ABSTRACT

The new stability-indicating high performance liquid chromatography (HPLC) method has been developed and validated with different parameters for atenolol (ATE) and nifedipine (NIFE) in the combined dosage form. The chromatographic conditions were optimized using a mobile phase of MeOH:OPA (70:30) with a flow rate of 0.7 mL/min. Column (C18) of 4.6 × 250 mm dimension was used as a stationary phase; the particle size capacity of the column was 5 µm. The detection was carried out at 233 nm. The method was validated according to ICH guidelines for linearity, precision, repeatability, the limit of detection (LoD), and limit of quantitation (LoQ). The response was found to be linear in the concentration range of 20 to 100 mcg/mL for ATE and 1 to 5 mcg/mL for NIFE. The developed method shows the minimum quantity of drugs to be identified (LoD) and minimum drug to be quantified (LoQ). The LoD and LoQ were found to be 0.1415 and 0.4289, respectively, for ATE, and 0.1834 and 0.5558, respectively, for NIFE. The method was linear, simple, precise, and accurate and, therefore, suitable for routine analysis of drugs in tablet form. The forced degradation studies were also done through the exposure of analyte solution to four different stress conditions.

Keywords: Atenolol, Development, Forced degradation, High performance liquid chromatography (HPLC), Nifedipine, Validation.

INTRODUCTION

The HPLC has two types, i.e., normal phase-high performance liquid chromatography (NP-HPLC) and RP-HPLC. In this chromatographic study, we have selected RP-HPLC. In the RP-HPLC method, the mobile phase is polar, and the stationary phase is non-polar. Chromatographic separation in HPLC is a result of the specific interaction of the drug with the mobile and stationary phases. The mobile phase runs the solution of drugs through the column. Column acts as a stationary phase. HPLC contains different parts from the mobile phase reservoir, degasser, and column to the detector for analyzing different samples.

ATE and NIFE are anti-hypertensive drugs. NIFE is a drug used to manage high blood pressure and angina. NIFE is one of the choices of drugs for prinzmetal angi. It may be used to treat high blood pressure in pregnancy. ATE is a selective β1 receptor antagonist, a drug belonging to the group of beta-blockers, a class of drugs used primarily in cardiovascular diseases. ATE works by slowing down the heart and reducing its workload.

MATERIALS AND METHODS

Reagents and Chemicals

ATE and NIFE reference standards were supplied by J. B. Chemicals, Ankleshwar, India. Pharmaceutical dosage form
Stability-Indicating RP-HPLC Method for Simultaneous Determination of Atenolol and Nifedipine

Standard Stock Solution of ATE and NIFE (Mixed)\textsuperscript{6-8}
Accurately weigh 50 mg ATE and 20 mg NIFE. Dissolve in methanol and make volume up to 10 mL. Standard solution contains 5,000 mg/mL of ATE and 2,000 mg/mL NIFE (stock solution-I).

- Take 0.05 mL from stock solution and make up volume 10 mL with MP = 25 µg/mL ATE and 10 µg/mL NIFE
- Take 0.1 mL from stock and make up volume 10 mL with MP = 50 µg/mL ATE and 20 µg/mL NIFE
- Take 0.15 mL from stock and make up volume 10 mL with MP = 75 µg/mL ATE and 30 µg/mL NIFE
- Take 0.2 mL from stock and make up volume 10 mL with MP = 100 µg/mL ATE and 40 µg/mL NIFE
- Take 0.3 mL from stock and make up volume 10 mL with MP = 150 µg/mL ATE and 60 µg/mL NIFE

Chromatographic Equipments
HPLC [ChemStation-Agilent (1100) gradient system UV detector] was used to develop and validate this method. The chromatographic separation was carried out at ambient temperature by using C\textsubscript{18} column (Agilent 4.6 × 250 mm). Class ‘A’ borosilicate glassware was employed for volumetric and general purpose in the study.

Preparation of Mobile Phase
A mixture of methanol and o-phosphoric acid (0.1% in water) in the ratio of 70:30 was prepared (filtered and degassed).

Preparation of Standard Solution
The standard stock solution of NIFE and ATE was prepared by dissolving 20 mg of NIFE and 50 mg of ATE in 10 mL MeOH to get a solution containing 2,000 µg/mL NIFE and 5,000 µg/mL ATE (stock solution-I). Then, this stock solution-I was diluted to get solutions, containing 10 to 60 µg/mL NIFE and 25 to 150 g/mL ATE.

Forced Degradation Sample Preparation
This study was carried out to check the effective separation of ATE and NIFE and their degradation product. Forced degradation study was performed to evaluate the stability-indicating properties of the method. Forced degradation study was carried out by treating samples with acid, alkali, hydrogen peroxide (oxidative degradation), and neutral (water). These are discussed below:

Acidic Degradation
A sample (0.2 mL) was taken from the stock solution-I and transferred in a 10 mL volumetric flask. To this, 5 mL 1 N hydrochloric acid (HCl) was added, the solution was made up to the mark with the mobile phase, and the solution was heated at 60°C for 30 minutes. After 30 minutes, flask was removed and cooled to room temperature. The chromatogram was recorded for this solution.

Alkaline Degradation
A sample (0.2 mL) was taken from stock solution-I and transferred in a 10 mL volumetric flask. To this, 5 mL 1 N sodium hydroxide (NaOH) was added, the solution was made up to the mark with the mobile phase, and the solution was heated at 60°C for 30 minutes. After 30 minutes, flask was removed and cooled to room temperature. The chromatogram was recorded for this solution.

Oxidative Degradation
A sample of 0.2 mL was taken from stock solution-I and transferred in a 10 mL volumetric flask. To this, 5 mL 10 % hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) was added, the solution was made up to the mark with the mobile phase, and the solution was heated at 60°C for 30 minutes. After 30 minutes, flask was removed and cooled to room temperature. The chromatogram was recorded for this solution.

Hydrolysis
A 0.2 mL sample was taken from stock solution-I and transferred in a 10 mL volumetric flask. To this, 5 mL water was added, the solution was made up to the mark with the mobile phase, and the solution was heated at 60°C for 30 minutes. After 30 minutes, flask was removed and cooled to room temperature. The chromatogram was recorded for this solution.

Method Validation\textsuperscript{9-10}
Method validation was done to show that method is suitable for assay and stability studies of ATE and NIFE. The method validation was carried out as per ICH guidelines for precision, linearity, accuracy, robustness, LoD, and LoQ, in analytical solution [ICH 1996, Q2 (R1) ICH, 2005].

LoD
The LoD value was calculated from the calibration curve. SD is the standard deviation of the response of the minimum detectable drug and slope of the calibration curve. The formula for LoD is given below—

\[ \text{LoD} = 3.3 \times \text{avg SD/slope} \]

LoQ
The LoQ value was calculated from the calibration curve. SD is the standard deviation of the response of the minimum detectable drug and slope of the calibration curve. The formula for LoQ is given below—

\[ \text{LoQ} = 10 \times \text{avg SD/slope} \]

RESULTS AND DISCUSSION\textsuperscript{2-5,12,13}
Optimization of Chromatographic Condition (Method Development)
Individual drugs and their mixture were taken in various combinations of mobile phase for chromatographic study. The proper selection of the method depends upon the nature of the sample (ionic/ionizable/neutral molecule, its molecular weight, and solubility). Here, the RP-HPLC method was selected for initial separation because of its simplicity and suitability.
Various mobile phases, such as acetonitrile and water, and acetonitrile and buffer, were tried. Finally, ortho-phosphoric acid (0.05%) and methanol were used in the ratio of 65:35 for further chromatographic study. The results of these trials are reported in Figures 3A to C.

**Optimised Chromatographic Condition**

HPLC: Agilent (1100) gradient system UV detector  
Column: C18 (4.6 × 250 mm id)  
Particle size packing: 5 mm  
Mobile phase: methanol-0.05% OPA with TEA (70:30)  
Detection wavelength: 233 nm  
Flow rate: 0.7 mL/min  
Temperature: ambient  
Sample size: 20 mL (Table 1)  

**LoD**

Table 2 shows the minimum detection limit of ATE and NIFE.  
LoD of ATE and NIFE were found to be 0.1415 and 0.1834, respectively. These LoD values indicate that the method is suitable to determine a lower concentration of ATE and NIFE, and it confirms that the developed method is sensitive for determination.

**LoQ**

Table 3 shows the minimum quantitation limit of ATE and NIFE.  
LoQ of ATE and NIFE was found to be 0.4289 and 0.5558, respectively. These LoQ values indicate that the method is suitable to determine a lower concentration of ATE and NIFE and confirms that the developed method is sensitive for determination.

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Chromatographic condition</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mobile phase-MeOH:water (70:30) (0.1% OPA in water) Flow rate-1 mL/min, λ <em>max</em> -233 nm</td>
<td>Not sharp peak Rejected</td>
</tr>
<tr>
<td>2</td>
<td>Mobile phase-MeOH:water (60:40) Flow rate-1 mL/min, λ <em>max</em> -233 nm</td>
<td>Not sharp peak Rejected</td>
</tr>
<tr>
<td>3</td>
<td>Mobile phase-MeOH:water (70:30) (0.1% OPA in water) Flow rate-0.7 mL/min, λ <em>max</em> -233 nm</td>
<td>Sharp peak Selected</td>
</tr>
</tbody>
</table>

Table 1: Various trials and optimization of chromatographic condition

Table 2: Limit of detection (LoD)

<table>
<thead>
<tr>
<th>Atenolol</th>
<th>Nifedipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula LoD = 3.3 × avg SD/slope</td>
<td>Formula LoD = 3.3 × avg SD/slope</td>
</tr>
<tr>
<td>Avg SD = 3.72</td>
<td>Avg SD = 2.27</td>
</tr>
<tr>
<td>Slope = 86.72</td>
<td>Slope = 40.84</td>
</tr>
<tr>
<td>LoD = 3.3 × 3.72 ÷ 86.72 = 0.1415</td>
<td>LoD = 3.3 × 2.27 ÷ 40.84 = 0.1834</td>
</tr>
</tbody>
</table>

Figures 3:A to C: Chromatograms of trial for method development (ATE and NIFE); A. MeOH:water (70:30) (0.1% OPA in water) flow rate-1 mL/min; B. MeOH:water (60:40); C. MeOH:water (70:30) (0.1% OPA in water) flow rate-0.7 mL/min
Forced Degradation Study
ATE and NIFE standard sample was undergone acidic, alkaline, oxidative, and hydrolytic degradation. The sample shows 13.5, 20, 16.5, and 16.5% degradation in acidic, alkaline, oxidative, and hydrolysis conditions, respectively. The degradation was under acceptance criteria. It shows stability-indicating the properties of the method. The chromatograms of the sample are shown in Figures 4-7.

CONCLUSION
The aim of the current chromatographic study was to develop a stability-indicating HPLC method for the estimation of ATE and NIFE in bulk and tablet dosage form. Hence, the stability-indicating RP-HPLC method has been developed and validated for LoD and LoQ. Forced degradation study was also performed under four different stress conditions.

From the chromatographic study, we concluded that the developed method is more linear, accurate, precise, reliable, and reproducible for routine analysis of ATE and NIFE in bulk and tablet dosage form. So one can perform validation and forced degradation study.

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