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New Validated RP-HPLC Method for Simultaneous Estimation of Valsartan and Nebivolol in Bulk and Dosage Forms

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Authors' contributions

This work was carried out in collaboration among all authors. Author BA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ST and YB designed the study, managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The main reason of this work to develop a sensitive, rapid, economic, precise reverse phase liquid chromatographic method to separate and assay the simultaneous determination of Valsartan and Nebivolol as a minor component in bulk and dosage forms. The separation was carried out using SHIMADZU UV-photo diode array detector at 200, 245 nm for Nebivolol and Valsartan respectively equipped with reverse phase C18 column Sunniest 5µm, 4.6 mm, 250 mm at 40˚C oven temperature, flow rate of 1 mL/min with mobile phase consist of 70:30 methanol: 10 mM phosphate buffer pH=3. The retention time of Nebivolol and Valsartan were to be found at 4.3 min and 8 min respectively. The method was validated according to ICH guidelines. The linearity of the proposed method was investigated in concentration range of 45.8-229% r=0.9997 for Nebivolol and the range 55-166%, r=0.9999 for Valsartan. Robustness in the case of little change of some chromatographic conditions. Validation of the proposed method was carried out for its linearity, accuracy, precision, robustness and assay. This method can be applied easily in routine work analysis to detrmine the estimation of nebivolol and valsartan in bulk and dosage forms.

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Keywords: Valsartan, Nebivolol, RP-HPLC, Antihypertensive drugs.

1. INTRODUCTION

Valsartan Fig. 1 is chemically known as (2S)-3-methyl-2-[N-({4-[2-(2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl) pentanamido] butanoic acid [1]. Valsartan is an antagonist of the angiotensin II receptor with similar actions to losartan. It is used to treat hypertension, to decrease cardiovascular mortality in patients with left ventricular dysfunction following myocardial infarction, and to manage heart failure [2].

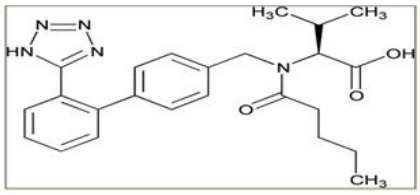

Fig. 1. Valsartan chemical structure

Nebivolol Fig. 2 is chemically known as 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 [3]. Nebivolol is a beta blocker that is cardio selective. It has vasodilating activity, which seems to be due to the endothelium's direct action, likely involving the release of nitric oxide. Nebivolol is used in the control of hypertension and in patients aged 70 years and older with stable chronic heart disease as an adjunct to standard care. It is given orally as the hydrochloride although doses are expressed in terms of the base; 5.45 mg of Nebivolol hydrochloride is equivalent to about 5 mg of base [2].

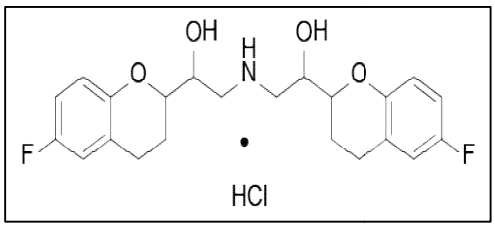

Fig. 2. Nebivolol chemical structure

Different analytical methods have been reported in the literature for the assay of Nebivolol and Valsartan in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS [4-20]. In this paper we present a simple HPLC method for the analysis of Nebivolol and Valsartan in bulk as well as in tablet dosage form. This method was validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1 MATERIALS

Valsartan and Nebivolol working standard manufactured by verdant life sciences PVT. Ltd.India. Multi-component tablet vitapress (Valsartan 80mg, Nebivolol 5mg) manufactured by Vita pharmaceutical industry, Syria. Batch Nr. 004 MFG 9/2019 EXP 9/2022. All samples, as received, were stored in the dark at ambient temperature and humidity. They were all analyzed within expiry dates. Isocratic HPLC grade methanol was purchased from Scharlau, Spain. Ortho-Phosphoric acid 85% extra pure was purchased from Scharlau, Spain. Potassium Hydroxide 85% from BELAMI fine chemicals PVT. LTD. India.

2.2 Instrumentation

A high performance liquid chromatography Shimadzu LCsolution version 1.25 equipped with a degasser DGU 20 A3, Shimadzu photo diode array detector SPD-M20A, an auto sampler SIL-20A, a pump LC-20AT, and an oven CTO-20A. Ultrasonic bath Hwashin technology co. Korea. A pH meter Crison, Spain. A Sartorius balance BP221S d = 0.1 mg, Spain. A micro pipette was from ISOLAB, Filters 0.45 µm.

2.3 Stock Solution Preparation

The stock of Valsartan and Nebivolol were prepared by weighed and transferred 100 mg of Nebivolol and 100 mg of Valsartan working standards into a 100 mL clean dry volumetric flask, and fill the flask to 100 mL with methanol, sonicated for 30 minutes. This was done for the standard mixture but for Valsartan stock Valsartan was weighed only and the same thing was done for Nebivolol stock.

2.4 Standard Solution Preparation

2.4.1 Standard solution of valsartan preparation

The standard solution was done by taken 1 mL of the Valsartan stock or the appropriate amount

according to the demand concentration and transfer it to a 10 mL clean dry volumetric flask, add 3 mL of phosphate buffer pH=3 and make it up to the final volume with methanol.

2.4.2 Standard solution of nebivolol preparation

The standard solution was done by taken 1 mL of the Nebivolol standard or the appropriate amount according to the demand concentration and transfer it to a 10 mL clean dry volumetric flask, add 3 mL of phosphate buffer pH=3 and make it up to the final volume with methanol.

2.4.3 Standard solution of the combination valsartan and nebivolol preparation

The standard solution was done by taken 1 mL of the mixture standard or the appropriate amount according to the demand concentration and transfer it to a 10 mL clean dry volumetric flask, add 3 mL of phosphate buffer pH=3 and make it up to the final volume with methanol.

2.4.4 Phosphate buffer preparation

To prepare 10 mM with pH=3 phosphate buffer. Add 0.2 mL of ortho phosphoric acid purity 85%, make up with deionized water and adjust the pH to 3 with 10% w/w potassium hydroxide solution the final volume 297 mL.

2.4.5 Sample solution preparation

20 tablets of valsartan and nebivolol product was crushed then the average was calculated then an equivalent amount to one tablet as labeled content added to 50 mL of methanol and sonicated for 20 minutes until the active ingredients was dissolved, then add 175 mL of methanol.

3. RESULTS AND DISCUSSION

3.1 Method Development

3.1.1 Selection of wavelength

After scanning the UV spectrum of the compounds the maximum absorbance of valsartan was 204 nm and there is a secondary peaks at 230 nm, 245 nm and the last one was chosen according to the best sensitivity and accuracy data see Fig. 3. And for nebivolol the maximum absorbance was 201 nm and secondary peaks at 215 nm, 281 nm and 200 nm was chosen for giving the best suitable results see Fig. 4.

3.1.2 Mobile phase composition

After trying different ratios of methanol:water 50:50 60:40 70:30 there was so much interaction and no good peak was detected.

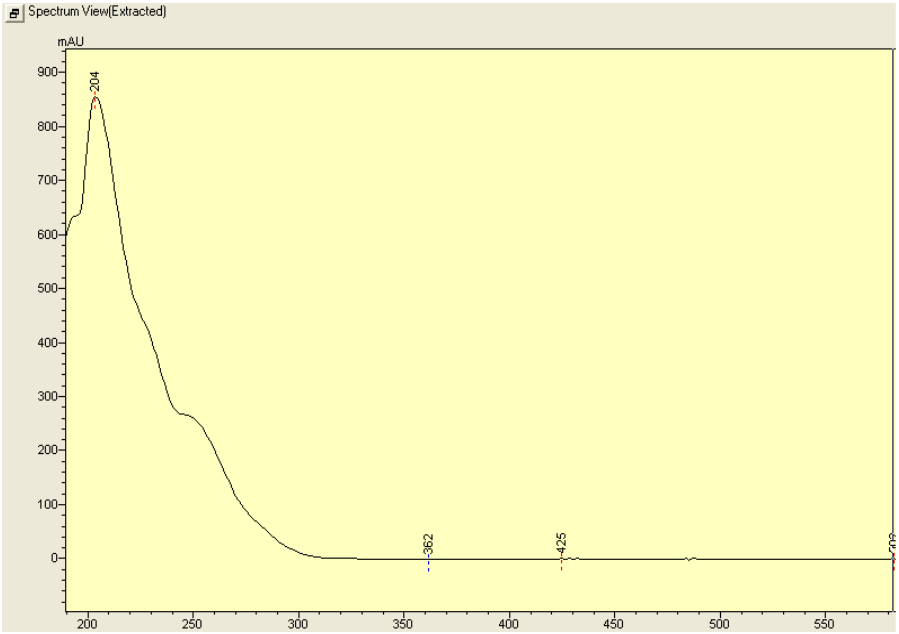

Fig. 3. Valsartan UV-spectrum

Then methanol:phosphate buffer with pH=3 was tested with different ratios as follow:

- 50:50 valsartan took very long time with very interaction.
- 60:40 bad resolution with tailing for valsartan and nebivolol.
- 70:30 good resolution and tailing it was the best.

3.2 The Optimal Chromatographic Conditions

The elution was isocratic. Separation was done by using a C18 column Sunniest 5µm, 4.6 mm, 250 mm. The mobile phase consists of 70:30 methanol: 10 mM phosphate buffer pH=3. The injection volume was 10 µl and the flow rate was 1 mL/min. prior to injection of the solutions, column was equilibrated for at least 40-min with mobile phase flowing through the system. The analytes were measured with a UV-photo diode array detector at 200, 245 nm for Nebivolol and Valsartan, respectively. Chromatographic separations were carried out at 40˚C see Figs. 5-6-7.

3.3 Method Validation

The analytical method was validated as per ICH guidelines with respect to parameters such as linearity, accuracy, precision, assay and robustness as follows.

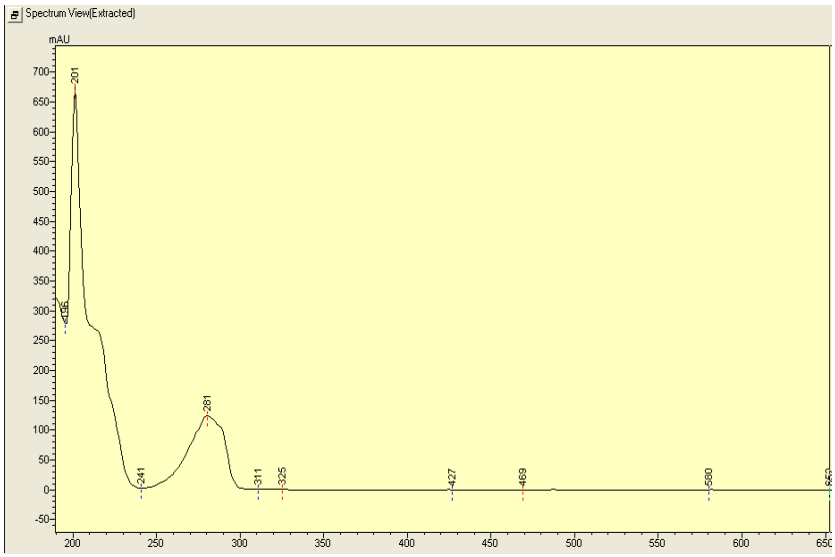

Fig. 4. Nebivolol UV-spectrum

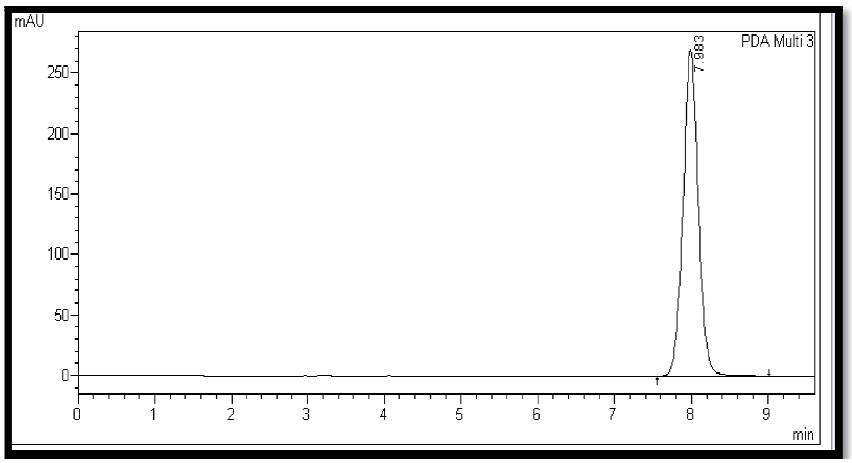

Fig. 5. Valsartan chromatogram by optimized condition

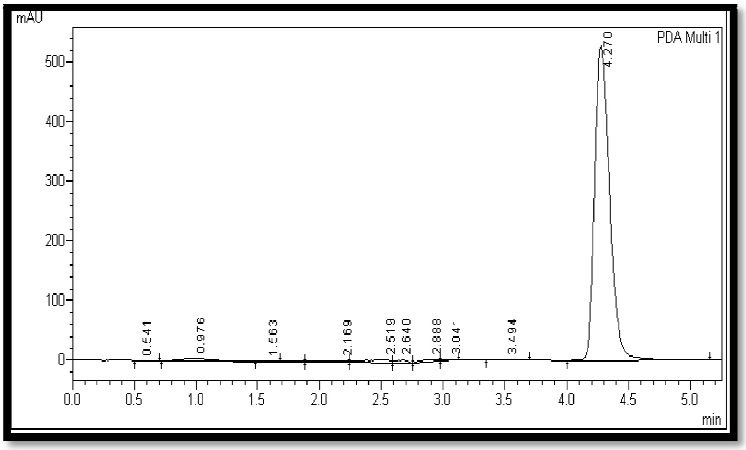

Fig. 6. Nebivolol chromatogram by optimized condition

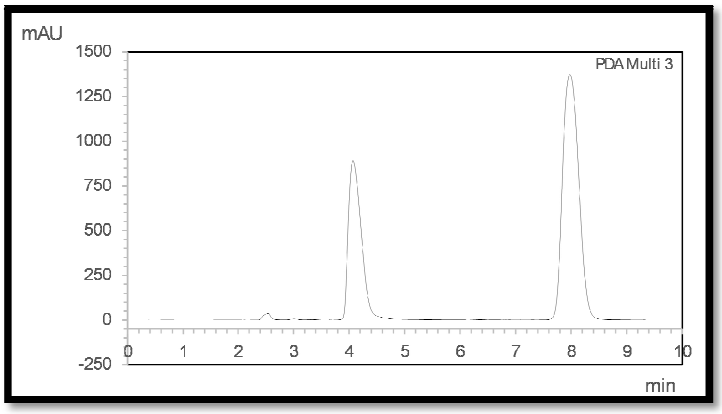

Fig. 7. Valsartan and nebivolol chromatogram by optimized condition

3.3.1 Linearity

Serial dilution of analyte were prepared from Valsartan stock solution and another serial dilution were prepared from Nebivolol stock solution, and the measurement done for every stock solution by taking suitable volume and diluted up to 10 mL as mentioned in the Valsartan sample preparation to get the desired concentrations 200, 300, 400, 500 and 600 µg/mL for linearity in the range of 55-166%, and the same steps were done for Nebivolol sample preparation to get the desired concentrations 10, 20, 30, 40, and 50 µg/mL in the range of 45.8-229%. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected three times into the column. Absorbance at 245 nm was measured and calibration curve for Valsartan was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). Absorbance at 200 nm was measured and calibration curve for Nebivolol was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis) as shown in Fig. 8 and Fig. 9, respectively. The developed method was found to be linear over the range of 55-166% for Valsartan and of 45.8-229% for Nebivolol because the correlation coefficient R^2 above 0.999.

3.3.2 Accuracy

The accuracy was evaluated by preparing triplicate of three concentration from the linearity range to Valsartan and Nebivolol and injected into the HPLC system per the test procedure and the average %recovery was calculated as shown in table 1. The developed method is accurate according to the %recovery of the studied concentration which was between 98-102% [21].

3.3.3 Precision

The intraday precision was studied by determining three concentrations from the linearity range for both Valsartan and Nebivolol. The interday precision was studied by determining the same three concentrations that was studied in the intraday precision but with new preparations [21] Table 2. The method found to be precise in the intraday study %RSD below 1% and in the interday study %RSD below 2% [22].

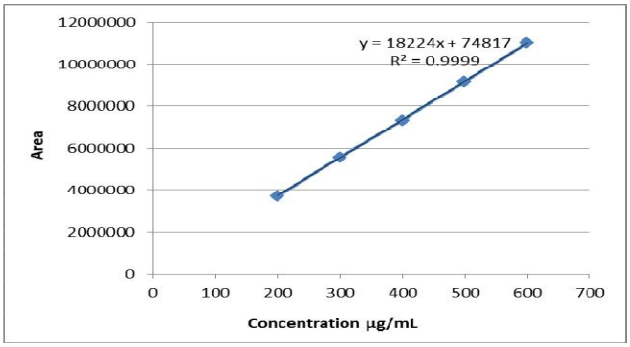

Fig. 8. Calibration curve of valsartan

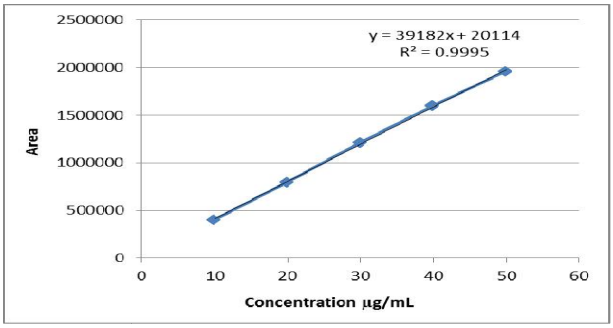

Fig. 9. Calibration curve of nebivolol

Table 1. Accuracy data

Substance	Conc. range (µg/mL) n=3	Amount recovered. range (µg/mL) n=3	%Recovery(Mean ±sd) n=3
Val	200	199.317	99.66±0.23
	400	399.243	99.81±0.15
	600	600.651	100.11±0.16
Neb	10	9.802	98.02±0.65
	30	30.417	101.39±0.43
	50	49.592	99.18±0.22

Table 2. Precision data

Parameter	Conc. range (µg/mL)	%RSD (Intra-day n=3)	%RSD (inter-day n=6)
Val	200	0.560	0.923
	400	0.375	1.710
	600	0.150	0.788
Neb	10	0.212	0.772
	30	0.134	1.688
	50	0.239	0.852

3.3.4 Robustness

The robustness was evaluated by making a small changes in the chromatographic conditions and calculate the %RSD for average peak area as it was below 2%. The selected factors are the mobile phase composition, the column temperature, the wavelength and buffer pH Table 3. For each parameter in robustness studies the relative standard deviation was found less than 2%, and the low %RSD value confirms the robustness of the method.

3.3.5 System Suitability Test

The system suitability was assessed by six replicate analysis of Valsartan and Nebivolol with concentration 100% to verify the chromatographic system adequate for the analysis to be done. This method was evaluated by analyzing the repeatability of retention time, peak area for Valsartan and Nebivolol tailing factor, theoretical plates of the column and resolution between the Valsartan and Nebivolol [23] Table 4. According to robustness results wavelength can't be changed. For this method the retention time should be 8.09±0.2 minutes for valsartan and 4.35±0.2 minutes for nebivolol, as for resolution between nebivolol and valsartan should not be less than 7, the tailing factor should be between 1.1-1.25 for both valsartan and nebivolol, and for number of plates should not be less than 3000 for both valsartan and nebivolol. The system suitability verify the repeatability of the chromatographic system for the selected factors %RSD below 1%.

3.3.6 Assay

The assay was done by injected the standard solution and the sample solution of Valsartan and Nebivolol as given in the chromatographic conditions. The amount of the sample solution was calculated by the linearity curve of Valsartan and Nebivolol. For vitapress label claim the amount is 80 mg for Valsartan and 5 mg for Nebivolol Table 5.

3.3.7 Specificity

With a good resolution and without interference between the peaks, the analyzed components were separated very well, meaning this method is specific. And there were no interfaces between the components analyzed and the excipients used in this table after applying it on a commercial table.

Table 3. Robustness data

		Valsartan		Nebivolol	
	Ratio	**Average peak area**	**RSD%**	**Average peak area**	**RSD%**
Mobile phase composition (meth:buff)	68:32	3555836	0.375	2091630	1.150
	70:30	3573103		2073703	
	72:28	3582182		2044467	
Column temperature	39˚C	3572362	0.020	2070767	1.129
	40˚C	3573103		2073703	
	41˚C	3571658		2112945	
Wavelength (val/neb)	243/198	3390424	2.673	2153184	2.8634
	245/200	3573103		2073703	
	247/202	3453225		2103873	
Buffer pH	2.9	3562201	0.334	2033765	1.001
	3.0	3573103		2073703	
	3.1	3586043		2045456	

Table 4. System suitability parameters (n=6)

Parameter	Val	Neb
Retention time (tR)	8.09	4.35
Resolution (RS)	9	-
Tailing factor (T)	1.17	1.20
Number of plates (N)	4358	3678
Precision (%RSD)	0.10	0.62

Table 5. Assay data

		Valsartan		Nebivolol	
Sample	**Label**	**Amount found**	**%Recovery**	**Amount found**	**%Recovery**
VITAPRESS	80/5mg	79.87	99.84	4.91	98.2

3.4 Comparative Study

In the literature review, there are many papers considering RP-HPLC determination of Valsartan and Nebivolol, each of them determining both drugs at low sensitivity UV-wavelength for valsartan [24-26]. Furthermore, the mobile phase used in this work containing methanol which is safer to the environment than acetonitrile used in many works before. Finally, there is no work before determining Valsartan and Nebivolol simultaneously with mobile phase containing methanol and phosphate buffer only [27].

4. CONCLUSION

The developed method was sensitive, rapid, economic and precise reverse phase liquid chromatographic method for the simultaneous determination of Valsartan and Nebivolol as a minor component in bulk and dosage forms. It has useful characteristics over other existing methods. This method can be applied easily in routine work to determinate Valsartan and Nebivolol in combined dosage forms.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the us and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of us.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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