DEVELOPMENT OF STABILITY-INDICATING HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL HYDROCHLORIDE AND VALSARTAN IN TABLET DOSAGE FORM

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ABSTRACT

The purpose of the present study is to develop a new, simple, rapid, accurate and precise HPLC method for simultaneous estimation of Nebivolol Hydrochloride and Valsartan in tablet dosage form. The chromatographic separation was done by YMC Pack Pro C-18 (4.6mm*150mm, 3µm) column and a mobile phase was ACN: Methanol: KH2PO4 (30:30:40). detection wavelength was 279 nm, flow rate was 1.0 ml/min, injection volume was 20 µl, temperature of column was 30°C and auto sampler compartment was ambient. The retention time of Nebivolol Hydrochloride and Valsartan were found to be 3.62 min and 7.56 min, respectively. The method was found to be simple, accurate, economical, Precise and reproducible. There was no interference of any degradants and excipient in the determination of drugs in marketed formulation. So the method can be successfully applied for routine analysis.

KEY WORDS: HPLC, Nebivolol Hydrochloride, Valsartan, Forced degradation, Stability indicating, ICH.
INTRODUCTION

Nebivolol Hydrochloride\textsuperscript{[1-3]}
Nebivolol Hydrochloride known as (IRS, 1'RS)-I, 1'-(2RS, 2'SR)-bis (6flurochroman- 2-yl))-2,2'-iminodiethanol hydrochloride. It is white to off white powder generally soluble in methanol. Nebivolol is a selective β1-receptor antagonist. Activation of β1-receptors by epinephrine increases the heart rate and the blood pressure and the heart consumes more oxygen. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure.

![Fig 1. Structure of Nebivolol Hydrochloride](image)

Valsartan\textsuperscript{[4-6]}
Valsartan is known as N-pentanonyl-N-[2'-(lH-tetrazol-5-yl) biphenyl4-ylmethyl]-L-valine. It is white to almost white powder generally soluble in methanol. Valsartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure.

![Fig 2. Structure of Valsartan](image)

Combination of these drugs containing Nebivolol Hydrochloride(5 mg) and Valsartan(80 mg) are available in market as tablet. The Literature survey reveals that these drugs have been
analysed individually and in combination by many analytical methods, but there is no method available for the estimation of Nebivolol Hydrochloride and Valsartan by force degradation study. Therefore it was thought of interest to develop and validate Stability-indicating HPLC Method for the Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan in tablet Dosage Form.

**Stability Study**[7-8]

A Stability Indicating Assay Method (SIAM) is a “Quantitative analytical procedure used to detect a decrease in the amount of the active pharmaceutical ingredient (API) present due to the degradation.”

According to FDA guidelines, a SIAM is defined as “A validated analytical procedure that accurately and precisely measures active ingredients (drug substance or drug product) free from potential interferences like degradation products, process impurities, excipient, or other potential impurities”, and the FDA recommends that all assay procedures for stability studies should be stability indicating.

**Purpose of Stability Testing of the Drugs**33

1. SIAM is developed routinely by stressing the API under conditions exceeding those normally used for accelerated stability testing.

2. In addition to demonstrating specificity in SIAM, stress testing, also referred to as forced degradation, also can be used to provide information about degradation pathways and products that could form during storage and helps to facilitate formulation development, manufacturing, and packaging.

3. Stressing the API in both solutions and in solid-state form generates the sample that contains the products most likely to form under most realistic storage conditions, which is in turn used to develop the SIAM.

4. In simplest terms, the goal of the SIAM is to obtain baseline resolution of all the resulting products (the API and all the degradation products) with no co-elutions.

5. Generally, the goal of these studies is to degrade the API 5-10 %. Any more than this and relevant compounds can be destroyed, or irrelevant degradation products produced.
Types of SIAM
1. Specific Stability Indicating Assay Method: It can be defined as “a method that is able to measure unequivocally the drugs in the presence of all degradation products, in the presence of excipients and additives, expected to be present in the formulation.”
2. Selective Stability Indicating Assay Method: It can be defined as “a method that is able to measure unequivocally the drugs and all the degradation products in the presence of excipients and additives which are expected to be present in the formulation.

MATERIALS AND METHOD

Instruments
The HPLC system used for forced degradation was Agilent technologies 1200 infinity series equipped with PDA detector. Cintex digital water bath was used for hydrolysis studies. Photo stability study was carried out in photo stability chamber. Thermal stability study was carried out in dry air oven. All weighing were done on analytical balance. Ultrasonicator and pH meter was used for preparation of solution.

Chemicals
Nebivolol Hydrochloride and Valsartan pure drug samples are obtained from Intas pharmaceuticals, ahmedabad and combination tablets containing 5 mg of Nebivolol Hydrochloride and 80mg of Valsartan which is obtained with trade name of Nebicard V are used. Acetonitrile [HPLC from Merck Pvt.Ltd], Methanol [HPLC from Merck Pvt Ltd.], KH₂PO₄ (Merck Pvt Ltd), Hydrochloric acid and hydrogen peroxide [Merck Pvt. ltd.], Sodium peroxide [Finar reagent] was used throughout the work.

HPLC Conditions
YMC Pack Pro C-18 (4.6mm*150mm, 3µm) column was used as stationary phase, which is maintained at 30ºC and mobile phase containing ACN: Methanol: Buffer taken in the ratio 30:30:40 with pH adjusted to 3, flow rate was 1mL/min. Mobile phase was filtered and degassed for 10 min by sonicator. Injection volume of 20 µl was injected into the system and the effluents were detected at wavelength 279 nm.

Preparation of Mobile Phase
Prepare 0.05M Potassium Dihydrogen Phosphate by dissolving 6.8 gm of Potassium Dihydrogen phosphate in 1000 ml of water. adjust pH 3.0 with OPA solution. This solution
was sonicated for 5 min for degassing and filtered through 0.45µ millipore filter. Prepare the ratio of ACN: Methanol: Potassium Dihydrogen Phosphate (30:30:40).

**Preparation of Standard Solution**

50 mg of Nebivolol Hydrochloride and 50 mg of Valsartan were taken and transferred to 50 ml volumetric flask separately and volume was made up with diluent (Stock solution-1000 µg/ml Nebivolol Hydrochloride and 1000 µg/ml Valsartan). Take 5 ml from Nebivolol stock solution into 50 ml volumetric flask and volume was made up with diluent (100 µg/ml Nebivolol). Make further dilution by taking 1 ml from 100 µg/ml Nebivolol solution into 10 ml volumetric flask and volume was made up with diluent (10 µg/ml Nebivolol). Similarly take 1.6 ml from 1000 µg/ml Valsartan solution into 10 ml volumetric flask and volume was made up with diluent (160 µg/ml).

**Preparation of Sample Solution**

The average weight of 20 tablets was determined and was ground in a mortar. An accurately weighed amount of powder equivalent to 5 mg of Nebivolol Hydrochloride or 80 mg Valsartan was transferred to 50 ml volumetric flask. About 30 ml of diluent was added and sonicated for 5 minutes to ensure complete solubilisation of drug. After sonication, volume was made up to the mark with diluent (stock solution). From this stock solution 1 ml of solution was pipetted out in 10 ml of volumetric flask and volume was made up to the mark with diluent. (10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan).

**Acid Degradation**

10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan was pipetted out from sample solution, 1 ml of 0.1 N hydrochloric acid was added and kept at room temperature for 2 hours in water bath. After that it was neutralized by adding 1 ml of 0.1 N NaOH & volume was made up to 10 ml with diluent. Sample solution was injected in HPLC and the peak area and peak shape was observed.

**Base Degradation**

10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan was pipetted out from sample solution, 1 ml of 0.1 N Sodium Hydroxide was added and kept at room temperature for 2 hours in water bath. After that it was neutralized by adding 1 ml of 0.1 N HCL & volume was made up to 10 ml with diluent. Sample solution was injected in HPLC and the peak area and peak shape was observed.
Peroxide Degradation
10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan was pipetted out from sample solution, 1 ml of 3% H₂O₂ was added and kept at room temperature for 2 hours in water bath. After that volume was made up to 10 ml with diluent. Sample solution was injected in HPLC and the peak area and peak shape was observed.

Photolytic Degradation
Tablet Powder equivalent to 10 mg Nebivolol Hydrochloride and 160 mg Valsartan was taken. It was exposed to direct sun light for 1 hour. After that powder transferred to the 50 ml volumetric flask and diluted to obtain final concentration of 10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan. Sample solution was injected in HPLC and the peak area and peak shape was observed.

Thermal Degradation
Tablet Powder equivalent to 10 mg Nebivolol Hydrochloride and 160 mg Valsartan was taken. It was kept in hot air oven at 60°C for 1 hour. After that powder transferred to the 50 ml volumetric flask and diluted to obtain final concentration of 10 µg/ml Nebivolol Hydrochloride and 160 µg/ml Valsartan. Sample solution was injected in HPLC and the peak area and peak shape was observed.

RESULT

Final chromatogram

![Fig. 3 Final Chromatogram of Nebivolol Hydrochloride (10mcg/ml) and Valsartan (160mcg/ml)]
Name | Retention | Area  | Asymmetry | Resolution | Theoretical plates
---|---|---|---|---|---
Nebivolol | 3.627 | 337948 | 0.750 | 0.00000 | 4515
Valsartan | 7.560 | 4222631 | 0.980 | 13.02060 | 6016

**Force Degradation**

![Fig. 4 Chromatogram of Sample for acid degradation](image1)

![Fig. 5 Chromatogram of Sample for base degradation](image2)

![Fig. 6 Chromatogram of Sample for peroxide degradation](image3)
Table 1 Degradation Summary

<table>
<thead>
<tr>
<th>Condition</th>
<th>NEBI</th>
<th>VAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Tab_0.1N HCL at RT for 2 hrs</td>
<td>23.24%</td>
<td>14.27%</td>
</tr>
<tr>
<td>Base Tab_0.1N NaOH at RT for 2hrs</td>
<td>10.87%</td>
<td>7.53%</td>
</tr>
<tr>
<td>( \text{H}_2\text{O}_2 ) Tab_3% H2O2 at RT for 2 hrs</td>
<td>20.18%</td>
<td>8.74%</td>
</tr>
<tr>
<td>Photo Tab_Sun light Exposure for 1 hr</td>
<td>9.82%</td>
<td>8.79%</td>
</tr>
<tr>
<td>Thermal Tab_Thermal at 60°C for 2hrs</td>
<td>12.57%</td>
<td>9.68%</td>
</tr>
</tbody>
</table>

CONCLUSION

Hence, we can conclude that the developed HPLC method is simple and rapid as it separates components with good chromatographic criteria. Method has short run time and all degradants are well separated from drug. Moreover, the method is quite sensitive, economic, fast and reliable to qualify as well as to quantify components in microgram quantities. Here, Specific method gives spectrally pure active peak. Nothing is co-eluted in drug peak and
there is no interference in peak purity. The simplicity of the method allows its application in the laboratory for routine quality check as well as for the stability studies for the formulated product. Stability indicating power of method has been proved.

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REFERENCES
1. Indian pharmacopoeia; Indian Pharmacopoeia Commission, Government of India ministry of health and family welfare, Controller of India, 2010; 6(3):1758-59.