CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1231031

Available online at: http://www.iajps.com Research Article

A NEW RP-HPLC METHOD FOR THE SIMULATANEOUS ESTIMATION OF NEBIVOLOL AND VALSARTAN IN TABLETS

Bhuvanagiri Krishna[1*], Gudimitla Raveendra Babu[1], Muddala Ramayyappa[1], Averineni Ravi Kumar[2]

[1]Department of Pharmaceutical Analysis, [2]Department of Pharmacognosy, Alluri Krishna Rao and Govindamma College of Pharmacy, Nallajerla-534112, Andhra Pradesh, India.

Abstract:

A simple RP-HPLC method was developed and validated for the simultaneous estimation of Valsartan and Nebivolol in tablets. The Valsartan and Nebivolol were analyzed by RP-HPLC using Hypersil BDS column (150 cm X 4.6 mm, 5 µm) with isocratic mobile phase consists of ammonium acetate buffer to pH 5.20 and acetonitrile (55:45, v/v), at a flow rate of 1 ml/min in the detection wavelength of 282 nm. The linearity ranges of Valsartan are 200-1200 µg/ml and 12.5-75 µg/ml for Nebivolol by RP-HPLC. The limit of detection and quantification for Valsartan and Nebivolol were found to be 0.25 µg/ml and 1.4 µg/ml, 0.77 µg/ml and 4.5 µg/ml respectively. The accuracy of this method evaluated by recovery measurements and good recovery results obtained from 99.61% to 101.65% for all parameters and the relative standard deviation is below 2 % were achieved.

Key words: *Valsartan, Nebivolol, Estimation, Accuracy, Tablets.*

Corresponding author:

B. Krishna,
Assistant Professor
AKRG College of Pharmacy,
Nallajerla- 534112,
Andhra Pradesh, India.
Krishnasri516@gmail.com
09666666516.

QR code

Please cite this article in press B. Krishna et al., ***A New RP-HPLC Method for the Simulataneous Estimation of Nebivolol and Valsartan in Tablets,*** *Indo Am. J. P. Sci, 2018; 05(04).*

INTRODUCTION:

Valsartan is chemically called as **(Figure 1)** (2S) -3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol5yl) phenyl] phenyl} methyl) pentanamido] butanoic acid. Valsartan is an angiotensin-receptor blocker and used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure [1]. Valsartan was competing with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin I. Nebivolol is chemically called as **(Figure 2)** 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. Nebivolol is a highly cardio selective vasodilatory beta1 receptor blocker and used in the treatment of hypertension [2]. Nebivolol blocks these receptors which reverses the effects of epinephrine, lowering the heart rate and blood pressure. Few analytical methods were reported for the estimation of Nebivolol and Valsartan as individually or other combination of drugs by UV [3] and HPLC [4-8] methods in its pharmaceutical dosage form. The present RP-HPLC method was validated as per ICH guidelines [9].

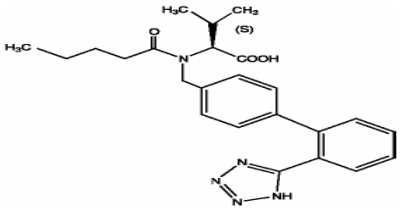

Fig 1: Structure of Valsartan

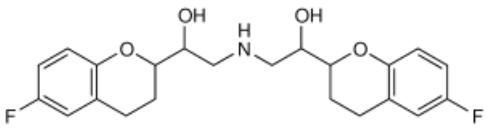

Fig 2: Structure of Nebivolol

MATERIALS AND METHODS:

Equipments and Conditions:

The chromatographic separation was conducted on a water liquid chromatographic system equipped with a Water 2695 isocratic solvent delivery system (pump), Water 2996 photo diode detector. A Hypersil BDS column (150 cm X 4.6 mm, 5 µm size) was used for the separation. The mobile phase consisted of a mixture of ammonium acetate buffer pH 5.2 adjusted with ortho phosphoric acid and acetonitrile (55:45, v/v). The mobile phase was prepared daily, filtered, sonicated before use and delivered at a flow rate of 1.0 ml/min at the detection wavelength of 282 nm.

Chemicals and Reagents:

The pure samples of Valsartan and Nebivolol obtained from Spectrum labs, Hyderabad. **NEBICARD-V** brand of Valsartan and Nebivolol procured from local market, Nuzuvid. Acetonitrile and methanol used were of HPLC grade; all analytical grade chemicals and solvents were obtained from E Merck (India) Ltd, Mumbai. Ammonium acetate and orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system.

Standard solution preparation:

Stock solutions for chromatographic measurement were prepared by dissolving standard Valsartan and Nebivolol in methanol of the 1 mg/min and for calibration, series of above solution containing Valsartan 200, 400, 600, 800, 1000 and 1200 µg/ml and Nebivolol 12.5, 25, 37.5, 50, 62.5, 75 µg/ml and were prepared by diluting the stock standard solutions of each drug with methanol in volumetric flask (10 ml) and all dilutions were scanned in the wavelength range of 200-400 nm. The detection wavelength was fixed at 282 nm.

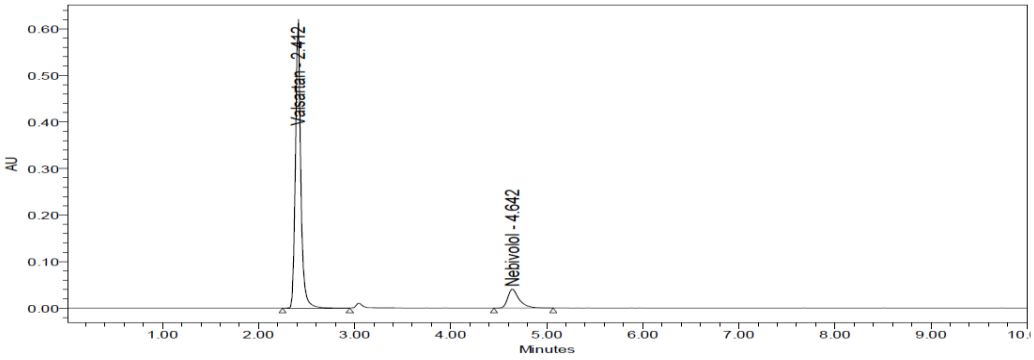

Fig. 3: Standard of Valsartan and Nebivolol chromatogram

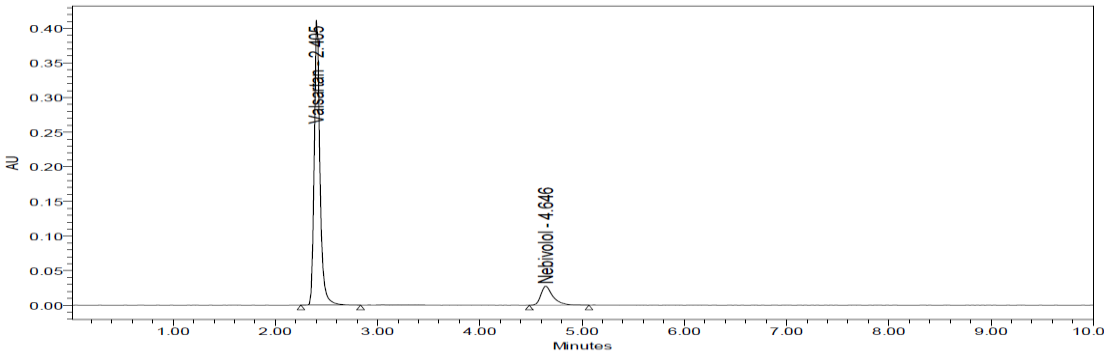

Fig. 4: Sample of Valsartan and Nebivolol chromatogram

Sample preparation:

Twenty tablets were weighed and finely powdered equivalent to 80 mg of Valsartan and 5 mg of Nebivolol and transferred in to 100 ml of volumetric flask. The drugs extracted with methanol, sonicated for 30 minutes and volume was made up to 100 ml with the same solvent. The solution was filtered through 0.45 µm nylon membrane filter and sonicated to degas. 2 mL of the solution was pipetted out and transferred in to 10 mL volumetric flask and make up to the volume with mobile phase and this solution was used for estimation. The retention times of sample Valsartan and Nebivolol were found to be 2.405 min and 4.646 min shown in Figure 4, respectively. The detection wavelength was fixed at 282 nm.

Method Validation:

Linearity: The linearity of Valsartan and Nebivolol were found to be 200-1200 µg/ml and 12.5-75 µg/ml, respectively. The linearity plot was constructed by plotting response factor against concentration of drug. The slope and intercept values of linearity plot for Valsartan Y = 1980 X + 25678 (R^2= 0.9990) and for Nebivolol Y= 4462 X+2016(R^2=0.9990), where Y represents the ratio of the peak area of analyte and X represents analyte concentration.

Precision: The precision of RP-HPLC method was obtained by analyzing on the same day (intra-day) and analyzing on the different day by triplicate analysis (inter-day) and expressed as percentage relative standard deviation (%.R.S.D).

Accuracy: The accuracy of the developed method was estimated using a mixture of Valsartan and Nebivolol solutions containing three concentrations of drug corresponding to 50%, 100% and 120% by determining the recovery of the added drug. Expressed as percentage relative standard deviation (%.R.S.D).

LOD and LOQ: The sensitivity of Valsartan and Nebivolol was determined as limit of detection (LOD) and limit of quantification (LOQ), they were calculated by use of the equations LOD = 3.3 x N/S and LOQ = 10 x N/S, where N is the standard deviation of the drug (n = 3), taken as a measure of the noise and S is the slope of the corresponding calibration plot.

***Results and Discussion*:**

Several attempts were performed in order to get satisfactory resolution of Valsartan and Nebivolol different mobile phases with various ratios of organic phase and buffer by using Hypersil BDS column. Initially the mobile phase used as a mixture of water and methanol fallowed by water and acetonitrile in different ratios. Other mobile phase tried was ammonium sulphate buffer pH 5.8 and acetonitrile (70:30, v/v) by isocratic elution which gave no satisfactory resolution. Ammonium acetate buffer pH 5.20 and acetonitrile (55:45, v/v) mobile phase was used by isocratic elution to obtain satisfactory and good resolution. The resolution of standard and sample solution for Valsartan and Nebivolol found reproducible and satisfactory.

UV wavelength selection: The detector wavelength was selected based on higher sensitivity in present study and the wavelength was fixed at 282 nm.

Method Validation:

Linearity: The linearity of RP-HPLC method was estimated at six concentration levels for Valsartan and Nebivolol and correlation coefficient shown in **Figure 5, Figure 6** and data presented in **Table 1**. The results shown that there is significant correlation exists between response factor and concentration of drugs within the concentration range indicated on Y-axis.

Precision: Intra-day precision was determined by analyze the solutions three times on the same day and inter-day precision was assessed by analyze the solution on three different days over a period of one week. The percentage relative standard deviation of both precision studies was reached within the limits.

The data of intra-day precision and inter-day precision presented in **Table 2**.

Accuracy: The accuracy of the method was estimated by the standard addition method at three different levels. The recovery studies were carried out for tablets by spiking standard of each drugs equivalent to 50%, 100% and 120% to the original amounts presents in each drug formulations. The average recoveries were as presented in **Table 3**.

LOD and LOQ: LOD and LOQ were indicating to the sensitivity of the method and the LOD and LOQ value were reported in **Table 4**.

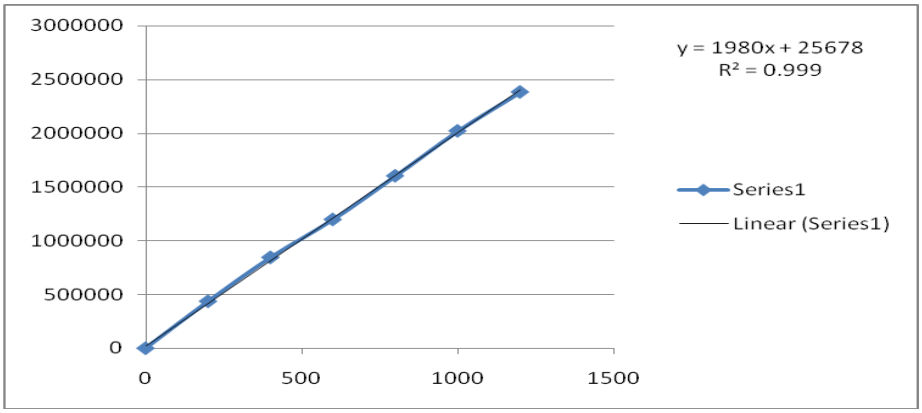

Figure 5: Linearity plot of Valsartan

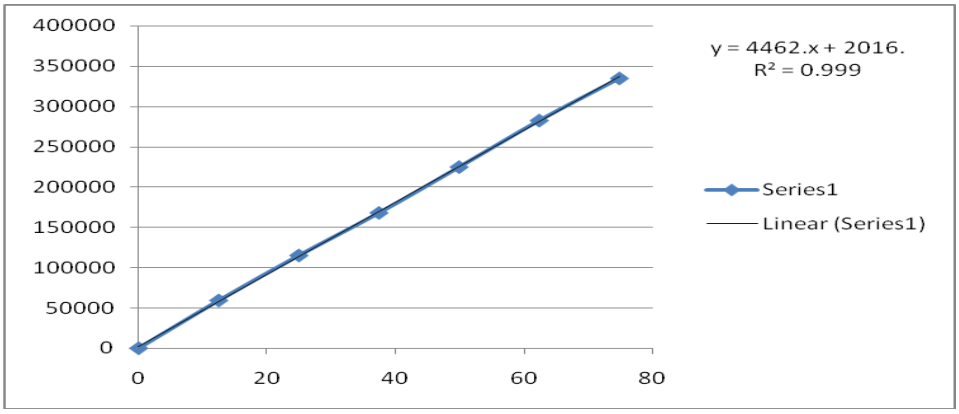

Figure 6: Linearity plot of Nebivolol

Table 1: Linearity data of Valsartan and Nebivolol

S. No	Valsartan		Nebivolol	
	Concentration levels (µg/mL)	Peak area	Concentration levels (µg/mL)	Peak area
1	200	437281	12.5	59389
2	400	845700	25	115200
3	600	1198897	37.5	168090
4	800	1605023	50	225013
5	1000	2023178	62.5	282758
6	1200	2460459	75	342147
Slope	1980		4462	
Y-intercept	25678		2016	
Correlation coefficient.	0.9990		0.9990	

Table 2: Precision data of Valsartan and Nebivolol

Sample number	Valsartan		Nebivolol	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	1684986	1684986	235978	235978
2	1684758	1684758	235075	235075
3	1684254	1684254	238097	238097
4	1686852	1686852	234626	234626
5	1707540	1707540	238325	238325
6	1686440	1686440	233712	233712
Mean	1689472	1689472	236102	236102
S.D	0.53	9071.0	0.778	1837.3
%RSD	0.533	0.53702	0.772	0.778

Table 3: Accuracy data of Valsartan and Nebivolol

Drug name	Level of addition (%)	Peak area	% Recovery	Average % recovery
Valsartan	50	849690	99.87	
	100	1694408	99.58	100.22± 0.23
	150	2583619	101.22	
Nebivolol	50	120530	101.29	101.09± 0.32
	100	241729	101.57	
	150	358474	100.42	

Table 4: LOD and LOQ data of Valsartan and Nebivolol

S. No	Parameter	Valsartan	Nebivolol
1	LOD (µg/mL)	0.25	1.4
2	LOQ (µg/mL)	0.77	4.5

CONCLUSION:

A new RP-HPLC method development and validation for the simultaneous estimation of Valsartan and Nebivolol in Tablets. The method assured the satisfactory precision and accuracy and has high analytical potential. The present method was found to be simple, accurate, economical and reproducible and can be applied for routine analysis in laboratories. RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies for same formulations.

ACKNOWLEDGEMENT:

The author thanks to Spectrum laboratories, Hyderabad for providing pure samples of Valsartan and Nebivolol. The author grateful to the management of Allure Krishna Rao and Govindamma College of Pharmacy for providing facilities to carry out this work.

REFERENCES:

1. Brixius KB, Bolck B, Nguyen Q, Schwinger RH. Eur J Pharmacol, 2003; 460:1-19.
2. The Martindale 35th ed., the complete drug reference, Pharmaceutical press, Lambeta high street, London SEI 75M, UK. 2006.
3. Arunadevi SB, Subramania NM and Suresh B. Simultaneous determination of Nebivolol and Valsartan in solid dosage form by spectrophotometric and RP-HPLC method. I J P S R, 2011; 2:424-431.
4. Bratty MdA, Gunasekar M, Hassan AA. Development and validation of RP-HPLC method for the simultaneous estimation of Amlodipine and Nebivolol in raw and tablet formulation. Int J Pharm Res Allied Sci, 2016; 54:37-44.
5. Sridevi RC, Badal KM and Sameer GN. Stability-indicating HPLC method for the simultaneous determination of Valsartan and

Ezetimibe in pharmaceuticals. Trop J Pharm Resea, 2014; 13:809-817.

6. Pani BD, Moorthy NT, Prakah DJ. Development and validation for the simultaneous quantification of Nebivolol hydrochloride and Hydrochlorothiazide by UV spectroscopy, RP-HPLC and HPTLC in tablets. E-Journal of Chemistry, 2010; 7: 341-348.
7. Kalpana N, Shanmukha VKJ, Ramachandran D, Ramanaiah G and Srinivas G. Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of Nebivolol and Valsartan in bulk and its pharmaceutical formulations. A J A D D, 2014; 2:624-637.
8. Text on Validation of Analytical Procedures Q2 (R1) in, I.C.H.Harmonised Tripartite Guidelines; Nov. 1996.