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DEVELOPMENT OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL AND VALSARTAN BULK AND IT'S FORMULATION

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ABSTRACT

The estimation of Nebivolol and Valsartan was done by RP-HPLC. The assay of Nebivolol and Valsartan was performed with tablets and the % assay was found to be 99.70 and 98.30 which shows that the method is useful for routine analysis. The linearity of Nebivolol and Valsartan was found to be linear with a correlation coefficient of 0.999 and 0.998. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.13% and 99.95% for Nebivolol and Valsartan.

Key words: Nebivolol, Valsartan, RP-HPLC

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. It is well known in the pharmaceutical industry that pharmaceutical analyst in research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed (1).

Nebivolol (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) is a highly cardioselectivevasodilatory beta1 receptor blocker

used in treatment of hypertension. In most countries, this medication is available only by prescription.

Valsartan ((2S)3methyl2[N({4[2(2H1,2,3,4tetrazol5yl)phenyl]phenyl}methyl)pentanamido] butanoic acid) is an angiotensin-receptor blocker (ARB) that may be used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy and heart failure. Valsartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Valsartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease.

The literature review (2-6) reveals that few HPLC methods for the estimation of Nebivolol and Valsartan alone and in combination with other drugs. Very few methods are reported for estimation of both drugs from formulation .We intends to develop RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity and faster elution.

MATERIALS AND METHODS

HPLC method development

Wave length selection

UV spectrum of 10 µg / ml Nebivolol and Valsartan in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 240. At this wavelength both the drugs show good absorbance.

Preparation of Phosphate buffer

Take 6.8gms of Potassium Di hydrogen Ortho Phosphate in 1000ml of water and sonicate for 2 minutes and adjusted the Ph for 3.0, Sonicate for 2 min.

Preparation of mobile phase

Mix a mixture of above buffer 500 ml (50%) and 500 ml Acetonitrile (50%) and degas in ultrasonic water bath for 5 minutes. Filter through 045 µ filter under vacuum filtration.

Assay

Standard Solution Preparation

Accurately weigh and transfer 5 mg of Nebivolol & 160 mg of Valsartan working standard into a 100ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1 ml of Nebivolol & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml of Nebivolol & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation

Accurately weigh and transfer equivalent to 5 mg of Nebivolol & 160 mg Valsartan equivalent weight of the sample into a 10ml clean dry volumetric flask add about 70ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 1 ml of Nebivolol & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3 ml of Nebivolol & Valsartan of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure

Inject 20 µl of the standard, sample into the chromatographic system and measure the areas for the Nebivolol & Valsartan peaks and calculate the %Assay by using the formulae (7)

Validation of the method using formulations was done by ICH guidelines (8-10).

RESULTS AND DISCUSSION

Optimized Method

Column: ODS C18 column (4.6×200mm)10µ, Mobile phase ratio : ACN: pH 3 phosphate buffer (50: 50 % v/v), Detection wavelength : 240 nm, Flow rate : 1.0ml/min, Injection volume : 20µl, Column temperature : Ambient, Auto sampler temperature: Ambient, Run time : 6min.

Assay

Standard and sample solution injected as described under experimental work. The corresponding chromatograms and results are shown below (Fig-1 and 2).

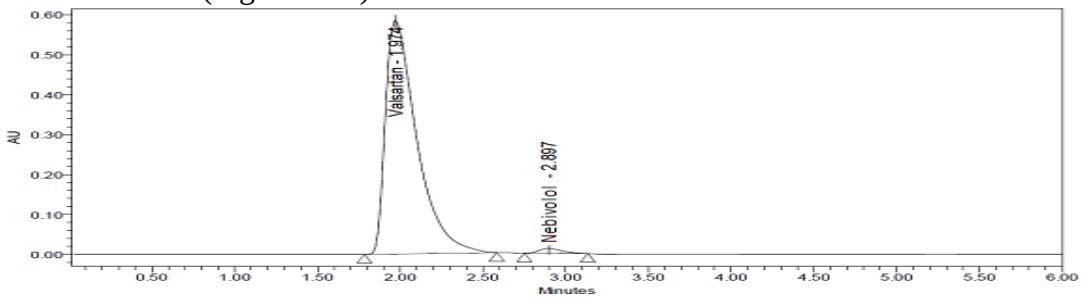

Figure 1- Chromatogram for Standard

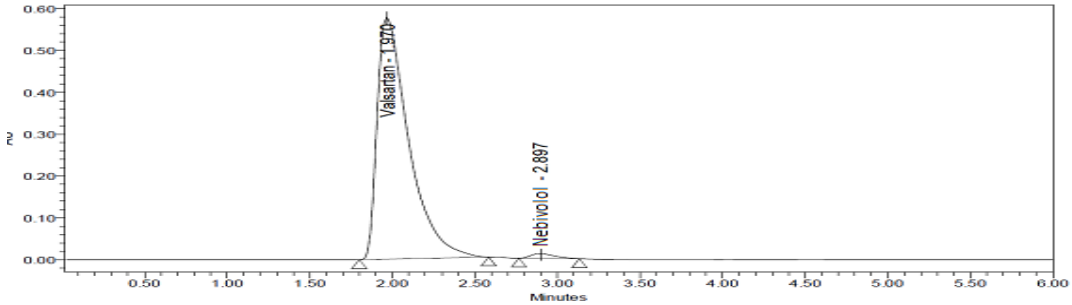

Figure 2- Chromatogram for Sample

Linearity

The linearity range was found to lie from 0.5µg/ml to 2.5µg/ml of Nebivolol (Table-1 and fig-3), 16µg/ml to 80µg/ml of Valsartan (Table-1 and fig-4).

Table 1- Area of different concentration of Nebivolol and Valsartan

S. No	Nebivolol		Valsartan	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	0.5	34657	16	2558079
2	1	75042	32	5042405
3	1.5	117770	48	7198342
4	2	161425	64	9471867
5	2.5	191811	80	11368323

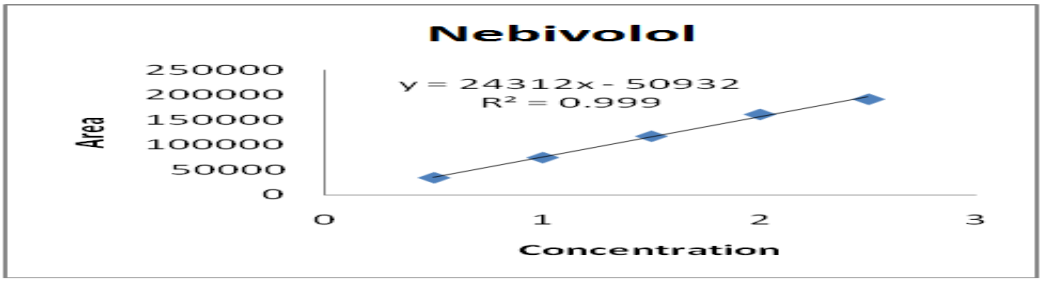

Figure 3- Calibration graph for Nebivolol

Figure 4- Calibration graph for Valsartan

Precision

Precision of the method was carried out for both sample solutions as described under experimental work. The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Accuracy

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated (Table-2 and 3)

Table-2 Accuracy (recovery) data for Nebivolol

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	61068	2.5	2.55	101.94	100.13
100%	118579	5	4.95	98.98	
150%	178732	7.5	7.46	99.46	

*Average of three determinations

Table-3 Accuracy (recovery) data for Valsartan

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3888916	80	80.92	100.10	99.95
100%	7609013	160	158.33	98.96	
150%	11626003	240	241.92	100.80	

*Average of three determinations

The percentage recovery was found to be within the limit (97-103%).The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

CONCLUSION

The estimation of Nebivolol and Valsartan was done by RP-HPLC. The assay of Nebivolol and Valsartan was performed with tablets and the % assay was found to be 99.70 and 98.30 which shows that the method is useful for routine analysis.The linearity of Nebivolol and Valsartan was found to be linear with a correlation coefficient of 0.999 and 0.998, which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 1.75 and 0.32 for Nebivolol and Valsartan which shows that the method is precise. The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 1.48 and 0.26 for Nebivolol and Valsartan which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.13% and 99.95% for Nebivolol and Valsartan. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Nebivolol was found to be 2.98 and 9.98 and LOD and LOQ for Valsartan was found to be 2.96 and 9.96. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

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