UV-absorbance difference method for simultaneous estimation of atenolol and amlodipine besylate in combined dosage forms

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ABSTRACT

A new UV-spectrophotometric method for the simultaneous and separate estimation of atenolol and amlodipine besylate in binary tablet formulations has been described. The method is based on the estimation of one drug in presence of another drug by absorbance method. In this method, two wavelengths 209 and 244.5 nm were selected for atenolol and at these wavelengths the absorbance difference was found to be almost zero. Similarly, the two wavelengths 265 and 278 nm were selected for amlodipine besylate and the absorbance difference was almost zero. In the mixture of atenolol and amlodipine besylate solution the absorbance values of four wavelengths 209, 244.5, 265 and 278 nm were measured. The amount of atenolol is directly proportional to the absorbance difference between 265 and 278 nm. Similarly, the amount of amlodipine besylate is directly proportional to the absorbance difference between 209 and 244.5 nm.

Keywords: Spectrophotometry, Atenolol, Amlodipine besylate, absorbance difference method.

INTRODUCTION

Atenolol [1] is an antihypertensive, anti IC anginal, and antiarrhythmic drug; chemically, it is 4-(2-hydroxy-3-isopropyl amino propoxy)-phenyl acetamide. Amlodipine besylate [2] is calcium antagonist and chemically, it is 3-ethyl-5-methyl-(4 RS)-2-[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3,5-dicarboxylate benzene sulphonate. The Indian Pharmacopoeia described a non-aqueous titration method for the assay of atenolol. The British Pharmacopoeia examines amlodipine besylate by liquid chromatography. HPLC [3], reversed phase HPLC [4], colorimetric method [5, 6, 7], HPTLC [8, 9], gas liquid chromatography [10], difference spectrophotometric estimation [11] are few of the methods reported in literature for the analysis of atenolol and amlodipine besylate from their respective formulations. HPTLC [12], reversed phase HPLC [13], HPTLC [14] and vierordt’s [15,16,17] methods are reported for simultaneous estimation of atenolol and amlodipine besylate in combined dosage form, but no method is reported for simultaneous estimation of these drugs in combination by absorbance difference method by spectrophotometry. The aim of the present work is to develop a simple, rapid, precise reproducible and economically viable method for the simultaneous analysis of the selected binary drug formulation by using absorbance difference method without any interference from each other.

MATERIALS AND METHODS

Spectronic-2080 double beam UV-VIS spectrophotometer with spectral band width of 0.1nm with a pair of 10 mm quartz cells used for all spectral and absorbance measurements. All chemicals used were of analytical grade.
Preparation of standard Atenolol solution. Pure Atenolol (50mg) was dissolved in 50 mL ethanol. Further the stock solution is diluted with ethanol to get a working concentration of 20 µg/mL.

Preparation of standard Amlodipine besylate solution. Pure Amlodipine besylate (50mg) was dissolved in 50 mL ethanol. Further the stock solution was diluted with ethanol to get a working concentration of 0.35 µg/mL.

Preparation of mixed solution. Two solutions, the first containing 50 µg/mL of Atenolol and the second containing 0.35 µg/mL of Amlodipine besylate were used as mixed solution. Four mixed standard solutions were made by taking 4, 3, 2 and 1mL of Atenolol solution in series of test tubes and the Amlodipine besylate solution was added to a series of test tubes to keep the total volume at 5mL.

Preparation of Atenolol curve. Various aliquots of (5, 6, 7 and 8mL) of Atenolol solution were transferred into a series of 10mL standard flasks and the volume in each flask was adjusted to 10mL with ethanol. The absorbance of these solutions was scanned over the range 200-320 nm. The absorbance spectrum of Atenolol was shown in Fig. 1.

Preparation of Amlodipine besylate curve. Various aliquots of (5, 6, 7 and 8mL) of Amlodipine besylate solution were transferred into a series of 10mL standard flasks and the volume in each flask was adjusted to 10mL with ethanol. The absorbance of these solutions was scanned over the range 200-300 nm. The absorbance spectrum of Amlodipine besylate was shown in Fig.2.
Two wavelengths 209 and 244.5 nm was selected for Atenolol; at these two wavelengths the absorbance difference was almost zero and in case of Amlodipine besylate there is maximum absorbance difference. A calibration curve was drawn between the absorbance difference values of Amlodipine besylate and the amount of Amlodipine besylate in µg/mL. The amount of Amlodipine besylate present in the sample was estimated from the calibration curve. Similarly, the two wavelengths 265 and 278 nm were selected for Atenolol; at these two wavelengths the absorbance difference was almost zero and in case of Atenolol there is a maximum absorbance difference. A calibration curve was drawn between the absorbance difference values of Atenolol and the amount of Atenolol in µg/mL. The amount of Atenolol present in the sample was estimated from the calibration curve.

Various aliquots of mixtures of Atenolol and Amlodipine besylate solutions in different proportions were transferred into a series of test tubes and the volume in each case was made 5mL (Table 1). The absorbance values were measured at two wavelengths 209 nm and 244.5 nm for Atenolol and two wavelengths 265 nm and 278 nm for estimation Amlodipine besylate. A calibration curve was drawn between the absorbance difference values of Atenolol and the amount of Atenolol in µg/mL. A calibration curve was drawn between the absorbance difference values of Amlodipine besylate and the amount of Amlodipine besylate in µg/mL. A linear curve in each case was obtained. The linearity of the curves obtained indicated that it obeys Beer’s law and the suitability of this method for the simultaneous determination of two drugs in a mixture.

![Fig 3: Over lain spectrum of Atenolol and amlodipine besylate](image)

**Table 1. Concentration of the two components in the four mixed standards**

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Volume of AT* (mL)</th>
<th>Amount of AT* (µg/mL)</th>
<th>Volume of AM+ (mL)</th>
<th>Amount of AM+ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>80</td>
<td>1</td>
<td>3.5</td>
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<tr>
<td>2</td>
<td>3</td>
<td>60</td>
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<td>7.0</td>
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<tr>
<td>3</td>
<td>2</td>
<td>40</td>
<td>3</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>20</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

*=Atenolol, += Amlodipine besylate

**Table 2. Estimation of atenolol and amlodipine besylate in pharmaceutical preparations**

<table>
<thead>
<tr>
<th>Sample Label</th>
<th>Claim (mg/tab)</th>
<th>Amount found by proposed method (mg)**</th>
<th>% Recovery by proposed method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet A</td>
<td>50</td>
<td>49.96</td>
<td>99.82</td>
</tr>
<tr>
<td>Tablet B</td>
<td>50</td>
<td>49.90</td>
<td>99.96</td>
</tr>
<tr>
<td>Tablet C</td>
<td>50</td>
<td>50.02</td>
<td>99.90</td>
</tr>
<tr>
<td>Tablet D</td>
<td>50</td>
<td>49.9</td>
<td>99.70</td>
</tr>
</tbody>
</table>

*a:amlodine AT marketed by mankind pharma, *B:stamloBeta marketed by Dr.Reddy’s Labs Ltd, *C:Angicam-Beta marketed by Blue Cross Labs LTD, *D:amlovas-AT marketed by macleods pharmaceuticals LTD ; Atenolol, Am: Amlodipine; *Average of five determinations; **determination of Atenolol and Amlodipine besylate in combined dosage form pharmaceutical preparations by proposed method.

**Estimation in the marketed formulation.** 20 Tablets were weighed and crushed into fine powder. An average weight of the tablets containing the two drugs atenolol and amlodipine besylate in the ratio 1:3 were taken and an amount of 50mg dissolved in 50mL ethanol by vigorously shaking and the volume was made up to the mark. The solution was then filtered through the Whatmann filter paper No.41 and the solution was diluted to get a final concentration.
of 20 µg/mL of Atenolol and 0.35 µg/mL of Amlodipine besylate. The sample solutions were measured at 209 and 244.5 nm for Atenolol and 265 and 278 nm for amloidipine besylate. The results are presented in table 2.

**Validation of the method.** The method was validated in terms of linearity, accuracy, precision, specificity and reproducibility of the sample applications. The linearity of the method was investigated by serially diluting the stock solutions of Atenolol (20 µg/mL) and Amlodipine besylate (0.35 µg/mL) and measured the absorbance values at 209 and 244.5 nm for Atenolol and 265 and 278 nm for amloidipine besylate. Calibration curves were constructed by plotting the absorbance difference values against the amount of drug in µg/mL.

![Fig. 4. Calibration curves of atenolol and amloidipine besylate](image)

**Statistical analysis.** A statistical analysis was performed on the statistically significant variables using the statistical software. The parameters determined were coefficient of variation, standard deviation and student t-test.

**Recovery experiment.** To ensure the accuracy and reproducibility of the results obtained, recovery experiments were performed by adding a known amount of standard drug to previously analysed pharmaceutical preparation. The results are recorded in table 2.

**RESULTS AND DISCUSSION**

The present study was carried out to develop a simple, rapid, sensitive, precise, reproducible and accurate spectrophotometric method for the estimation of Atenolol and Amlodipine besylate in combined dosage forms. The proposed absorbance difference method was simple, less time consuming, low cost, found to be one of the best analytical techniques employed for routine analytical purposes like assay and pharmaceutical formulations. At the initial stage in the development of the method the Atenolol solution was first scanned over the range 200-320 nm and two wavelengths 209 and 244.5 nm were selected for Atenolol having the absorbance almost zero at these wavelengths. In case of Amlodipine besylate there should be a considerable absorbance difference; Amlodipine besylate solution was scanned over the range 200-300 nm and similarly, two wavelengths 265 and 278 nm were selected for estimation and the absorbance difference was almost zero at these wavelengths. The results obtained by the proposed method are in good agreement with the label claim of the tablets. The additive and excipients usually present in tablets do not found interfered. As a check on accuracy of the method, recovery experiment was performed and percent recovery values also tabulated in table 2.

![Table 3. Statistical analysis of estimation of atenolol and amloidipine besylate](table)

<table>
<thead>
<tr>
<th>Sample</th>
<th>S.D.</th>
<th>C.V.**</th>
<th>t cal*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATb</td>
<td>AMc</td>
<td></td>
</tr>
<tr>
<td>Tablet 1</td>
<td>0.3049</td>
<td>0.4560</td>
<td>0.2934</td>
</tr>
<tr>
<td>Tablet 2</td>
<td>0.4764</td>
<td>0.2701</td>
<td>0.3755</td>
</tr>
<tr>
<td>Tablet 3</td>
<td>0.2449</td>
<td>0.3193</td>
<td>0.2503</td>
</tr>
<tr>
<td>Tablet 4</td>
<td>0.1788</td>
<td>0.2073</td>
<td>0.1993</td>
</tr>
</tbody>
</table>

*Standard deviation, **Coefficient of variation; *Calculated ‘t’ value by proposed method; Theoretical values at 95% confidence limit, ‘t’ 2.78; ATenolol, AMlodipine

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The statistical analysis was studied by the proposed method (table 3). The values of standard deviation and coefficient of variation were satisfactorily low, indicating the accuracy and the reproducibility of the method. Student t-test showed that the calculated ‘t’ values are less than the theoretical value 2.78 with 4 degrees of freedom at 5% level of significance, indicating that there is no significant difference between the proposed and official values.

CONCLUSION

In Conclusion, the results indicate that the proposed absorbance difference method was found to be simple, rapid, precise and accurate, and it can be used for the routine analysis of simultaneous estimation of Atenolol and Amlodipine besylate.

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REFERENCES