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Research Article

# Estimation of Valsartan and Nebivolol in pharmaceutical dosage forms by absorption ratio method

**Chaudhary Ankit B*[1], Patel Rakesh K[2], Chaudhary Sunita A[1]**

[1]Saraswati Institute of Pharmaceutical science, Dhanap, Gandhinagar, Gujarat, India.

[2]Shree S. K. Patel College of Pharmaceutical Education and Research, Ganapat University, Mehsana, Gujarat, India.

**ABSTRACT**

A new Absorption Ratio method was developed and validated for the determination of Valsartan and Nebivolol in tablets. Calibration curves for Valsartan and nebivolol over concentration range of 4 - 80 µg/ml were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 281 nm (λ-max of nebivolol) and 276.5 nm (iso- absorptive point). The results of analysis have been validated statistically and by recovery studies. The value of standard deviation was satisfactory and recovery studies ranging from 98.74-101.27 % for Valsartan and 98.06-102.96% for nebivolol were indicative of the accuracy and precision of the proposed method. The results of the assay are in good agreement with the label amount. The method was found to be simple, rapid, and accurate and can be adopted in routine analysis of these drugs in formulations. Due to these attributes, the proposed method could be used for routine analysis of these drugs in combined dosage forms.

**Keywords:** Nebivolol; Valsartan; Iso-absorptive point.

## INTRODUCTION

Valsartan (VAL), N-(1-Oxopentyl)-N-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-L-valine, is a potent angiotensin receptor blocker( Budavari S,1997).

Literature survey revealed that VAL is not yet official in any of the pharmacopoeia. Methods such as HPLC (Koçyiğit, 2006; Daneshtalab, 2002; González,2002; Tian,2008; Chitlange, 2008; Kadam, 2007) LC-MS (Koseki, 2007; H. Li, 2007; Selvana,2007) Protein precipitation (Macek, 2006) Capillary Electrophoresis (Hillaert, 2003) and simultaneous UVspectrophotometric methods (Satanaa,2001; Tatar,2002) are reported for estimation of VAL alone or in combination with other agents.

Nebivolol (NEB) is chemically, $\alpha,\alpha^1$ -[imino bis (methylene)]bis[6-fluoro-3,4-dihydro-2 *H* -1-benzopyran-2-methanol]( Budavari,2001) which is a selective $\beta_1$ -receptor antagonist without partial agonist activity (Brian,2001)

It is official in martindale (Sean, 2005) the extra pharmacopoeia. Literature assessment showed that HPLC (Sahoo, 2009) HPTLC (Patel, 2007) and liquid chromatography-mass spectroscopy (LC-MS) (Mario, 2001; Ramakrishna, 2005; Maurer, 2004) methods are reported for estimation of NEB in dosage formulations and in biological fluids.

A literature survey revealed HPLC method for analysis of VAL and NEB in pharmaceutical preparations (Kokil, 2009).A literature survey has revealed there is no absorption ratio method for analysis of VAL and NEB in pharmaceutical preparations. The present work describes a validated absorption ratio method for simultaneous determination of these drugs in tablets.

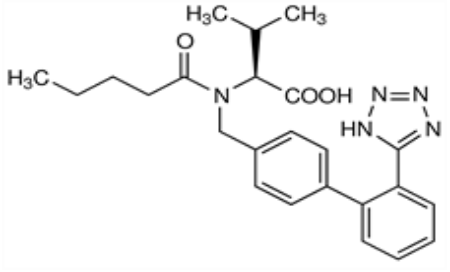


**Figure 1: Structure of Valsartan**

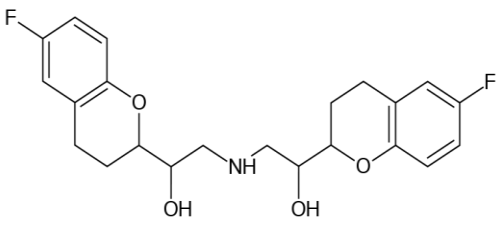


**Figure 2: Structure of Nebivolol**

* Corresponding Author
Email: ankit_mqa@yahoo.co.in
Contact: +91-9825448865
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## EXPERIMENTAL

### Material

A double-beam UV-Visible spectrophotometer, model UV-1800 (Shimadzu, Japan) having two matched cells with 1-cm light path wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10 mm quartz cells. A Sartorius electronic analytical balance (CP224S) was used for weighing the sample. An ultrasonic cleaner (Frontline FS 4) was used for sonicating the tablet powder. Valsartan (VAL) and Nebivolol (NEB) from Torrent Pharmaceuticals Ltd and Methanol -AR grade (Finar laboratories) were used in the study.

### Methods

#### Preparation of stock solutions

Standard Stock solutions (100 µg/ml) of VAL and NEB were prepared by dissolving separately, 5 mg of drug in 50 ml volumetric flask and dilute up to the mark with methanol.

#### Determination of iso-absorptive point and wavelength of maximum absorbance

The working standard stock solutions of VAL and NEB were scanned in the range of 200 to 400 nm against methanol as a blank. Iso- absorptive point was found at 276.5 nm. (Fig. 3) as at this wavelength, there is minimum interference of the other drug and another wavelength used is 281 nm which is λ-max of NEB.

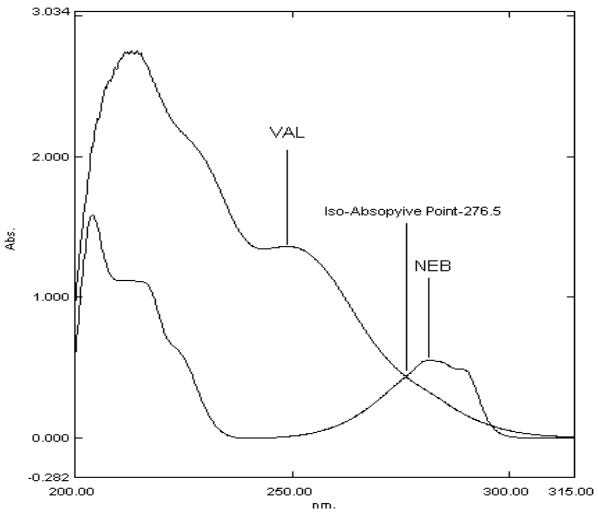


**Figure 3: Overlay spectra of VAL and NEB Showing iso-absorptive point at 276.5 nm**

#### Preparation of Sample solution from tablet dosage form

Weigh and powder 20 tablets. Weigh accurately a quantity of the powder equivalent to about 80 mg of VAL and 5 mg of NEB into 100 ml volumetric flask and diluted to 100 ml with methanol. This solution is sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41. Transfer 1 ml of solution into 10 ml volumetric flask and dilute to the mark with methanol to get a final concentration 80 µg/ml of VAL and 5 µg/ml of NEB.

#### Validation studies

#### Calibration curve (Linearity)

A calibration curve was plotted over a concentration range of 4-100 µg/ml for both VAL and NEB. Accurately measured standard stock solution of VAL (0.4, 1, 2, 4, 6, 8, 10 ml) and standard stock solution of NEB (0.4, 1, 2, 4, 6, 8, 10 ml) were transferred to a separate series of 10 ml of volumetric flasks and diluted to the mark with methanol. The absorbance of each solution was measured at both the wavelength 276.5 nm and 281 nm. Calibration curves were constructed for VAL & NEB by plotting absorbance versus concentrations at both wavelengths. Each reading was average of three determinations.

#### Accuracy (% Recovery)

It is defined as closeness of agreement between the actual (true) value and analytical value and obtained by applying test method for a number of times. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (VAL 80 µg/ml and NEB 5 µg/ml) with three different concentrations of standards (VAL 5,10,15 µg/ml and NEB 5,10,15 µg/ml).

**Table 1: Data of recovery study of VAL and NEB**

| Drug | Amount taken (µg/ml) | Amount added (µg/ml) | Amount found (µg/ml) | % Recovery ± S.D (n=3) |
|---|---|---|---|---|
| VAL | 80 | 5 | 86.08 | 101.27 ± 1.12 |
| | 80 | 10 | 90.96 | 101.06 ± 0.83 |
| | 80 | 15 | 93.81 | 98.74 ± 1.74 |
| NEB | 5 | 5 | 9.91 | 99.10 ± 1.32 |
| | 5 | 10 | 14.71 | 98.06 ± 0.75 |
| | 5 | 15 | 25.74 | 102.96 ± 1.37 |

#### Method Precision

For evaluation of precision, repeatability of the results for a concentration of 20 µg/mL was evaluated by 6 replicate determinations. For evaluation of intermediate precision, the results over the concentration range 4 - 100 µg/ml for VAL & NEB were evaluated by 4 replicate determinations to estimate intraday variation and another replicate determination on different 4 days to estimate interday variation. The coefficients of variation (CV) values at these concentration levels were calculated.

**Limit of Detection and Limit of Quantification**

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where $\sigma$ = the standard deviation of the response and S = Slope of calibration curve.

**Estimation of VAL and NEB from pharmaceutical dosage form**

The absorptivity coefficients of these two drugs were determined using calibration curve equation. The concentration of VAL and NEB were determined using the following simultaneous equations.

$$C_X = \frac{(Q_M - Q_Y) \times A_1}{(Q_X - Q_Y) \times aX_1} \quad \text{AND} \quad C_Y = \frac{A_1}{aX_1 - C_X}$$

Where, A1& A2 are the absorbance of the mixture at 276.5 nm & 281 nm respectively; $aX_1$ and $aY_1$ are absortivities of VAL and NEB respectively at 276.5 nm; $aX_2$ and $aY_2$ are absortivities of VAL and NEB respectively at 281 nm; $Q_M = A_2/A_1$, $Q_X = aX_2/aX_1$ and $Q_Y = aY_2/aY_1$.

**RESULT AND DISCUSSION**

In this method, the standard stock solutions of VAL and NEB were prepared in methanol. Calibration curves for VAL and NEB over concentration range of 4 -100 µg/ml were plotted and molar absorptivity for both the drugs were calculated at both the wavelengths of 281 nm (λ-max of NEB) and 276.5 nm (iso- absorptive point). It is evident from the spectra of VAL and NEB that these drugs obey the Lambert-beer's law at all the wavelength. Calibration curve of VAL and NEB at 281 are shown in figure 4 and 5 respectively, while calibration curve at 276.5 nm (iso- absorptive point) is shown in figure 6. The optical and regression characteristics and validation parameters are reported in Table 2.

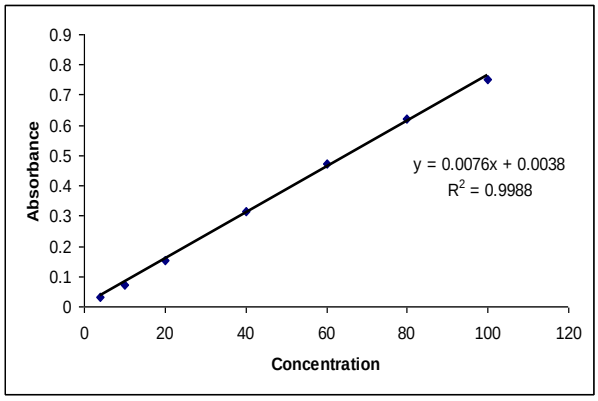


**Figure 4: Calibration curve of VAL at 281 nm**

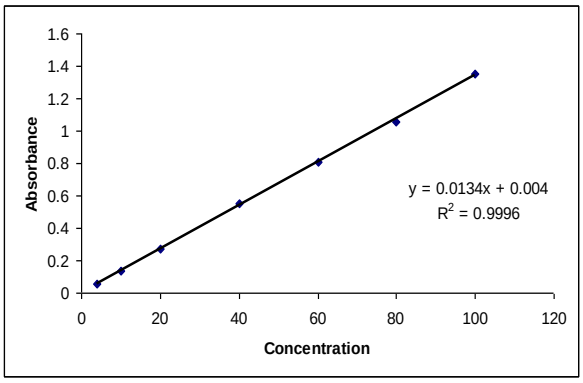


**Figure 5: Calibration curve of NEB at 281 nm**

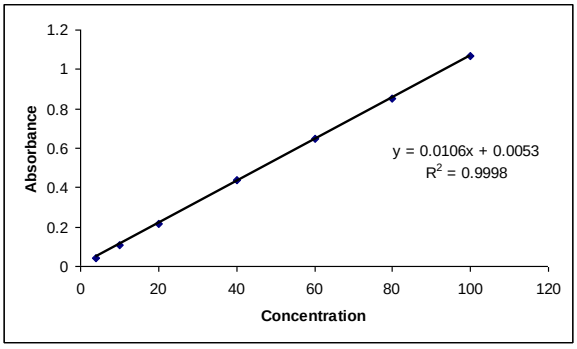


**Figure 6: Calibration curve of at 276.5 nm (Iso-absorptive point)**

**Table 2: Optical and Regression characteristics and validation parameters of Q Absorbance ratio method for analysis of VAL and NEB**

| Parameters | Iso absorptive Point | VAL (281) | NEB (281) |
|---|---|---|---|
| Beer's Law Limit (µg/ml) | 4 -100 | 4 -100 | 4 -100 |
| Absorptivity | 108 | 135 | 76 |
| Regression equation (y* = mx + c)<br>Slope (m)<br>Intercept (c) | <br>0.0106<br>0.0503 | <br>0.0076<br>0.0038 | <br>0.0134<br>0.004 |
| Correlation Coefficient ($r^2$) | 0.9998 | 0.9988 | 0.996 |
| Standard Deviation (S.D) | 0.0026 | 0.0021 | 0.0042 |
| Relative Standard Deviation (RSD or %CV) | 1.3658 | 1.2329 | 0.9969 |
| LOD (µg/ml) | 0.809 | 0.911 | 0.46 |
| LOQ (µg/ml) | 2.452 | 2.763 | 3.134 |
| Precision<br>Intra-day (n=5) (% CV)<br>Inter-day (n=5) (% CV) | <br>0.72-2.73<br>0.89-1.97 | <br>0.91-2.16<br>0.78-2.06 | <br>0.64-1.87<br>0.78-1.76 |

**Table 3: Application of the proposed method to the pharmaceutical dosage forms**

| Formulation | VAL | | | NEB | | |
|---|---|---|---|---|---|---|
| | Amount labeled (mg) | Amount found (mg) | % Amount Found S.D. (n=3) | Amount labeled (mg) | Amount found (mg) | % Amount Found S.D. (n=3) |
| Brand I | 80 | 81.08 | 101.35 ± 1.37 | 5 | 5.13 | 102.60 ± 1.86 |
| Brand II | 80 | 80.59 | 100.73 ± 1.58 | 5 | 4.91 | 98.23 ± 1.56 |

**Application to the pharmaceutical dosage form**

The proposed validated method was successfully applied to determine VAL and NEB in bulk powder and in tablet dosage forms. Results are given in Table 3. No interference of the excipients with the peaks of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of VAL and NEB in pharmaceutical dosage forms.

**CONCLUSION**

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of VAL and NEB in bulk and in pharmaceutical formulations without interference and with good sensitivity.

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