Development of High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Atenolol and Nefidipine in Combined Dosage Form

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An accurate and precise high pressure thin layer chromatographic method for simultaneous estimation of atenolol and nefidipine in their combined dosage form has been developed. The study employs kieselghur 60, GF254 on aluminium foil and a mobile phase comprising cyclohexane:methanol:ethyl acetate:ammonia (5:1.5:3:0.5 v/v). The detection was carried out at 230 nm. The linear detector response for atenolol was observed between 5.7-18.9 µg/mL while for nifedipine 2.3-7.0 µg/mL. The recovery study was carried out by standard addition method. The results of recovery were 99.76 ± 0.216, 100.72 ± 0.216 for atenolol and 100.04 ± 1.069, 99.89 ± 1.058 for nifedipine.

Key Words: Atenolol, Nefidipine, HPTLC, Validation.

INTRODUCTION

Atenolol is an antihypertensive, antianginal and antiarrhythmic drug and chemically is 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenylacetamide. Nifedipine is antianginal and antihypertensive and chemically is dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol and nefidipine. Gas liquid chromatography, reversed phase high performance liquid chromatography, ultra violet spectrophotometry, colorimetric estimation, miscellar electrokinetic chromatography, HPLC and RP-HPLC are few methods reported in literature for the analysis of atenolol and nefidipine from their respective formulations. HPLC and RP-HPLC method is also reported for simultaneous estimation of atenolol and nefidipine in combined dosage form.

EXPERIMENTAL

All chemical and reagents were of AR/HPLC grade. The instruments used in the present study was Camag-HPTLC system comprising of Camag Linimat IV automatic sample applicator, Camag TLC Scanner III with CATS 4 software, Camag twin trough glass chamber were used.

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Chromatographic conditions

Stationary phase: The stationary phase used was of kieselghur 60, GF254 TLC precoated aluminium foiled plates and the mobile phase selected was cyclohexane: methanol:ethyl acetate:ammonia (5:1.5:3:0.5 v/v). The saturation time was 15 min, thickness of plate was 200 µm, sample application: 6 mm band, separation technique: ascending, temperature: 20 ± 5 °C, relative humidity: 50-60 %, migration distance: 70 mm, scanning mode: absorbance, detection wavelength: 230 nm. The detection wavelength was selected from overlain spectra of both the drugs in methanol.

Selection of wavelength: The separated bands on HPTLC plates were scanned over the wavelength of 200-400 nm.

Calibration curve response: Standard solution ranging from 3-21 µL was applied on TLC plates by microlitre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 230 nm. Peak height and areas were recorded for each concentration of drugs and the calibration curves were constructed.

Preparation of standard and sample solutions

Standard solutions: An accurately weighed quantity of about 100 mg each of atenolol and nifedipine were transferred to two separate 100 mL volumetric flasks. Both drugs were dissolved in about 50 mL of methanol and then volume was made up to the mark with the same solvent (concentration-1 mg/mL of atenolol and nifedipine, respectively).

Mixed standard solution: Accurately weighed quantities of 50 mg atenolol and 20 mg of nifedipine were transferred to a 50 mL volumetric flask. The drugs were dissolved in 30 mL of methanol and finally the volume was made up to the mark with methanol [concentration 1 mg/mL (1 µg/µL) concentration of atenolol and 0.4 mg/mL (0.4 µg/µL) of nifedipine, respectively].

Analysis of laboratory mixture

Preparation of standard laboratory mixture: Accurately weighed quantities of 50 mg of atenolol and 20 mg of nifedipine was transferred to 50.0 mL volumetric flask. The drugs were dissolved in 30 mL of methanol and finally the volume was made up to mark.

Preparation of sample mixtures: Three laboratory mixtures of atenolol and nifedipine were prepared by appropriately weighing the quantities of drug samples so as to get the concentration in the range of 1 and 0.4 mg/mL of atenolol and nifedipine, respectively.

On the HPTLC plates one spot of standard and six spots of sample were applied, developed and scanned densitometrically at 230 nm. The per cent estimates of both the drugs were calculated using the formula:

\[
\text{% Estimated} = \frac{\text{Amount estimated}}{\text{Amount applied}} \times 100
\]

The results are given in Table-1.
TABLE-1
PER CENT ESTIMATION OF DRUG FROM LABORATORY MIXTURE, MARKETED FORMULATION AND THEIR RECOVERY STUDIES

<table>
<thead>
<tr>
<th>Sample</th>
<th>Statistics</th>
<th>Estimate of labeled claim (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ATN &amp; NFD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>By height &amp; area</td>
<td>By height &amp; area</td>
</tr>
<tr>
<td>Laboratory mixture</td>
<td>Mean</td>
<td>100.71 &amp; 99.99</td>
<td>100.72 &amp; 100.30</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.221 &amp; 0.529</td>
<td>0.265 &amp; 0.233</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>0.219 &amp; 0.529</td>
<td>0.263 &amp; 0.232</td>
</tr>
<tr>
<td>Marketed preparation</td>
<td>Mean</td>
<td>100.44 &amp; 99.48</td>
<td>99.98 &amp; 100.11</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.538 &amp; 2.790</td>
<td>0.678 &amp; 1.811</td>
</tr>
<tr>
<td></td>
<td>CV</td>
<td>1.531 &amp; 1.809</td>
<td>0.678 &amp; 0.391</td>
</tr>
</tbody>
</table>

Each % labeled claim is the mean of five readings.

**Estimation of drugs in marketed preparations:** Twenty tablets were weighted and finely powdered. An accurately weighed quantity of tablet powder equivalent to 50 mg of atenolol was transferred in 50 mL volumetric flask and 25 mL methanol was added to it. The content was shaken for about 10 min and then the volume was adjusted up to the mark with methanol. The solution was filtered using Whatmann paper 1. The filtrate was diluted further to get the final concentration of standard solution as 1.0 and 0.4 mg/mL of atenolol and nifedipine, respectively. The per cent labeled claim of drug estimated in the marketed formulation was calculated by using the formula:

\[
\text{% Labeled claim} = \frac{\text{Amount estimated}}{\text{Amount applied (on labeled claim basis)}} \times 100
\]

The results are shown in Table-1.

**Validation of proposed method:** The proposed method was validated by considering the following parameters:

**Accuracy:** The accuracy of the proposed method was ascertained by carrying out the recovery studies by standard addition method. The recovery study was performed to determine the possible interference due to the excipients present in the marketed formulation. The method was found to be accurate on the basis of the results shown in Table-1 indicating no interference of excipients in the recovery of both the drugs.

**Precision:** Standard deviation and relative standard deviation of the observation were determined and results shown in Table-2 were found to be within the prescribed standard limits. The results are shown in Table-2.

**RESULTS AND DISCUSSION**

Various pure solvents of varying polarity like methanol, ethyl acetate, chloroform, toluene, cyclohexane, etc. and their mixtures in different proportions were tried as mobile phase for development of chromatogram. The mobile phase found to be most appropriate was cyclohexane:methanol:ethyl acetate:ammonia (5:1.5:3.0:}
TABLE-2
RESULTS OF THE PRECISION STUDY BY PROPOSED METHOD

<table>
<thead>
<tr>
<th>Weight of tablet powder (mg)</th>
<th>Atenolol</th>
<th>Nefidipine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak height</td>
<td>Peak area</td>
</tr>
<tr>
<td>162.69</td>
<td>100.78</td>
<td>102.06</td>
</tr>
<tr>
<td>164.00</td>
<td>98.76</td>
<td>99.88</td>
</tr>
<tr>
<td>164.22</td>
<td>101.78</td>
<td>96.52</td>
</tr>
<tr>
<td>Mean</td>
<td>100.44</td>
<td>99.48</td>
</tr>
<tr>
<td>SD</td>
<td>1.538</td>
<td>2.790</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.531</td>
<td>2.805</td>
</tr>
</tbody>
</table>

Each % estimation is the mean of five observations.

0.5 v/v). It gave the good resolution of two compounds with \( R_f \) values as 0.07 for atenolol and 0.69 for nefidipine. The densitometric evaluation of the chromatogram was done on 230 nm as both the drugs have sufficient absorbance and better sensitivity at the specified wavelength. The linearity response was observed in the concentration range of 5.7-18.9 µg/mL for atenolol and 2.3-7.6 µg/mL for nefidipine. The per cent estimate of drugs in laboratory mixture were found to be 100.71 ± 0.221, 99.99 ± 0.529, 100.72 ± 0.265 and 100.30 ± 0.233 by peak height and peak area for atenolol and nefidipine, respectively. The per cent estimates of drugs in marketed formulation were 100.44 ± 1.538, 99.48 ± 2.790, 99.98 ± 0.678 and 100.11 ± 1.811 for both the drugs. The accuracy of the method was determined by recovery studies using standard addition method. Results of the estimation by recovery studies of both the drugs were ca. 99-100 % indicating the non interference of excipients.

The replicate estimation of atenolol and nefidipine in the same batch of tablet as analyzed by the proposed method gave concurrent results indicating the reliability of the method. The values of SD and RSD and coefficient of correlation were within the prescribed limit of < 2 %, showing high precision of the method.

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