Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan and Nebivolol Hydrochloride and Hydrochlorothiazide in Pharmaceutical Formulations by UV Spectrophotometric Methods

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Abstract
Two simple, precise and reproducible UV spectrophotometric methods, simultaneous equation method and Q-value analysis method, have been developed and validated for the simultaneous estimation of nebivolol hydrochloride and valsartan used as cardiovascular drugs available in capsule dosage form and nebivolol hydrochloride with hydrochlorothiazide used as antihistaminic H$_1$ blocker available in tablet dosage form. The methods are based on the measurement of absorbance of nebivolol hydrochloride and hydrochlorothiazide at 270.4 nm, 280.2 nm and 270 nm and measurement of absorbance of nebivolol Hcl and valsartan at 246.6 nm, 280.2 nm and 275 nm respectively. These methods obeyed Beer's law in the concentration range of 0.5 - 2.5 µg/ml for nebivolol Hcl, 1.0 – 20 µg/ml for valsartan and 1.0–3.0 µg/ml for hydrochlorothiazide. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed methods. These methods were successfully applied to the determination of these drugs in pharmaceutical dosage forms.

Keywords : Nebivolol hydrochloride; Valsartan; Hydrochlorothiazide; Simultaneous determination; Q-Analysis method; UV Spectrophotometry.

INTRODUCTION
Nebivolol hydrochloride (NEB) is (±) [2R* R* R* (S *)] α, α [imino bis (methylene)] bis- [6- fluoro-3,4 - dihydro - 2H - 1 - benzopyran - 2 - methanol] hydrochloride is an antihypertensive drug. It is a racemate of two enantiomers with four chiral centres. The SRRR-enantiomer (d-nebivolol) is a potent and cardio selective 1-adrenergic blocker. The RSSS-enantiomer (nebivolol) has a favourable hemodynamic profile$^{1-3}$. Valsartan (VAL) (N-valeryl-N-[2-(1H-tetrazol-5-yl)biphenyl-4-yl][methyl] valine, is an orally active, potent and specific competitive angiotensin II antagonist acting at the AT1 receptor, which mediates all known effects of angiotensin II on the cardiovascular system. Valsartan is widely used in the treatment of hypertension$^{14}$. Hydrochlorothiazide (HCT) is 6-chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonylamine 1,1-dioxide one of the popular thiazide diuretics. HCT is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones$^{1-3}$.

Literature survey reveals that a few methods have been reported for estimation of Valsartan, Nebivolol hydrochloride individually$^{6,7}$ as well as for the combination of Valsartan and Hydrochlorothiazide with other drugs were reported$^{8,9}$. The present paper describes two methods of two different combinations, NEB with VAS and NEB with HCT. To the best of our knowledge, no study has been described for the simultaneous determination of both combinations in pharmaceutical formulations by UV spectrophotometric methods. Therefore it is desirable to develop a simple and reproducible analytical methods.

MATERIALS AND METHODS
Materials
Pharmaceutical grade NEB from Cadila pharmaceutical Ltd. Ankleshwer, VAS from Hetero Labs. Hyderabad and HCT from Franco-Indian Pharma. Mumbai, India was used. All analytical grade chemicals and solvents were supplied by Merck, India.

Equipment
The Shimadzu UV-Visible Spectrophotometer-160A with data processing system was used. The sample solution was recorded in 1cm quartz cells against solvent blank over the range 200–400 nm. The optimal conditions
PROCEDURE

Standard solutions and calibration curves
Stock solutions for spectrophotometric measurements were prepared by dissolving NEB, VAS and HCT in methanol to obtain concentration of 1 mg/ml for each compound. For calibration, a series of above solutions were prepared containing NEB 0.5, 1.0, 1.5, 2.0, 2.5 µg/ml, VAS 1.0, 4.0, 8.0, 12.0, 16.0, 20.0 µg/ml and HCT 1.0, 1.5, 2.0, 2.5, 3.0 µg/ml by diluting the stock standard solution with methanol in standard volumetric flasks (10ml). The dilutions were scanned in the wavelength range of 200-400 nm. Two wavelengths selected for each formulation for the use of simultaneous equation were 280.2 & 246.6 nm for NEB with VAS combination and 280.2 & 270.4 nm for NEB with HCT combination as shown in Figure 1 and Figure 2, respectively. (A1%, 1cm) \( \varepsilon \) was calculated for each standard drug by measuring absorbance of 1% solution at 1cm path length. Similarly, mixed standard solutions were used for UV-spectrometric analysis by Simultaneous equation and Q-analysis Methods.

Simultaneous equation method
Twenty capsules were weighed, powdered and weighed accurately equivalent to 5 mg of NEB and 80 mg VAS. The twenty tablets were weighed, powdered and weighed equivalent to 5 mg NEB and 12.5 mg HCT were transferred to a 100 ml volumetric flask separately, dissolved in 50ml of methanol by ultrasonication for 20 min. The solutions were diluted with the same solvent, filtered through Whatmann No. 41 filter paper. The filtrate was diluted with methanol to get final dilution 1.0 µg/ml and 16.0 µg/ml of NEB and VAS or 1.0 µg/ml and 2.5 µg/ml NEB and HCT respectively. Absorbance of these solutions were measured at selected wavelengths as \( A_1 \) and \( A_2 \) and concentrations of the two drugs in each sample were calculated by using following equations. The method employed solving of simultaneous equations using Cramer’s rule and matrices.

The simultaneous equations NEB and VAS analysis were
\[
A_1 = C_{iv} \times C_i + C_{iv} \times C_2 \quad \text{(1)}
\]
\[
A_2 = C_{iv} \times C_i + C_{iv} \times C_2 \quad \text{(2)}
\]
The simultaneous equations NEB and HCT analysis were
\[
A_1 = C_i \times C_i + C_{iv} \times C_2 \quad \text{(1)}
\]
\[
A_2 = C_i \times C_i + C_{iv} \times C_2 \quad \text{(2)}
\]
\( C_{iv} \) and \( C_{iv} \) absorptivity values of NEB at 280.2 nm wavelength
\( C_i \) and \( C_{iv} \) absorptivity values of VAS at 246.6 nm wavelength
\( C_{iv} \) and \( C_{iv} \) absorptivity values of HCT at 270.4 nm wavelength

Q value analysis method
From the overlain spectrum shown in Figure 1 for VAS and NEB two wavelengths were selected one at 275 nm as isoabsorptive point for both the drugs and at 246.6 nm. Similarly from the overlain spectrum shown in Figure 2 for HCT and NEB two wavelengths were selected one at 270 nm as isoabsorptive point for both the drugs and at 280.2 nm. The absorbance of the standard and sample solutions were prepared and measured in the same manner as in the previous method. The absorptivity values for both standard drugs at the selected wavelength obtained for simultaneous equation method were employed for determination of Q values and another wavelength used was isoabsorptivite point for each combination. The concentrations of drugs in sample solution were determined by using the following formula.

For estimation of VAS
\[
C_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of sample at 246.6 nm}
\]
\[
Q_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of sample at 275 nm}
\]

For estimation of NEB
\[
C_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of VAS at 246.6 nm}
\]
\[
Q_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of VAS at 275 nm}
\]
\[
Q_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of NEB at 246.6 nm}
\]
\[
Q_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of NEB at 275 nm}
\]

\( A = \) Absorbance of sample at isoabsorptive point
\( a_1 \) and \( a_2 \) = Absorptivities values of VAS and NEB respectively at iso-absorptivite point

For estimation of HCT
\[
C_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of sample at 280.2 nm}
\]
\[
Q_i = \frac{Q_i - Q_{av}}{Q_i - Q_{av}} \quad \text{Absorptivity of sample at 270 nm}
\]
For estimation of NEB
\[
C = \frac{Q_2 - Q_1}{a_2} \times \frac{Q_1}{Q_2 - Q_1} \times \frac{\text{Absorptivity of HCT at 280.2 nm}}{\text{Absorptivity of HCT at 270 nm}}
\]
\[
Q_2 = \frac{\text{Absorptivity of NEB at 280.2 nm}}{\text{Absorptivity of NEB at 270 nm}}
\]

A = Absorbance of sample at isoabsorptive point.
a_1 and a_2 = Absorptivity values of HCT and NEB respectively at iso-absorptivite point.

RESULTS AND DISCUSSION
The NEB, VAS and HCT were sparingly soluble in water so methanol was used as solvent for all standard and sample solution preparation. UV absorption spectrum exhibited maximum absorbance for NEB, VAS and HCT at 280.2, 246.6 and at 270.4 respectively. Standard 1% solutions were prepared and measured absorbance at both the wavelengths of each respective content of formulations for calculating C_1v, C_2v, and C_1H, C_2H values. The absorbance of sample solutions was noted at the respective wavelengths for both formulations.

Similarly by calculating Q-values for each drug and then put these values in formula and determine content of each drug in formulation. Statistical parameters obtained were reported in Table 1. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines. The results of the recovery analysis are reported in Table 2.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 12 h at room temperature. The retention time and peak area of NEB and VAS remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 12 h, which was sufficient time to complete the whole analytical process.

CONCLUSION
The proposed two spectrophotometric methods assured required precision and accuracy. Both the methods were found to be simple, accurate, economical, reproducible and rapid. Hence, it can be employed for routine analysis.

ACKNOWLEDGEMENT
The authors are thankful to Cadila pharmaceutical Ltd. Ankleshwer, Hetero Labs.Hyderabad and Franco-Indian Pharma, Mumbai for generous gift samples of Pharmaceutical grade Nebivolol Hcl, Valsartan and Hydrochlorothiazide respectively. The authors also thank Mr. Supe (Drug Inspector), Miss Krishnveni N., Mr. S. Rajan Mr. S. Murlidharan (CADRAT) for their cooperation. The authors are grateful to “His Holiness Jagadguru Sri Sri Shivarathree Deshikendra Mahaswamigalavaru” of Sri Suttur Mutt, Mysore and AICTE (QIP) cell for providing facilities to carry out this work.

Fig. 1: Overlaid Spectra for Valsartan and Nebivolol hydrochloride
A: - Absorbance of Valsartan
B: - Absorbance of Nebivolol hydrochloride
C: - Absorbance at isoabsorptive point

Fig. 2: Overlaid Spectra for Hydrochlorothiazide and Nebivolol hydrochloride
A: - Absorbance of Hydrochlorothiazide
B: - Absorbance of Nebivolol hydrochloride
### Table No.1: Statistical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>UV-Simulation equation method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NEB</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td>1.48</td>
</tr>
<tr>
<td>coefficient of variation (R.S.D.)</td>
<td>1.49</td>
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<tr>
<td>Standard error</td>
<td>0.40</td>
</tr>
<tr>
<td>Correlation co-efficient®</td>
<td>0.998</td>
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<tr>
<td>Linearity Range (µg/ml)</td>
<td>0.5 - 2.5</td>
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<tr>
<td>Accuracy (%)</td>
<td>101.2</td>
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</table>

NEB - Nebivolol hydrochloride, VAS - Valsartan, HCT - Hydrochlorothiazide

### Table No.2: Analysis of Tablet formulations

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulation</th>
<th>Label claim mg /dose</th>
<th>Amount found mg /dose</th>
<th>%Recovery ± SD*</th>
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</thead>
<tbody>
<tr>
<td>Simultaneous</td>
<td>T₁</td>
<td>5</td>
<td>80</td>
<td>5.10 ± 80.2</td>
</tr>
<tr>
<td>Equation Method</td>
<td>T₂</td>
<td>5</td>
<td>12.5</td>
<td>4.99 ± 12.5</td>
</tr>
<tr>
<td>Q-Value analysis Method</td>
<td>T₁</td>
<td>5</td>
<td>80</td>
<td>5.12 ± 80.5</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>5</td>
<td>12.5</td>
<td>5.01 ± 11.9</td>
</tr>
</tbody>
</table>

* = Average of 6 determinations

**T₁** = Capsule formulation containing NEB 5 mg and VAS 80 mg per dosage.

**T₂** = Tablet formulation containing NEB 5 mg and HCT 12.5 mg per dosage.

### REFERENCES