SIMULTANEOUS DETERMINATION OF NEBIVOLOL AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT: A Simple, reverse phase high performance liquid chromatography (HPLC) method was developed for the determination of Nebivolol and Hydrochlorothiazide in combined tablet dosage from. A octadecyl silane (ODS) C$_{18}$ column (250 X 4.6 mm), with mobile phase methanol: water (60:40 v/v) adjusted to pH 3:2 with o-phosphoric acid was used. The flow rate was 1.0 ml /min and the effluent was monitored at 281.0 nm. The retention times for Nebivolol and Hydrochlorothiazide were 2.628 and 7.042 minutes, respectively. The linearity range was found to be 5 to 50 µg/ml for Nebivolol and 12.5 to 125 µg/ml for Hydrocholothiazide. The propose method was also validated.

KEY WORDS : NEBIVOLOL, HYDROCHLOROTHIAZIDE, RP-HPLC

INTRODUCTION
Hydrochlorothiazide (HCTZ), 6-chloro-3,4-dihydro-7-sulfamoyl-2H-1,2,4-benzothia-di-azine-1,1-dioxide, is a thiazide diuretic. It increases sodium and chloride excretion in distilled convoluted tubule. Nebivolol (NBV), α,α'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzpyran-2-methanol], is used for treatment of hypertension through vascular endothelial nitric oxide releasing capabilities and β$_1$- antagonist action.

Literature survey reveals many analytical methods, including spectroscopic and chromatographic methods, for the quantitative determination of HCTZ alone or in combination with other antihypertensive drugs. While NBV has been reported to be estimated using spectrophotometric and HPLC method. However no method is reported for simultaneous estimation of these two drugs in tablet. Hence the present work was attempted to develop accurate simple and sensitive method for simultaneous estimation of NBV and HCTZ.

MATERIALS AND METHODS
Reagents and Chemicals
Methanol, water of HPLC grade and o-phosphoric acid AR grade were procured from Merck Co., Mumbai. The drug samples, NBV and HCTZ were obtained from Hetero Drugs limited, Medak and Golden Cross, Daman respectively.

Chromatographic conditions
A liquid chromatographic sepration was performed on Shimadzu isocratic liquid chromatographic system model LC-10 AD, equipped with Rheodyne injector model 7725 with 20 µl fixed loop, UV-visible detector mode SPD-10A, Mayur analytical Pvt. Ltd. The C$_{18}$ column intersil (250 X 4.6 mm, particle size 5µ) was used as stationary phase. The mobile Phase used was a mixture of methanol and water in the ratio (60:40v/v) adjusted to pH 3.2 with o-phosphoric acid and was filtered before use through membrane filter of 0.2µ size. The elution was carried out at the flow rate of 1ml /min. Detection was carried out at 281 nm at ambient temperature.

Preparation of standard stock solutions
Standard stock solutions for NBV and HCTZ were prepared by dissolving 25 mg of both drugs separately in methanol and volume was made up to 25 ml.

Preparation of working standard solutions
Working standard solutions were prepared by diluting 10 ml of the stock solution to 100 ml with mobile phase (100µg/ml).

Standard calibration curve
Various dilutions were prepared by taking 1 to 10 ml and volumes were adjusted to 10 ml with mobile phase. The calibration curve was constructed by plotting peak
area against the corresponding drug concentrations. NBV was found to be linear in the concentration range of 5 to 50 µg/ml, while HCTZ was found to be linear in the concentration range of 12.5 to 125 µg/ml.

**Estimation of drug in commercial tablet formulation**

For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Accurately weighed tablet powder equivalent to 5mg of NBV and 12.5mg of HCTZ was dissolved in methanol and shaken for 15 minutes. The volume was made up to 100ml and filtered through syringe membrane filter. Aliquot portion of this solution was diluted with mobile phase to produce concentration of 5 µg/ml for NBV and 12.5 µg/ml for HCTZ and filtered through syringe membrane filter. Standard solution of same concentrations was prepared from working stock solutions. Equal volumes of 20 µl of standard and sample solutions were injected separately after the equilibration of stationary phase and area under curve noted.

Amount of drug in the tablet formulation was calculated by using the following formula,

\[
\text{Percentage Labeled Claim} = \frac{A_t}{A_s} \times \frac{D_s}{D_t} \times \frac{W_t}{W_s} \times \frac{A}{LC} \times 100
\]

Where,

- \( A_t \) = Area under the curve for tablet sample solution
- \( A_s \) = Area under the curve for standard solution
- \( W_s \) = Weight of standard (g)
- \( W_t \) = Weight of tablet sample (g)
- \( D_t \) = Dilution factor for tablet sample
- \( D_s \) = Dilution factor for standard sample
- \( A \) = Average Weight of tablet

**VALIDATION OF METHOD**\(^{9,10,11}\)

**Accuracy**

The accuracy of the experiment was established using recovery technique i.e. by external standard addition method. The result of recovery analysis is presented in Table No 3. The result of recovery was well within the acceptable limit. Hence the method is accurate.

**System suitability**

For system suitability, six replicates of standard solutions were injected and parameters studied were number of theoretical plates, peak area, resolution, retention time and asymmetry of peak. The relevant data is shown in Table No 1.

**Precision**

Replicate estimation of both NBV & HCTZ in the same batch of tablet were done by the proposed method, which yielded quite concurrent results, indicating reliability of the method. The value of SD and RSD were within the prescribed limit of 2% showing high precision of the method. (Table No 2).

**Ruggedness**

The proposed method was repeated under different condition like at different time intervals, on different days and by different analyst. The results are shown in Table No 4, proved that the method was reproducible.

**Linearity and range**

During linearity study, it was observed that the absorbance values of NBV and HCTZ in the marketed formulation were linear in the range of 80-120% of test concentration with \( R^2 \) close to one for this method of analysis (Fig. 3).

**RESULTS AND DISCUSSION**

Estimation of NBV and HCTZ in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The typical chromatogram of NBV and HCTZ for standard drug is shown in Fig 1. The overline UV spectrum of NBV and HCTZ is shown in Fig. 2. The Percentage of individual drug found in formulations, along with SD value is shown in Table 1. The results of analysis show that the amount of drugs were in good agreement with the label claim of the formulation.

**CONCLUSION**

The present study comprises of RP- HPLC method to determine NBV and HCTZ from tablet dosage form. During the study of validation parameters namely accuracy, precision (SD and RSD), ruggedness (interday, intraday and different analyst), linearity and range, it was observed that the proposed method was accurate, precise, rapid, rugged and reproducible for the simultaneous determination of NBV and HCTZ in tablet dosage form. The developed method could be conveniently adopted for routine analysis in quality control laboratories.

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Fig. 1: Chromatogram obtained for laboratory mixture of NBV and HCTZ. Showing retention time for HCTZ = 2.628 min and NBV = 7.042.

Fig. 2: Overlaid Spectra of NBV and HCTZ

Fig. 3: Linearity and range study for NBV and HCTZ
Table No. 1- System suitability parameters

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameter</th>
<th>Value</th>
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<td></td>
<td>NBV</td>
<td>HCTZ</td>
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</tr>
<tr>
<td>1</td>
<td>Retention time (min)</td>
<td>7.0464</td>
<td>2.6302</td>
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<td>2</td>
<td>Asymmetry</td>
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<tr>
<td>4</td>
<td>Peak area</td>
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<td>5</td>
<td>Resolution</td>
<td>8.371</td>
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Table No. 2- Results of analysis of tablet formulation and statistical data

<table>
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<tr>
<th>Sample</th>
<th>Label claim mg/tablet</th>
<th>% Label claim*</th>
<th>SD</th>
<th>RSD</th>
<th>SE</th>
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<tr>
<td>Brand I Nebistar-H</td>
<td>NBV 5 mg</td>
<td>99.794</td>
<td>0.3988</td>
<td>0.0040</td>
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<td></td>
<td>HCTZ 12.5 mg</td>
<td>99.67</td>
<td>0.3242</td>
<td>0.00325</td>
<td>0.1449</td>
</tr>
<tr>
<td>Brand II Nebi-H</td>
<td>NBV 5 mg</td>
<td>100.074</td>
<td>0.4036</td>
<td>0.00403</td>
<td>0.1805</td>
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<tr>
<td></td>
<td>HCTZ 12.5 mg</td>
<td>100.006</td>
<td>0.2959</td>
<td>0.00296</td>
<td>0.1323</td>
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</table>

*Results are mean of five replicates

Table No. 3- Results of recovery study

<table>
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<tr>
<th>Quantity of drug added (µg/ml)</th>
<th>% Recovery* for brand I Nebistar-H</th>
<th>% Recovery* for brand II Nebi-H</th>
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<tr>
<td>NBV</td>
<td>HCTZ</td>
<td>NBV</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>100.19</td>
</tr>
<tr>
<td>4</td>
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<td>6</td>
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<td>99.80</td>
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*Results are mean of five replicates

Table No. 4- Results of ruggedness study

<table>
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<th>Conditions</th>
<th>% Label claim*</th>
<th>SD</th>
<th>RSD</th>
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<tr>
<td></td>
<td>NBV</td>
<td>HCTZ</td>
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<tr>
<td>Intraday study</td>
<td>99.85</td>
<td>99.82</td>
<td>0.7559</td>
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<tr>
<td>Interday study</td>
<td>99.85</td>
<td>100.13</td>
<td>0.7670</td>
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<tr>
<td>Different analyst study</td>
<td>100.32</td>
<td>99.89</td>
<td>0.4255</td>
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</table>

*Results are mean of five replicates

REFERENCES


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10. ICH Q2B; Guidelines on validation of analytical procedure; Methodology, Federal Register, 1996, 60, 27464.


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