

## SIMULTANEOUS ESTIMATION AND VALIDATION OF NEBIVOLOL AND VALSARTAN IN TABLET DOSAGE FORM BY RP-HPLC

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### ABSTRACT

**Objective:** The objective of this work is to develop a rapid, precise, accurate and sensitive reverse phase liquid chromatographic method for the simultaneous estimation of Nebivolol and Valsartan in tablet dosage form.

**Method:** The chromatographic method was standardized using C<sub>18</sub> Inertsil ODS column (250×4.6mm, 5µm particle size) with UV detection at 278nm and flow rate of 1ml/min. The mobile phase consists of ACN : Buffer( pH adjusted to 3.5 with dilute Ortho Phosphoric acid) in the ratio of 60:40 v/v.

**Results:** The linearity of proposed method was investigated in the range of 2.5-7.5µg/ml(R<sup>2</sup>=0.999) for Nebivolol and 40-120µg/ml(R<sup>2</sup>=0.999) for Valsartan respectively. The limit of detection (LOD) was found to be 0.05µg/ml and 0.81µg/ml for Nebivolol and Valsartan respectively. The limit of quantification (LOQ) was found to be 0.15µg/ml and 2.44µg/ml for Nebivolol and Valsartan respectively. The retention time of Nebivolol and Valsartan were found to be 3.233min and 5.056min respectively.

**Conclusion:** The method was statistically validated and %RSD was found to be less than 2 indicating high degree of accuracy and precision. Hence proposed method can be successfully applied for the simultaneous estimation of Nebivolol and Valsartan in marketed formulation.

**Keywords:** Nebivolol, Valsartan, RP-HPLC, ICH guidelines, Validation.

### INTRODUCTION

Nebivolol is chemically 1-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-[[2-(6-fluoro-3,4-dihydro-2H-1-benzopyran-2-yl)-2-hydroxyethyl]amino]ethan-1-ol, an anti-hypertensive drug. It is selective β<sub>1</sub> receptor antagonist. Valsartan is chemically (2S)-3-methyl-2-[N-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl)methyl}pentanamido]butanoic acid, an anti-hypertensive drug. It is an angiotensin receptor blocker that selectively inhibits binding of angiotensin II to type I angiotensin II receptor subtype. The combination is used for the treatment of high blood pressure and particularly demonstrated significant decrease in diastolic blood pressure.

Very few RP-HPLC and UV spectrophotometric methods have been reported for this combination. The RP-HPLC methods were reported by SU Kokil[1], Birajdar [2] and Shinde Sachin[3]. The spectrophotometric methods were reported by Jagadish S.Modiya[4], and Arunadevi S. Birajdar[5]. But the retention of these methods are too large. Hence present method is developed and found advantageous over the existing methods.

### MATERIALS AND METHODS

#### Chemicals and reagents

API of Nebivolol and Valsartan were received as gift sample from Dr.Reddy labs (Hyderabad AP., India). Acetonitrile and water of HPLC grade were procured from Rankem lab ltd. Ortho Phosphoric acid AR grade was purchased from E.Merck chemicals Mumbai, India. All other reagents were of AR grade. The commercial sample of Nebicard-V containing Nebivolol 5mg and Valsartan 80mg is purchased from local market.

#### Instrumentation

The LC system consisted of Waters model 2695, UV-Visible detector. The output signals were monitored and integrated using Empower2 software. Melter Electronic Balance and Solomon pH meter were used.

#### Chromatographic conditions

The chromatographic separation was achieved on C<sub>18</sub> Inertsil ODS (250×4.6mm, 5µm) column using mobile phase consisting of ACN: Buffer in ratio of 60:40v/v. The buffer (0.01N disodium hydrogen phosphate) pH is adjusted to 3.5 with dilute ortho phosphoric acid and filtered through 0.45µm membrane filter. The column was

maintained at room temperature (25°C) and the flow rate is 1ml/min. Prior to inject of the solutions the column is stabilised for atleast 30 minutes with the mobile phase flowing through the system. The volume of sample injected was 20µl. The UV-Visible detector was set at wavelength 278nm. Under described experimental conditions, all the peaks were well defined and free from tailing. A typical chromatogram of Nebivolol and Valsartan sample is shown in figure 1.

#### Preparation of Standard solution

5mg of Nebivolol working standard and 80mg of Valsartan working standard was weighed and transferred into 100ml volumetric flask, to it 50 ml of diluent (mobile phase) was added and sonicated to dissolve. Then the solution was made upto mark with diluent. Further the solution was diluted to get a concentration of 5µg/ml of Nebivolol and 80µg/ml of Valsartan. The solutions of concentration range 2.5-7.5µg/ml of Nebivolol and 40-120µg/ml of Valsartan were prepared and linearity was determined.

#### Preparation of Sample solution

About 20 tablets were taken and their average weight is calculated. These pre-weighed 20 tablets are finely grinded. Of the grinded powder, sample quantitatively equivalent to 5mg Nebivolol and 80mg Valsartan is transferred into 100ml volumetric flask and 50ml of diluent is added, sonicated to dissolve for 10 minutes. Then the solution is made upto mark with diluent. Further the solution is filtered through 0.45µm membrane filter. 10ml of above filtrate is diluted to 100ml with diluent to get a concentration of 5µg/ml of Nebivolol and 80 µg/ml of Valsartan[6].

#### Validation of the method

The analytical method was validated as per ICH guidelines[7-9] with respect to parameters such as linearity, accuracy, precision, assay, ruggedness, robustness, limit of detection and limit of quantification as follows.

#### Linearity

Linearity of this method was evaluated by linear regression analysis, calculated by least square method and studied by preparing standard solutions of Nebivolol and Valsartan at different concentrations. The peak areas of Nebivolol or Valsartan were plotted versus their respective concentrations. The response was

found to be linear over the concentration range of 2.5-7.5µg/ml for Nebivolol and 40-120µg/ml for Valsartan. Typically, the regression equation were  $y=54248x+198.2$  for Nebivolol and  $y=18941x+822$  for Valsartan. The correlation coefficient( $R^2$ ) for both Nebivolol and Valsartan were found to be 0.999. The data is given in table1.

**Accuracy**

Accuracy was performed in triplicate for various concentrations of Nebivolol and Valsartan equivalent to 50%, 100% and 150% of standard amount, was injected into the HPLC system as per test procedure. The average % recovery of Nebivolol and Valsartan was calculated. The data was given in the table 2.

**Precision**

**A) Method repeatability**

Six sample solutions of the same concentration were prepared and injected into the HPLC system as per test procedure.

**B) Intermediate Precision**

Two analysts as per test procedure conducted the study. For Analyst-1 Method Repeatability and for Analyst-2 six sample solutions of same concentration were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

**Limit of Detection(LOD) and Limit of Quantification(LOQ)**

LOD and LOQ were performed on samples containing concentration of analytes, based on calibration curve method. Standard solution of

Nebivolol and Valsartan were injected in six replicates. Average peak area of six analytes were plotted against concentration.LOD and LOQ were calculated. The LOD and LOQ were found to be 0.05µg/ml, 0.81µg/ml and 0.15µg/ml, 2.44µg/ml for Nebivolol and Valsartan respectively.

**Assay**

The validated method was applied for the determination of Nebivolol and Valsartan in commercially available Nebicard-V tablets. The results of assay (n=3) undertaken yielded 99.99% (%RSD=0.07) of Nebivolol and 99.79 % ( %RSD= 0.18%) of Valsartan. The data is given in table 4.

**Robustness and Ruggedness**

Robustness was done by small deliberate changes in chromatographic conditions and retention time of Nebivolol and Valsartan was noted. The factors selected were flow rate and mobile phase. The results remained unaffected by small variation in these parameters as shown in table 5 and 6. Ruggedness of method was checked by using different instruments. The relative standard deviation of the results obtained from different instruments was <2.0%. The results were given in table 7.

**System Suitability**

System suitability and chromatographic parameters were validated such as number of theoretical plates, asymmetry factor and tailing factor. The results are given in the table 8.

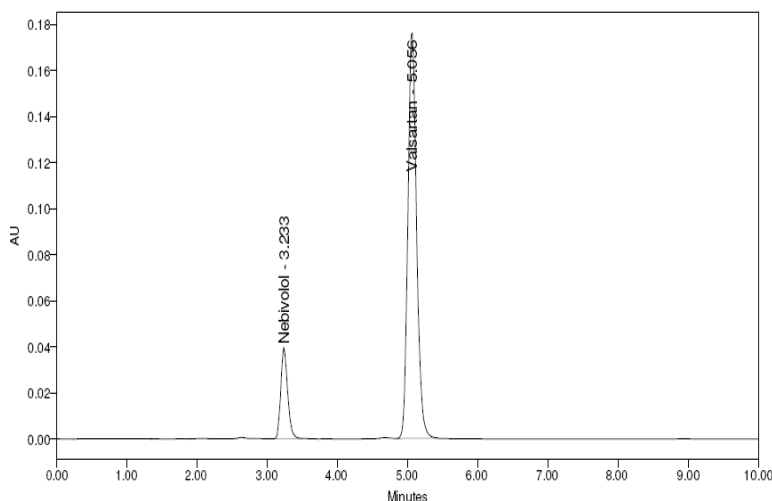


Fig. 1: Chromatogram of Nebivolol and Valsartan sample

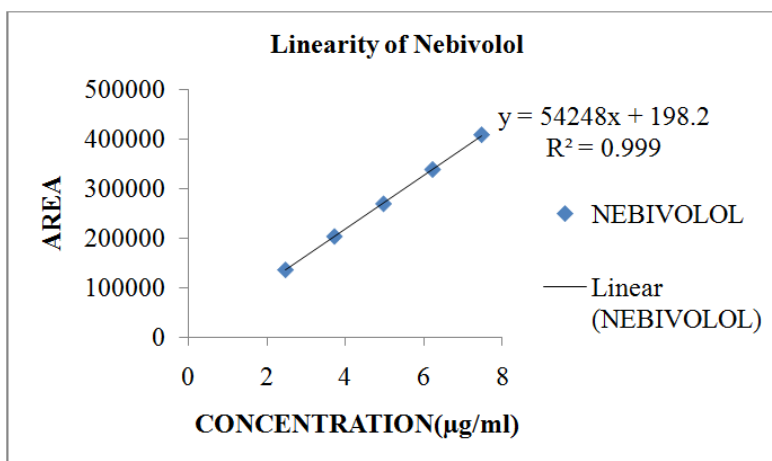


Fig. 2: Linearity of Nebivolol

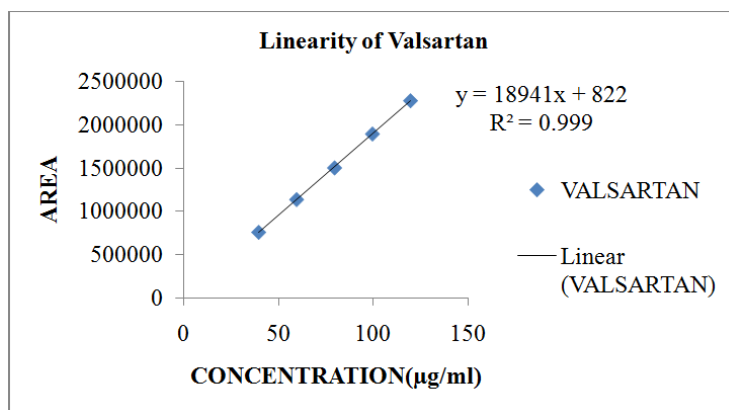


Fig. 3: Linearity of Valsartan

Table 1: Linearity data for Nebivolol and Valsartan (n=3)

S. No.	Concentration(µg/ml)	Injection	Retention time(mins)	Area
Nebivolol				
1	2.5	1	3.233	136683
2	3.75	1	3.233	203937
3	5	1	3.233	269585
4	6.25	1	3.232	338572
5	7.5	1	3.233	408416
$y=54248x+198.2$ $R^2=0.999$				
Valsartan				
1	40	1	5.056	762271
2	60	1	5.056	1139511
3	80	1	5.055	1505029
4	100	1	5.056	1895352
5	120	1	5.056	2278485
$y=18941x+822$ $R^2=0.999$				

Table 2: Accuracy (%recovery) data for Nebivolol and Valsartan

S. No.	Spiked level	Amount Added (µg/ml) (n=3)	Amount Found (µg/ml) (n=3)	% Recovery	% Recovery±RSD
Nebivolol					
1	50%	2.5	2.49	99.80	99.80±0.34
2	100%	5	4.99	99.90	99.90±0.05
3	150%	7.5	7.49	99.94	99.94±0.10
Valsartan					
1	50%	40.0	39.8	99.40	99.40±0.62
2	100%	80.0	79.9	99.88	99.88±0.03
3	150%	120.0	119.9	99.91	99.91±0.54

n= no. of sampling

Table 3: Precision data for Nebivolol and Valsartan

S. No.	Concentration(µg/ml)	Injection	Retention time(mins)	Area
Nebivolol				
1	3.75	1	3.233	203937
2	3.75	1	3.233	205102
3	3.75	1	3.223	204485
4	3.75	1	3.233	205214
5	3.75	1	3.231	203558
6	3.75	1	3.233	205863
Mean				204693
Std Dev				861
%RSD				0.42
Valsartan				
1	60	1	5.056	1139511
2	60	1	5.055	1138654
3	60	1	5.056	1135548
4	60	1	5.056	1133485
5	60	1	5.054	1127546
6	60	1	5.056	1139652
Mean				1135732
Std Dev				4696
%RSD				0.41

Table 4: Assay data for Nebivolol and Valsartan in marketed formulation

S. No.	Tablet sample	Label claim mg/tablet	%Avg Assay (n=3)	Std Dev	%RSD
1	Nebivolol	5	99.99	0.07	0.07
2	Valsartan	80	99.79	0.18	0.18

Table 5: Robustness data for Nebivolol and Valsartan relating to change in flow rate (1ml/min)

S. No.	Flow rate(ml/min)	Average*	Std Dev	%RSD
Nebivolol				
1	Flow rate 1-(0.9ml/min)	203227	65.31	0.03
2	Flow rate 2-(1.1ml/min)	230718	176.53	0.08
Valsartan				
1	Flow rate1-(0.9ml/min)	1138564	5371.09	0.47
2	Flow rate2-(1.1ml/min)	1141827	1534.6	0.13

\*n=3(average no. of determinations)

Table 6: Robustness data for Nebivolol and Valsartan relating to change in mobile phase composition (ACN:Buffer :: 60:40v/v)

S. No.	Mobile phase	Average*	Std Dev	%RSD
Nebivolol				
1	m.p-1(61:39v/v)	203127	67.11	0.03
2	m.p-2(59:41v/v)	203553	97.33	0.04
Valsartan				
1	m.p-1(61:39v/v)	1140630	2358.41	0.02
2	m.p-2(59:41v/v)	1144820	858.86	0.07

\*n=3(average no. of determinations)

Table 7: Ruggedness data for Nebivolol and Valsartan

S. No.	Instrument	Average*	Std Dev	%RSD
Nebivolol				
1	Instrument-1	200828	575.24	0.28
2	Instrument-2	201459	442.47	0.21
Valsartan				
1	Instrument-1	1143416	3413.19	0.29
2	Instrument-2	1139506	4958.28	0.43

\*n=3(average no. of determinations)

Table 8: System suitability data for Nebivolol and Valsartan

System suitability parameters	Nebivolol	Valsartan
Resolution	-	9.15
Theoretical plates	5445	8098
Retention time(min)	3.233	5.056
Asymmetry factor	0.88	0.76

## RESULTS AND DISCUSSION

The proposed method was found to be linear over concentration range of 2.5-7.5µg/ml and 40-120µg/ml for Nebivolol and Valsartan respectively. System suitability parameters indicate good resolution for both the peaks >2. The method was found to be accurate and precise as indicated by the results of recovery studies and precision studies whose %RSD is not more than 2%. There were no marked changes in the chromatograms which confirmed the ruggedness of the method. The standard deviation of %assay for sample was calculated, for each parameter in robustness studies the relative standard deviation was found less than 2%. The low %RSD value confirms the robustness of method.

## CONCLUSION

The proposed method was found to be rapid, precise, accurate and sensitive. It makes use of fewer amounts of solvents and has shorter retention times than existing methods. Many samples can be suitably analysed by this method. Hence developed method can be used for routine analysis of Nebivolol and Valsartan in tablet dosage form.

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