Spectrophotometric Simultaneous Determination of Amlodipine Besylate and Bisoprolol Fumarate in Combined Tablet Dosage Form by Dual Wavelength and Absorbance Corrected Method

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ABSTRACT

Two methods for the simultaneous determination of Amlodipine Besylate (AMLO) and Bisoprolol Fumarate (BISO) in combined tablet dosage form by UV spectrophotometry have been developed. These methods are dual wavelength (method A) and absorption corrected (method B). From a solvent effect studies and the spectral behaviors of AMLO and BISO, methanol was selected as solvent.

Beer’s law was obeyed in the concentration range of 10-30 µg/ml for AMLO and 5-15 µg/ml for BISO, respectively. In methanol AMLO and BISO shown λmax values 230 nm, 223.02 nm and 271 nm, 360 nm respectively. In method A two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Wavelengths selected for method B for estimation of AMLO was 234.69 nm; similarly wavelength selected for estimation of BISO was 232.59 nm. Both the methods were applied for formulation analysis and assay result were found in the range of 98.27-99.87%. These methods were validated with respect to linearity, accuracy, precision and robustness as per ICH analytical method validation guidelines. The recovery by proposed methods was found in the range of 98.81-100.94 for both the analytes. The linear, accurate, precise and robust methods have been developed for the simultaneous estimation of AMLO & BISO in combined dosage form.

Keywords: Absorbance corrected, Amlodipine Besylate, Bisoprolol Fumarate, Dual wavelength, UV spectrophotometry

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INTRODUCTION

Amlodipine Besylate (AMLO) is a long-acting 1,4-dihydropyridine calcium channel blocker used to treat hypertension and chronic stable angina. AMLO is chemically 3-Ethyl-5-methyl (±)-2-{2-aminoethoxy} methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate.

Bisoprolol Fumarate (BISO) is a synthetic, β1-selective (cardio selective) adrenoceptor blocking agent used for secondary prevention of myocardial infarction, heart failure, angina pectoris and mild to moderate hypertension. BISO is chemically (±)-1-[4-[[2-{1-Methylethoxy} ethoxy] methyl] phenoxy]-3[(1-methylethyl) amino]-2-propanol(E)-2-butenedioate [1-3]. The fixed dose combination therapy of AMLO and BISO is used to control blood pressure of essential hypertensive patient, when monotherapy does not work. It is one of the preferred fixed dose combination for hypertension [4-7]. If we estimate AMLO and BISO simultaneously in a combined dosage form with proper analytical method without prior separation, it is really worth [8-12]. Review of literature revealed that there are few methods have been reported for estimation of AMLO and BISO individually and in combination with other drugs [13-20]. However, dual wavelength and absorbance corrected methods have not been reported for simultaneous estimation of these drugs in combined dosage form. Therefore, the aim of the present work was to develop simple, accurate, robust spectrophotometric dual
wavelength and absorbance corrected methods and validate the same [21] for the analysis of BISO and AMLO in bulk and pharmaceutical formulations with good economical objective.

**MATERIALS AND METHODS**

**Instrumentation**

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10 mm matched quartz cells were used for spectrophotometric methods. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

**Reagents and chemicals**

Spectroscopy grade methanol was used throughout the study. Pure drug sample of AMLO (98.28% purity) and BISO (99.83% purity) were kindly supplied as a gift sample by Emcure Pharmaceuticals Pvt. Ltd., Pune and Nulife Pharmaceuticals Pvt. Ltd., Pune, respectively. Tablet used for analysis was Concor AM (batch No. BLMP09019 (F1), BLMP09183 (F2)) manufactured by Merck (India) Ltd, was procured from local pharmacy, each enteric coated tablet contains AMLO 5 mg and BISO 2.5 mg.

**Preparation of Standard Stock Solutions and Calibration Curve**

Standard stock solution of 1000 µg/mL of both the drugs was prepared separately in methanol. For verification of Beer’s Law, a series of diluted solutions of AMLO and BISO ranging from 10-30 µg/mL (series A) and 5-15 µg/mL (series B) respectively were prepared and mixture of both the drugs in (series C) same concentration range in methanol and scanned in the range of 200-400 nm [Figure 1].

**Preparation of Sample Solution and Formulation Analysis:**

For preparation of sample stock solution, twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 50 mg of AMLO (25 mg of BISO) was weighed and dissolved in the 40 mL of methanol with the aid of ultrasonication for 5 min and solution was filtered through Whatmann filter paper No. 41 into a 50 mL volumetric flask. Filter paper was washed with methanol, adding washings to the volumetric flask and volume was made up to the mark with methanol. The sample stock solution was suitably diluted further to get required final concentration of AMLO (20 µg/mL) and BISO (10 µg/mL).

**Method Validation:**

Method was validated as per ICH guidelines, and parameters used for validation were Linearity, Range, Method Sensitivity, Solution Stability, Recovery Studies, Precision, Specificity, Robustness, Ruggedness etc and are describes as follows.

Method Validation was done by following parameters that given in brief.

**Linearity range and method sensitivity:**

The values of LOD and LOQ were calculated by using σ (standard deviation of response) and b (slope of the calibration curve) and by using equations, LOD = (3.3×σ)/b and LOQ = (10×σ)/b.

**Solution stability:**

Method stability was checked by analyzing solution kept in fridge and at room temperature by both methods. Solution at room temperature was stable for 12 hours and solution in fridge was stable for 30 days.
Recovery studies: The Accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to pre analyzed sample solution at three different concentration levels (80%, 100% and 120%) within the range of Linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drug standard solution was 10 μg/mL of AMLO and 5 μg/mL of BISO for both the methods.

Precision of the Method: Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined [Table 1]. Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Specificity: Specificity is a procedure to detect quantitatively the analytes in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of drug.

Robustness: The robustness was tested by changing parameters such as wavelength range, slit width, temperature, shaking time etc. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

Ruggedness: Ruggedness of the proposed methods was determined by analyzing aliquots from homogenous lot in different under graduate laboratories using same operational and environmental conditions.

RESULTS AND DISCUSSION

Theoretical Aspects of Method A (Dual wavelength method):
The spectrum of AMLO [Figure 1] show that absorbance of BISO is identical at 218.33 nm (λ₁) and 226.51 nm (λ₂) therefore these two wavelength were selected for the analysis of AMLO. All the solutions of series A were scanned to ensure that the difference of absorbance between λ₁ and λ₂ is zero. Similarly, the BISO solutions were scanned to determine the two wavelengths, where absorbance is same. These two wavelengths were found to be 230.74 nm (λ₃) and 242.31 nm (λ₄). All the solutions of series B was scanned to ensure that difference of absorbance between (λ₃) and (λ₄) is zero. Thereafter, the solution of series C was scanned to ensure that varying concentration of AMLO and BISO are not affecting the absorbance at selected wavelength. Difference in absorbance's between 218.33 nm (λ₁) and 226.51 nm (λ₂) of series C solutions was used for preparation of calibration curve for AMLO. Similarly difference in absorbance between 230.74 nm (λ₃) and 242.31 nm (λ₄) of mixed standard solutions was used for preparation of calibration curve for BISO.

Figure 2: Overlay spectra of individual standards, standard mixture and Formulation
Theoretical Aspects of Method B (Absorbance Corrected Method):
The solutions were scanned in UV Spectrophotometer in the range 200-400 nm at 0.5 band width and 600 nm/min scan speed for the determinations of $\lambda_{\text{max}}$ of AMLO & BISO and was found to be at 234.69 nm and 232.59 nm respectively. AMLO showed absorbance at 234.69 nm, while BISO did not show any interference at 234.69 nm [Figure 1]. To construct Beer's plot for AMLO and BISO, stock solutions of 1000 μg/mL of both the drugs were prepared in methanol and working standard dilutions were made in methanol using stock solution of 1000 μg/mL. Also Beer’s plot was constructed for AMLO and BISO in solution mixture at different concentration (10:5, 15:7.5, 20:10, 25:12.5, 30:15 μg/mL) levels. Both the drugs followed linearity individually and in mixture within the concentration range 10-30 μg/mL and 5-15 μg/mL for AMLO and BISO, respectively. Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. Using appropriate dilutions of standard stock solution the two solutions was scanned separately. Critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in [Table 1]. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and Precision. Beer's law is obeyed in the concentration range of 10-30 μg/mL and 5-15 μg/mL for AMLO and BISO, respectively. Correlation coefficient was always greater than 0.9940 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets containing AMLO and BISO. Percent labeled claim and % RSD was calculated and the results are presented in [Table 2]. Results of accuracy study are presented in [Table 1]. The accuracy is evident from the data as results are close to 100% and standard deviation is low.

<table>
<thead>
<tr>
<th>Table 1: Results of linearity, precision, formulation, sensitivity &amp; ruggedness study</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>Wavelength (nm)</td>
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<td>Beer's law limit (μg/mL)</td>
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<td>Regression Equation*</td>
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<tr>
<td>Correlation coefficient (r)</td>
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<tr>
<td>Precision (%RSD)</td>
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<tr>
<td>Formulation Analysis (%Assay, %RSD, n=6)</td>
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<tr>
<td>LOD (μg/mL)</td>
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<td>LOD (μg/mL)</td>
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<tr>
<td>Ruggedness (%RSD)</td>
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RSD = Relative Standard Deviation, $Y^*$ = $mx + c$, where $Y$ is the absorbance and $X$ the concentration in micrograms per milliliter.
Table 2: Results of accuracy study

<table>
<thead>
<tr>
<th>Recovery Level</th>
<th>Name of Analyte</th>
<th>Amount Spiked (μg/mL)</th>
<th>Method A % Mean Recovery, % RSD, n=6</th>
<th>Method B % Mean Recovery, % RSD, n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 %</td>
<td>AMLO</td>
<td>8</td>
<td>100.94, 0.74</td>
<td>98.67, 0.38</td>
</tr>
<tr>
<td></td>
<td>BISO</td>
<td>4</td>
<td>100.36, 0.45</td>
<td>100.03, 0.28</td>
</tr>
<tr>
<td>100 %</td>
<td>AMLO</td>
<td>10</td>
<td>99.08, 0.19</td>
<td>98.95, 0.48</td>
</tr>
<tr>
<td></td>
<td>BISO</td>
<td>5</td>
<td>99.17, 1.34</td>
<td>100.29, 0.29</td>
</tr>
<tr>
<td>120 %</td>
<td>AMLO</td>
<td>12</td>
<td>99.89, 1.57</td>
<td>99.63, 0.93</td>
</tr>
<tr>
<td></td>
<td>BISO</td>
<td>6</td>
<td>99.64, 0.67</td>
<td>99.02, 1.38</td>
</tr>
</tbody>
</table>

CONCLUSION
The validated spectrophotometric Dual Wavelength and Absorbance Corrected Method employed simultaneous determination for AMLO and BISO proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of AMLO and BISO in tablet dosage form.

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1. URL: https://pubchem.ncbi.nlm.nih.gov/compound/


