A Validated RP-HPLC Method for Simultaneous Estimation of Nebivolol Hydrochloride and S-Amlodipine Besylate in Tablet Dosage Forms

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase high-pressure liquid chromatographic method has been developed for the simultaneous estimation of S(-) Amlodipine besylate and Nebivolol Hydrochloride for tablet formulations. The chromatographic separation was achieved on Zorbax SB CN column (250 × 4.6 mm, 5µ particle size) in isocratic mode with mobile phase consisting of Ammonium acetate buffer (pH 4.5): acetonitrile (50:50 v/v). The flow rate was 1ml / min and effluent was monitored at 274 nm PDA detection. The retention times of S(-) Amlodipine besylate and Nebivolol Hydrochloride were 9.590 ± 0.04 and 13.56 ± 0.05 minutes respectively. The linearity of the method was studied over the concentration range of 240-640 µg/ml for S(-) Amlodipine besylate and 120-320 µg/ml for Nebivolol Hydrochloride. The limit of detection and the limit of quantification for S(-) Amlodipine besylate and Nebivolol Hydrochloride were found as 3µg/ml and 0.7µg/ml and 10µg/ml and 2µg/ml respectively. The proposed method was applied for the quantitative determination of S(-) Amlodipine besylate and Nebivolol Hydrochloride in commercial combination formulations.

Keywords: S(-) Amlodipine Besylate, Nebivolol Hydrochloride, RP-HPLC, Validation, Simultaneous Estimation.

1. INTRODUCTION

Nebivolol hydrochloride[1-2] (NBH) is chemically, α,α-[iminobis(methylene)] bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2- methanol] hydrochloride which is a highly selective β1 receptor antagonist without partial agonist activity. It is official in Martindale, the extra pharmacopeia. Amlodipine besylate[3] (AMB) is chemically R.S-2-[(2- aminoethoxy)methyl]-4-(2-chloroethyl)-3-ethoxy carbonyl-5-methoxy carbonyl-6-methyl-1,4-dihydro pyridine benzene sulphonate used in the treatment of hypertension and congestive heart failure. It is official in British pharmacopoeia[4]. Many methods have been described in the literature for the determination of Nebivolol hydrochloride and Amlodipine besylate individually and in combination with other drugs.[5-16] So far, no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing Nebivolol...
hydrochloride (2.5 mg) and Amlodipine besylate (5 mg) is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Nebivolol hydrochloride and Amlodipine besylate in pharmaceutical dosage forms. The present study describes a precise, accurate, specific and sensitive RP-HPLC method as per ICH guidelines for the simultaneous estimation of NBH and AMB in tablets.\[17\]

2. EXPERIMENTATION

2.1. Equipment

Chromatographic separation was performed on HPLC waters alliance 2695, having 2996 photo diode array detector and Rheodyne injector with 20µl loop volume. Empower software was applied for data collecting and processing.

2.2. Reagents and chemicals

Acetonitrile HPLC grade was procured from E Merck (India) Ltd., Mumbai. Working standard of NBH was provided by Cadila Health care pvt Ltd., Ahmadabad and AMB was provided by Emcure Pharmaceutical Ltd., Pune. Potassium di hydrogen phosphate and triethyl amine were A.R grade from Merck chemicals Mumbai, India. Water HPLC grade was obtained from a Milli-Q RO water purification system. Tablets of two different brands, T1 (Cipla) and T2 (Cadila pharmaceuticals) having combination of NBH (2.5 mg) and AMB (5 mg) were used.

2.3. Optimized chromatographic Condition

Zorbax SB CN column (250 × 4.6 mm, 5µ particle size) Agilent column was used as the stationary phase. The mobile phase comprised of acetonitrile and 0.01M ammonium acetate buffer and in proportion of 50:50 (v/v) with pH adjusted to 4.5±0.5 by using triethyl amine. Injection volume was 20µl and run time was 20min and flow rate 1.0ml/min. The column was maintained at ambient temperature and the eluent was detected at 274 nm.

2.4. Standard preparation

Standard stock solution (1000µg/ml) of Nebivolol hydrochloride and Amlodipine besylate were prepared separately in mobile phase comprised of acetonitrile and 0.01M ammonium acetate buffer and in proportion of 50:50 (v/v) with pH adjusted to 4.5±0.5 by using triethyl amine. The working standard solutions were prepared and further diluted in mobile phase to Nebivolol hydrochloride and Amlodipine besylate contain a mixture of in over the linearity ranges from 120-320 µg/ml and240-640 µg/ml.

2.5. Sample preparation

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 5 mg of NBH and 10mg of AMB was weighed and transferred to a 25 ml volumetric standard flask and added 10 ml of mobile phase. The sample was kept in an ultrasonic bath for 20 min and further diluted to 25 ml by using mobile phase to get 200µg/ml of NBH and 400µg/ml of AMB. Then it is filtered through 0.22µ membrane filter paper. 20µl of this solution was injected in to HPLC system and chromatograms were recorded. Concentrations of NBH and AMB in the tablet formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table 1.

Table 1: Table for Assay

<table>
<thead>
<tr>
<th>Tablet formulation</th>
<th>Drug</th>
<th>Amount present (mg)</th>
<th>Amount found* (mg/tab)</th>
<th>% label claim*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>NBH</td>
<td>2.5</td>
<td>2.49</td>
<td>99.6%</td>
</tr>
<tr>
<td></td>
<td>AMB</td>
<td>5.0</td>
<td>5.02</td>
<td>102.0%</td>
</tr>
<tr>
<td>T2</td>
<td>NBH</td>
<td>2.5</td>
<td>2.52</td>
<td>100.8%</td>
</tr>
<tr>
<td></td>
<td>AMB</td>
<td>5.0</td>
<td>4.98</td>
<td>99.6%</td>
</tr>
</tbody>
</table>

T1 and T2 are two different brands of
tablet formulations. NBH and AMB denotes Nebivolol hydrochloride and S-Amlodipine Besylate respectively.*Each value is average of six determinations.

3. RESULTS AND DISCUSSION

The proposed HPLC method required fewer reagents and materials and it is simple and less time consuming. This method could be used in quality control test in Pharmaceutical industries. The chromatograms sample and standard solution of NBH and AMB were shown in (Fig.1) and (Fig2). There was clear resolution between Amlodipine besylate and Nebivolol hydrochloride with retention time of 9.51 and 13.56 minutes respectively.

3.1. VALIDATION OF THE METHOD

3.1.1. System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions (Table.2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ±3% standard deviation range during performance of the method. Here tailing factor for peaks of NBH and AMB was less than 2% and resolution was satisfactory. The peaks obtained for NBH and AMB were sharp and have clear base line separation.

3.1.2. Linearity

The response for the detector was determined to be linear over the range of 120-320µg/ml (120,160,200,240,280,320) of NBH and 240-640µg/ml (240,320,400,480,560,640) for AMB. Each of this concentration was injected in six times to get reproducible response. The calibration curve was plotted as concentration of the respective drug versus the response at each level. The proposed method was evaluated by its correlation coefficient and intercept value calculated in the statistical study. The results show that an excellent correlation exits between response factor and concentration of drugs within the concentration range indicated above. (Table 3)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Nebivolol Hydrochloride</th>
<th>Amlodipine Besylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Capacity factor</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Theoretical plate</td>
<td>2873</td>
<td>4140</td>
</tr>
<tr>
<td>3</td>
<td>Asymmetry of the peak</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>Retention time (min)</td>
<td>13.85</td>
<td>9.75</td>
</tr>
<tr>
<td>5</td>
<td>Resolution</td>
<td>4.96</td>
<td></td>
</tr>
</tbody>
</table>

Table No2: System Suitability
Table 3: Summary of Analytical Method Validation

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Acceptance criteria</th>
<th>Nebivolol Hydrochloride</th>
<th>Amlodipine Besylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity</td>
<td>$r^2=0.995$ to 1.0</td>
<td>0.9986</td>
<td>0.9992</td>
</tr>
<tr>
<td>2</td>
<td>Specificity</td>
<td>No interference with placebo</td>
<td>Specific</td>
<td>Specific</td>
</tr>
<tr>
<td>3</td>
<td>Accuracy (Recovery studies)</td>
<td>Recovery 98.0-102.0%</td>
<td>99.98%</td>
<td>101.02%</td>
</tr>
<tr>
<td>4</td>
<td>Precision</td>
<td>RSD NMT 2.0%</td>
<td>0.1052</td>
<td>0.4622</td>
</tr>
<tr>
<td>5</td>
<td>Robustness</td>
<td>NMT±1%</td>
<td>0.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>6</td>
<td>Limit of detection µg/ml</td>
<td>--------------------</td>
<td>0.7µg/ml</td>
<td>3µg/ml</td>
</tr>
<tr>
<td>7</td>
<td>Limit of Quantification µg/ml</td>
<td>---------------------</td>
<td>2µg/ml</td>
<td>10µg/ml</td>
</tr>
</tbody>
</table>

3.1.3. Precision and Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard NBH and AMB corresponding to 80%, 100%, and 120% of the label claim was added to pre-analyze sample of tablet dosage form separately. The recovery studies were carried out six times at each level of recovery. From the data obtained, recoveries of standard drugs were found to be accurate (Table 3). The %RSD of interday and intraday precision obtained was less than 2% for both the drugs. The intraday and interday precision of AMB was 0.1052 and 0.2526 and AMB was 0.4622 and 0.2723 respectively. From the data obtained, the developed HPLC method was found to be precise and accurate.

3.1.4. Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/ml was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both NBH and AMB from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

3.1.5 LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as $3 \hat{\sigma}/S$ and $10 \hat{\sigma}/S$, respectively as per ICH guidelines, where $\hat{\sigma}$ is the standard deviation of the response (y-intercept) and $S$ is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for NBH and AMB was found to be 0.7µg/ml and 3µg/ml, respectively. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 2µg/ml and 10µg/ml for NBH and AMB respectively. (Table 3)

3.1.6. Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instrument like Waters HPLC and Agilent HPLC by different operators using different columns of similar type like hypersil Cyano, Zorbax CN column. Robustness of the method was determined by making slight changes in the experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the
mobile phase and the chromatographic characteristics were evaluated. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust.

4. CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Nebivolol hydrochloride and S-Amlodipine Besylate in combined dosage forms is accurate, precise, linear, rugged, robust, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolutions studies.

5. ACKNOWLEDGEMENTS

The authors are thankful to Cadila Health care Pvt Ltd., Ahmadabad for providing gift samples of Nebivolol hydrochloride and Emcure Pharmaceutical Ltd., Pune for providing gift samples of S-Amlodipine Besylate.

6. REFERENCES


Dosage Form by Q-Analysis Method. 


