INTRODUCTION

The simple, accurate and precise method for simultaneous quantification of Nebivolol hydrochloride (NEB-H) and Amlodipine Besylate (AML) in tablets by HPTLC methods were developed. In this method, the chromatograms were developed using a mobile phase of Methylene Chloride : Methanol : Ammonia (8.5:1:0.5 v/v) on precoated plate of silica gel 60 F254 and quantified by densitometric absorbance mode at 285 nm. The Rf of AML and NEB-H were 0.19 and 0.41 respectively. Recovery studies of 99.96 – 102.11%, percentile relative standard deviation of not more than 0.8 and correlation coefficient (linearity range) of 0.9984 – 0.9998 shows that developed methods were accurate and precise for AML and NEB-H respectively. The LOD and LOQ values were found to be 30ng/ml, 30ng/ml and 80ng/ml, 80ng/ml for AML and NEB-H respectively. The mean percentage recovery values close to 100% it indicates there is no interferences of additives with Nebivolol hydrochloride (NEB-H) and Amlodipine Besylate (AML) present in tablet dosage forms. The method has been validated as per ICH guide lines. This method can be employed for the routine analysis of tablets containing AML and NEB-H.

Keywords: Nebivolol hydrochloride (NEB-H), Amlodipine Besylate (AML), High Performance Thin Layer Chromatography (HPTLC)

ABSTRACT

The simple and accurate method for simultaneous estimation of Nebivolol hydrochloride (NEB-H) and Amlodipine Besylate (AML) in tablets by HPTLC methods were developed. In this method, the chromatograms were developed using a mobile phase of Methylene Chloride : Methanol : Ammonia (8.5:1:0.5 v/v) on precoated plates of silica gel 60 F254 and quantified by densitometric absorbance mode at 285 nm. The Rf of AML and NEB-H were 0.19 and 0.41 respectively. Recovery studies of 99.96 – 102.11%, percentile relative standard deviation of not more than 0.8 and correlation coefficient (linearity range) of 0.9984 – 0.9998 shows that developed methods were accurate and precise for AML and NEB-H respectively. The LOD and LOQ values were found to be 30ng/ml, 30ng/ml and 80ng/ml, 80ng/ml for AML and NEB-H respectively. The mean percentage recovery values close to 100% it indicates there is no interferences of additives with Nebivolol hydrochloride (NEB-H) and Amlodipine Besylate (AML) present in tablet dosage forms. The method has been validated as per ICH guidelines. This method can be employed for the routine analysis of tablets containing AML and NEB-H.

Keywords: Nebivolol hydrochloride (NEB-H), Amlodipine Besylate (AML), High Performance Thin Layer Chromatography (HPTLC)
NEB-H (5 mg) and AML (5 mg) were used for the study. Water, Methylene Chloride, methanol, used was of HPLC grade (E. Merck, Mumbai, India). All the other chemicals used were of analytical grade (E. Merck, India).

Preparation of Stock Solutions

A stock solution was prepared by dissolving 10 mg and 10 mg of NEB-H and AML in 100 ml of mobile phase. The stock solution were further diluted with methanol to obtain various concentration of 100-500 ng/ml and 100 - 500 ng/ml for NEB-H and AML respectively.

The drugs were resolved using a mobile phase of Methylene Chloride : Methylene : Ammonia (8.5:1:0.5v/v), 10 mins time saturation with filter paper was selected because it gave compact spots and good resolution between analytes and good separation from solvent front and sample application positions. Development chamber (20 X 10cm), migration distance (80mm), band length (8mm), slit dimension (6 X 0.30mm), temperature 26.4°C, humidity 61% and UV detection was carried out at 285 nm Fig: 1. All measurements were repeated six times for each concentration and calibration curve was constructed by plotting peak area Vs the corresponding drug concentration.

Analysis of Formulation

The sample prepared as that of HPLC method and filtered through Whatmanns filter paper. The sample solution was suitably diluted and used for analysis. Two microlitres of standard and sample solutions were applied as band 8mm at 8mm interval under stream of nitrogen. The developed chromatograms were evaluated by scanning in densitometric mode at 285 nm. The amount of NEB-H and AML present per tablet was calculated by comparing peak area Vs the corresponding drug concentration.

Table 1: Recovery Studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount added (mcg/ml)</th>
<th>% Recovery 100% level</th>
<th>% RSD* 100% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB-H</td>
<td>2.5</td>
<td>102.11</td>
<td>0.2456</td>
</tr>
<tr>
<td>AML</td>
<td>2.5</td>
<td>99.96</td>
<td>0.2374</td>
</tr>
</tbody>
</table>

NEB-H – Nebivolol hydrochloride, AML - Amlodipine Besylate*Each value is a mean of six observations.

Recovery studies

Recovery studies were carried out by adding known quantities of standard at different levels to the pre-analysed sample to study the linearity, accuracy and precision of the proposed methods. The recovery studies also reveals whether there is a positive or negative influence on the quantification parameters by the additives usually present in dosage forms. The recovery study data are presented in Table 1.

Table 2: Validation Parameters of the Proposed HPTLC Method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Amlodipine</th>
<th>Nebivolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng/spot)</td>
<td>100 - 500</td>
<td>100 - 500</td>
</tr>
<tr>
<td>Precision (%CV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Intraday (n = 3)</td>
<td>1.15 – 2.48</td>
<td>0.58 – 1.53</td>
</tr>
<tr>
<td>- Interday (n=5)</td>
<td>0.47 – 2.62</td>
<td>0.72 – 1.69</td>
</tr>
<tr>
<td>% Recovery</td>
<td>99.96 – 101.85</td>
<td>100.56 –102.11</td>
</tr>
</tbody>
</table>

Table 3: Analysis of formulation

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount(mg / Tablet)</th>
<th>% Label claim</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB-H</td>
<td>5</td>
<td>99.72</td>
<td>0.2456</td>
</tr>
<tr>
<td>AML</td>
<td>5</td>
<td>101.16</td>
<td>0.2374</td>
</tr>
</tbody>
</table>

*Each value is a mean of six observations.

Fig. 1: Spectrum of Standard Amlodipine Besylate on TLC plate

Fig. 2: Spectrum of Standard Nebivolol hydrochloride on TLC plate
RESULTS AND DISCUSSION

AML and NEB-H are soluble in methylene chloride therefore, methylene chloride was selected as the solvent. The formulation was dissolved in methylene chloride with sonication for 20 min to assure complete release of the drug from the formulation matrix. The mixture of Methylene chloride: Methanol: Ammonia (8.5:1:0.5v/v), could resolve AML and NEB-H spots with a better peak shape. The combination of Methylene Chloride and methanol offered the optimum migration (Rf, values of 0.19 ± 0.02 for AML and 0.41 ± 0.02 for NEB-H) and resolution of AML from other components of the formulation matrix (Fig. 1) and the peak purity overlay spectra of AML and NEB-H shown in Fig 2 & 3 it indicates no interferences of other substances present in the formulation. On the other hand, an ammonia solution helped to sharpen the peak. Even saturation of the TLC chamber mobile phase with filter paper for 10 min assured better reproducibility and better resolution. Scanning of the same spot (100 ng/spot for AML and 100 ng/spot for NEB-H) of both drugs seven times without changing the position of the plate; the %CV for measuring the peak area was found to be 0.82% for AML and 0.23% for NEB-H. The repeatability of the method was checked by spotting 5 ng/ml of a combined standard solution seven times on the TLC plate (n = 7); the %CV for the peak area was found to be 0.98% % for AML and 0.68 for NEB-H. Both the %CV, for measuring the peak area and sample applications (less than 1% and 3%, respectively), ensuring proper functioning of the HPTLC system. The accuracy of the method was evaluated by calculating the recovery of AML and NEB-H by the standard addition method at different levels of the calibration curve (n = 6). Results are shown in Table-1.

Different validation parameters for the proposed HPTLC method for determining the AML and NEB-H content are summarized in Table 2 This method was applied to determine the content of AML and NEB-H in two different combined market samples of AML and NEB-H tablets. The content and percentage of AML and NEB-H in market samples are presented in Table 3. The results indicate that the proposed HPTLC method is simple, specific, rapid, precise and accurate for the simultaneous estimation of AML and NEB-H in its combined formulations.

REFERENCES

17. Prabhakar AH, Giridhar R, A spectrophotometric method for the determination of nebivolol besylate in pure form and in tablets, Indian Drugs 39, 2002, 204-08.

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