluted to produce solution having concentration of 5\( \mu \)g/ml of AMB and 20\( \mu \)g/ml of OLME. The absorbance of sample solution was measured at selected wavelengths and the concentrations of the two drugs were estimated using simultaneous equation method and absorbance ratio method. The analysis procedure was repeated six times and the results are depicted in Table 1.

VALIDATION:
The methods were validated with respect to linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy and ruggedness. To study accuracy of the developed methods, recovery studies were carried out using standard addition method at three different
levels. Percent recovery and low relative standard deviation for six replicates of sample solution was less than 2%, which met the acceptance criteria established for spectrophotometric methods. Ruggedness of the proposed method was determined by analysis of sample solution prepared by proposed methods between different days. The percent relative standard deviation was found to be less than 2% showed ruggedness of the spectrophotometric methods. The results obtained are summarized in Tables

Table 1 Linear regression analysis of calibration curves with their respective absorptivity values:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMB</td>
<td>OLME</td>
</tr>
<tr>
<td>Beer’s law limit (µg ml−1)</td>
<td>2.5-30</td>
<td>4.32</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9993</td>
<td>0.9995</td>
</tr>
<tr>
<td>Molar absorptivity (lit/mole/cm)</td>
<td>18882.77</td>
<td>19941.49</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0333</td>
<td>0.0335</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0113</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

Table 2 Results of recovery studies:

<table>
<thead>
<tr>
<th>Level of recovery</th>
<th>Amount of pure drug added (mg)</th>
<th>Simultaneous equation method % recovery</th>
<th>Absorbance ratio method % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMB</td>
<td>OLME</td>
<td>AMB</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>8</td>
<td>100.09</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>10</td>
<td>100.10</td>
</tr>
<tr>
<td>120</td>
<td>6</td>
<td>12</td>
<td>99.09</td>
</tr>
<tr>
<td>Mean recovery</td>
<td></td>
<td></td>
<td>99.76</td>
</tr>
<tr>
<td>SD*</td>
<td></td>
<td></td>
<td>0.5861</td>
</tr>
<tr>
<td>CV**</td>
<td></td>
<td></td>
<td>0.5875</td>
</tr>
</tbody>
</table>

*SD = Standard deviation ** CV = coefficient of variance

Table 3 Results of analysis of tablet formulation:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Simultaneous equation method % ± SD(n=6)</th>
<th>Absorbance ratio method % ± SD(n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>100.18±0.3421</td>
<td>99.95±0.4521</td>
</tr>
<tr>
<td>OLME</td>
<td>100.26±0.7422</td>
<td>99.84±0.8372</td>
</tr>
</tbody>
</table>

Table 4 Results of intermediate precisions:

<table>
<thead>
<tr>
<th>Day</th>
<th>Method I</th>
<th>Method II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Label claim estimated (Mean ±%R.S.D.*)</td>
<td>% Label claim estimated (Mean ±%R.S.D.)</td>
</tr>
<tr>
<td></td>
<td>AMB</td>
<td>OLME</td>
</tr>
<tr>
<td>Intraday</td>
<td>98.34±0.5465</td>
<td>99.28±0.8232</td>
</tr>
<tr>
<td>Interday</td>
<td>99.35±0.4532</td>
<td>100.89±0.8743</td>
</tr>
</tbody>
</table>

*R.S.D. = Relative standard deviation

RESULTS AND DISCUSSION

The overlain spectra of AMB and OLME exhibit λ max of 239 nm and 255 nm for AMB and OLME respectively which are quite separated from each other. Additionally one is absorptive point was observed at 245 nm. This wavelength was selected for simultaneous estimation of
AMB and OLME for Q value analysis and it is assumed to be sensitive wavelength. Standard calibration curves for AMB and OLME were linear with correlation coefficients (r) values in the range of 0.9993 – 0.9998 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.2465 – 0.7126. The calibration curves were repeated three times in a day and the average % RSD was found to be 0.4532 for AMB and 0.8321 for OLME; similarly the method was repeated for three different days and average % RSD was found to be 0.5298 for AMB and 0.9312 for OLME. The accuracy of the methods was conformed by recovery studies from tablet at three different levels of standard additions; recovery in the range of 99.76 – 100% justifies the accuracy of method.

CONCLUSION

The proposed UV spectrophotometric methods are a simple, accurate, precise, rapid and economical for the simultaneous estimation of AMB and OLME in tablet dosage form. The proposed methods use inexpensive reagents, solvents and instruments that are available in laboratories. Hence, these methods can be conveniently adopted for the routine analysis in quality control laboratories.

Acknowledgements

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