SIMULTANEOUS ESTIMATION OF NEBIVOLOL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN BULK AND IN A TABLET DOSAGE FORM BY MULTICOMPONENT AND SIMULTANEOUS ESTIMATION METHOD


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ABSTRACT

Novel, simple, sensitive, rapid, accurate and economical spectrophotometric methods have been developed for simultaneous estimation of Nebivolol Hydrochloride and Hydrochlorothiazide. These methods involve solving of simultaneous equations based on measurement of absorbances at two wavelengths 290 nm and 317 nm. Both the drugs obey the Beer’s law in the concentration ranges employed for these methods. Results of the methods were validated statistically and by recovery studies.

Key words: Nebivolol Hydrochloride, Hydrochlorothiazide, Thiazide diuretics, antihypertensive, β–blockers, Spectrophotometric analysis, Simultaneous equation method, Multicomponent

INTRODUCTION

Nebivolol Hydrochloride (NEB), chemically α, α’- [Iminobis (methylene)] bis [6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-methanol] is a beta-adrenergic blocker. Hydrochlorothiazide (HCTZ), chemically 6-Chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2,4-benzothiadiazine 1,1-dioxide is thiazide diuretics. Nebivolol selectively blocks the β1-adrenoceptor. Nebivolol reduces heart rate, rate of myocardial contractility, decrease systemic blood pressure and increase diastolic pause. The β - blockers are useful prophylactic agents in stable and unstable types of angina. Nebivolol is preferable in patients with bronchospasm, diabetes, peripheral vascular disease or Raynaud’s phenomenon.
Thiazide diuretics inhibit NaCl transport in the distal convoluted tubule (DCT). Furthermore, the renal cortex has a high affinity receptor for thiazide diuretics, and binding of thiazides localizes to the DCT. DCT is the primary site of action of thiazide diuretics. Hydrochlorothiazide initially induces a Na+ and water loss, leading to a fall in plasma volume and extra cellular fluid (ECF), which in turn lowers the cardiac output and blood pressure. On prolonged thiazide treatment the plasma volume and ECF return to normal, but their hypotensive action continues. This is probably due to the reduced sensitivity of the vascular bed to circulating catecholamines and angiotensin. This, in addition to reduced arteriolar oedema, decreases the TPR and lowers arterial BP.\[^5^,\][^6^]

Hydrochlorothiazide is inexpensive, as efficacious as other classes of antihypertensive agents, well tolerated and side effects are also mild. Hydrochlorothiazide has additive or synergistic effects when combined with beta-blockers. The combination of NEB and HCTZ are choice drugs for many low renin hypertensions.\[^4^\]

Literature survey reveals that few HPLC and colorimetric\[^7^,\][^8^,\][^9^,\][^10^,\][^11^,\][^12^,\][^13^,\][^14^] methods have been reported for determination of antihypertensive as single component in bulk, in formulations and also in biological fluids. Also, not a single UV and HPTLC method is reported for simultaneous analysis of NEB and HCTZ. Combination of 5mg of Nebivolol and 12.5 mg of Hydrochlorothiazide are now becoming available to the market by some companies. So to develop new methods to analyze the drugs simultaneously is at most need. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis. The objective of the investigation is to develop and validate a methodology for the estimation of the combined dosage form by simultaneous UV spectroscopic method.

**MATERIALS AND METHODS**

A Shimadzu UV/Visible spectrophotometer model 1601 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the tablet powder. Nebivolol Hydrochloride (Torrent Pharmaceuticals, Ahmedabad), Hydrochlorothiazide (Li-taka Pharmaceuticals,
Pune) and Methanol -AR grade (Qualigens Fine Chemicals, Mumbai) were used in the study.

Method-A

Standard Stock solutions (100 µg/ml) of NEB and HCTZ were prepared by dissolving separately 10 mg of drug in methanol. NEB exhibited \( \lambda_{\text{max}} \) at 282.5 nm and HCTZ showed \( \lambda_{\text{max}} \) at 227,271.5 and 317 nm, but for NEB, 290nm and for HCTZ 317 nm is taken as working \( \lambda \), because at these wavelength there is minimum interference. Absorbance of NEB and HCTZ was obtained at 290nm (\( \lambda_1 \)) and 317 nm (\( \lambda_2 \)), respectively against methanol as blank. NEB and HCTZ showed linearity with absorbances in the range 0-80 g /ml and 0-100 g /ml at their respected selected wavelength. Co-efficients of correlation were found to be 0.9996 for NEB and 0.9998 for HCTZ. The optical characteristics and regression values for the calibration curve are presented in Table 1.

For simultaneous estimation of NEB and HCTZ, 20 g /ml solution of NEB and 50 g/ml solution of HCTZ were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of the solutions of NEB and HCTZ were carried out in the range of 200 to 400 nm for obtaining the overlain spectra (fig.1). Absorbances and absorptivities of standard solution were recorded at selected wavelengths \( \lambda_1 \) (290nm) and \( \lambda_2 \) (317nm). Mixed standard for the pure drug was prepared from the stock solutions. Mixed standards containing 20 g/ml for NEB and 50 g/ml for HCTZ were prepared and their absorbances were recorded at selected wavelengths \( \lambda_1 \) and \( \lambda_2 \).

Concentration of NEB and HCTZ in the powder mixture is found by using equation (i) and (ii).

The method employed simultaneous equations\(^{[15],[16]}\) using Cramer’s rule and matrices

\[
C_{\text{NEB}} = A_1 a_{y_2} - A_2 a_{y_1} / a_{x_1} a_{y_2} - a_{x_2} a_{y_1} \quad (\text{i})
\]

\[
C_{\text{HCTZ}} = A_2 a_{x_1} - A_1 a_{x_2} / a_{x_1} a_{y_2} - a_{x_2} a_{y_1} \quad (\text{ii}).
\]

Where (1) \( a_{x_1} \) and \( a_{x_2} \) are absorptivities of NEB at \( \lambda_1 \) and \( \lambda_2 \) respectively.

(2) \( a_{y_1} \) and \( a_{y_2} \) are absorptivities of HCTZ at \( \lambda_1 \) and \( \lambda_2 \) respectively.

(3) \( A_1 \) and \( A_2 \) are absorbances of mixtures at \( \lambda_1 \) and \( \lambda_2 \) respectively.

Twenty tablets\(^{[17]}\) (brand name Nebicard-H and manufactured by Torrent Pharmaceuticals Ltd., Ahmedabad) were weighed and crushed to a fine powder. An
accurately weighed powder sample equivalent to 10 mg was transferred to a 100ml volumetric flask and dissolved in about 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with methanol. The solution was kept for sonication for about 20 minutes. The solution was filtered through Whatmann filter paper No.41 and was diluted to prepare the concentration of 20 g/ml NEB and 50 g/ml HCTZ.

The absorbances were recorded at selected wavelengths 290nm(λ₁) and 317nm(λ₂) and the amount of drug present in the sample solution were obtained by using equation in the same manner as that was used with pure mixed standards.

Method -B

The multicomponent mode of the instrument was used to calculate the concentration of the individual component in a mixture. Stock solution was prepared in the same manner as that of “simultaneous equation method”. Suitable dilutions of both the drugs (20 g/ml NEB & 50 g/ml HCTZ) were prepared separately; each solution was scanned between 400 nm to 200 nm. The wavelength for plotting calibration curves of two drugs were obtained from the spectra of the drugs recorded in case of Method-A (i.e. 290 nm for NEB and 317 nm for HCTZ).

Pure standards of NEB and HCTZ prepared earlier were scanned on multicomponent mode in range of 400 nm to 200 nm and were filled in the memory and the mixed standards were analyzed. The results were directly displayed on screen.

Tablet analysis was also carried out by preparing the stock solution of tablet powder in the same manner as that of method A.

Accuracy of the analysis was determined by performing recovery studies of NEB and HCTZ at 80%, 100% and 120% level for both the methods.

The results of the analysis of pure drug and statistical validation data are given in Table-2 for both the methods.

The results of the tablet analysis, recovery studies and statistical validation data are given in Table-3 and 4 for both the methods.

The proposed method for simultaneous estimation of NEB and HCTZ in combined sample solutions was found to be simple, accurate and reproducible. Once the equations are determined, analysis required only the measuring of the absorbances of the
sample solution at the two wavelengths selected, followed by a few simple calculations. Multicomponent mode is giving results directly on the screen and it does not require any calculations, so it is very rapid method. It is a new and novel method and can be employed for routine analysis in quality control R and D laboratories.

RESULTS AND DISCUSSION

Proposed method for simultaneous estimation of NEB and HCT in combined sample solutions was found to be simple, accurate and reproducible. Table.1 shows data for optical characteristics. Data for validation and recovery studies are given in Table no. 2, 3 and 4. Once the equations are determined, analysis required only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by a few simple calculations. Multicomponent mode method is very speedy, easy and accurate, that does not require any calculations. It is a novel method that can be employed for routine analysis in quality control or R & D lab.

TABLE 1: REGRESSION AND OPTICAL CHARACTERISTICS OF NEBIVOLOL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NEB</th>
<th>HCTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working λ (in Methanol)</td>
<td>290 nm</td>
<td>317 nm</td>
</tr>
<tr>
<td>Beer’s Law range</td>
<td>0-80 μg/ml</td>
<td>0-100 μg/ml</td>
</tr>
<tr>
<td>Molar absorptivity (1 / mole.cm)</td>
<td>5.3997 X 10^4</td>
<td>3.2818 X 10^4</td>
</tr>
<tr>
<td>Regression Values:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Slope</td>
<td>0.016</td>
<td>0.0099</td>
</tr>
<tr>
<td>ii. Intercept</td>
<td>0.0017</td>
<td>0.001</td>
</tr>
<tr>
<td>iii. Regression coefficient (r^2)</td>
<td>0.9996</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

TABLE 2: STATISTICAL VALIDATION OF PURE DRUGS

<table>
<thead>
<tr>
<th>Name of Component</th>
<th>Amount Present (μg/ml)</th>
<th>Method</th>
<th>Mean*</th>
<th>Standard Deviation</th>
<th>% Co-efficient of Variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB</td>
<td>20</td>
<td>Method-A</td>
<td>99.95</td>
<td>1.053</td>
<td>1.054</td>
<td>0.4297</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Method-B</td>
<td>100.58</td>
<td>1.814</td>
<td>1.080</td>
<td>0.7407</td>
</tr>
<tr>
<td>HCTZ</td>
<td>50</td>
<td>Method-A</td>
<td>99.98</td>
<td>1.290</td>
<td>1.290</td>
<td>0.5267</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Method-B</td>
<td>100.34</td>
<td>1.408</td>
<td>1.403</td>
<td>0.5748</td>
</tr>
</tbody>
</table>

NEB is Nebivolol Hydrochloride and HCTZ is Hydrochlorothiazide, Method-A is Simultaneous equation method and Method-B is Multicomponent Method. * Here Mean is the average of (n=6) results.
### TABLE 3: STATISTICAL VALIDATION OF TABLET

<table>
<thead>
<tr>
<th>Name of Component</th>
<th>Amount Present (μg/ml)</th>
<th>Method</th>
<th>Mean*</th>
<th>Standard Deviation</th>
<th>% Co-efficient of Variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEB</td>
<td>20</td>
<td>Method-A</td>
<td>99.95</td>
<td>0.9832</td>
<td>0.9836</td>
<td>0.4014</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Method-B</td>
<td>100.77</td>
<td>0.7870</td>
<td>0.7810</td>
<td>0.3213</td>
</tr>
<tr>
<td>HCTZ</td>
<td>50</td>
<td>Method-A</td>
<td>100.15</td>
<td>2.061</td>
<td>2.0579</td>
<td>0.8416</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Method-B</td>
<td>100.51</td>
<td>0.7260</td>
<td>0.7223</td>
<td>0.2964</td>
</tr>
</tbody>
</table>

NEB is Nebivolol Hydrochloride and HCTZ is Hydrochlorothiazide, Method-A is Simultaneous equation method, method –B is Multicomponent Method. * Here Mean is the average of (n=6) results.

### TABLE 4: RECOVERY STUDIES

<table>
<thead>
<tr>
<th>Level of % Recovery</th>
<th>Name of Drug</th>
<th>Amount Present (mg/tab)</th>
<th>Amount of Standard added (mg/tab)</th>
<th>% Recovery* ±SD</th>
<th>% Co-efficient of Variation</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>NEB</td>
<td>5</td>
<td>4</td>
<td>98.57 ±0.652</td>
<td>101.27 ±0.900</td>
<td>0.661 0.902 0.376 0.520</td>
</tr>
<tr>
<td></td>
<td>HCTZ</td>
<td>12.5</td>
<td>10</td>
<td>99.80 ±0.232</td>
<td>100.89 ±0.352</td>
<td>0.229 0.349 0.134 0.203</td>
</tr>
<tr>
<td>100</td>
<td>NEB</td>
<td>5</td>
<td>5</td>
<td>99.88 ±0.913</td>
<td>99.90 ±1.389</td>
<td>0.914 1.390 0.527 0.821</td>
</tr>
<tr>
<td></td>
<td>HCTZ</td>
<td>12.5</td>
<td>12.5</td>
<td>100.53 ±0.426</td>
<td>100.25 ±0.902</td>
<td>0.424 0.900 0.246 0.521</td>
</tr>
<tr>
<td>120</td>
<td>NEB</td>
<td>5</td>
<td>6</td>
<td>99.51 ±0.318</td>
<td>99.07 ±0.878</td>
<td>0.319 0.886 0.183 0.507</td>
</tr>
<tr>
<td></td>
<td>HCTZ</td>
<td>12.5</td>
<td>15</td>
<td>100.76 ±0.191</td>
<td>100.39 ±0.283</td>
<td>0.189 0.282 0.110 0.163</td>
</tr>
</tbody>
</table>

* Here % recovery is average of three results at each level.

A is Simultaneous equation method and B is Multicomponent Method.
CONCLUSION

The method was successfully used to estimate the amount of Nebivolol hydrochloride and Hydrochlorothiazide in marketed tablet formulation containing 5 mg of Nebivolol hydrochloride and 12.5 mg of Hydrochlorothiazide. The results obtained were comparable with the corresponding labeled amounts, indicating non-interference of excipients in the estimation.

By observing validation parameters, method was found to be specific, accurate, precise, repeatable and reproducible. This method is simple in calculation, hence can be employed for routine analysis of tablet for assay as well as dissolution testing.

ACKNOWLEDGEMENTS

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