An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING RP-UPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF NEBIVILOL AND VALSARTAN IN BULK AND PHARMACEUTICAL FORMULATION

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ARTICLE INFO

Key Words
ICH guidelines ,Nebivolol and Valsartan
Reverse phase Ultra performance liquid Chromatography

ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of the Nebivolol and Valsartan in Bulk and pharmaceutical formulation. Chromatogram was run through HSS C18 50 x 2.6 mm, 1.8µ. Mobile phase containing 0.01N KH2PO4: Acetonitrile taken in the proportions 65:35 was pumped through column at a flow rate of 0.3ml/min. Temperature was kept up at 30°C. Optimized wavelength selected was 260nm. Retention time of Valsartan and Nebivolol were observed to be 1.290 min and 1.554 min. %RSD of the Valsartan and Nebivolol were and observed to be 0.9 and 0.7 respectively. %Recovery was obtained as 100.27% and 99.63% for Valsartan and Nebivolol respectively. LOD, LOQ values obtained from regression equations of Valsartan and Nebivolol were 0.25, 0.77 and 0.02, 0.07 respectively. Regression equation of Valsartan is y = 14801x + 9449 , and y = 6612 x + 836 . 8 of Nebivolol Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular Quality control test in Industries.

INTRODUCTION

Valsartan and Nebivolol is a combination medicine used to treat hyper tension (High blood pressure) in adult [(1-4)]. High blood pressure adds to the work load of the heart and arteries. If it countinues for a loading time, the heart and arteries may not function properly. Lower blood pressure will reduse the risk of strokes and heart attacks. Indicated for hypertension, to lower blood pressure and reduce the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarction. Nebivolol is a beta-blocker. It works by affecting the response to nerve impulses in certail part of the body, like the heart. Valsartan is an angiotensin II receptor blocker (ARB). It works by blocking a substance in the body that causes blood vessels to tighten.

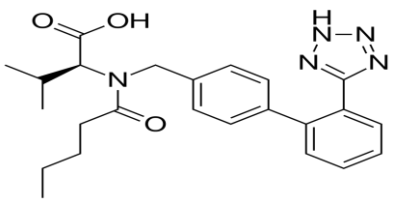

Fig 1: Structure of Valsartan

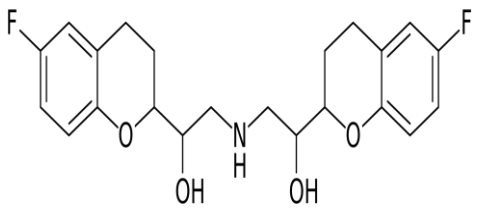

Fig 2: Structure of Nebivolol

As far as literature is concerned, there are No reported methods for the determination of Nebivolol and valsartan in combined pharmaceutical dosage form by using RP-UPLC methods. All the UPLC methods lack stability indicating nature.However, none of the reported analytical methods describe a stability indicating method by UPLC for the simultaneous determination of Nebivolol and Valsartan in a combined dosage form. To our knowledge, this was the first report of a stability indicating method for the simultaneous determination of both Nebivolol and valsartan in Pharmacuetical dosage forms by UPLC. The present manuscript describes a simple, rapid, precise and accurate isocratic reversed-phase stability-indicating UPLC method for the simultaneous determination of Nebivolol and valsartan in the same Pharmacuetical dosage form and validated as per ICH guideline [5-11].

MATERIALS AND METHODS:

Preparation of buffer:

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 3.40 with dil. Orthophosphoric acid solution.

Preparation of mobile phase: Mix a mixture of above buffer 650ml (650%) and acetonitrile 350ml (HPLC grade 60%)and degassed in ultrasonic water bath in 20min.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Stock solution:

Preparation of Standard stock solutions: Accurately weighed 20mg of Valsartan, 1.25mg of Nebivolol and transferred to 25ml volumetric flask and 3/4 the of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (800µg/ml of Valsartan and 50µg/ml of Nebivolol)

Preparation of Sample stock solutions: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (800µg/ml of Valsartan and 50µg/ml of Nebivolol)

Working solution:

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (80µg/ml Valsartan of and 5µg/ml of Nebivolol)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (80µg/ml of Valsartan and 5µg/ml of Nebivolol)

Procedure: 10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Valsartan and Nebivolol the peaks were used for calculating the % assay by using the for the formula.

Results and discussion: method validation: specificity, linearity range, accuracy, precision, repeatability, intermediate precision, limit of detection, limit of quatification, rubustness.

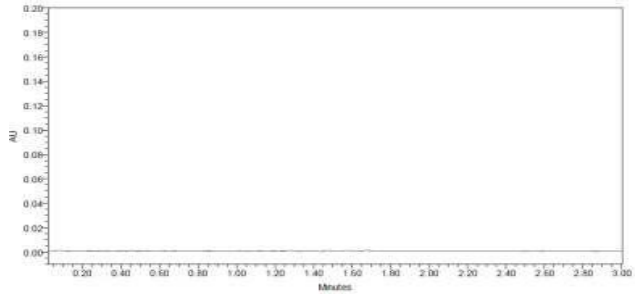

Fig. No. 3: Chromatogram showing Blank

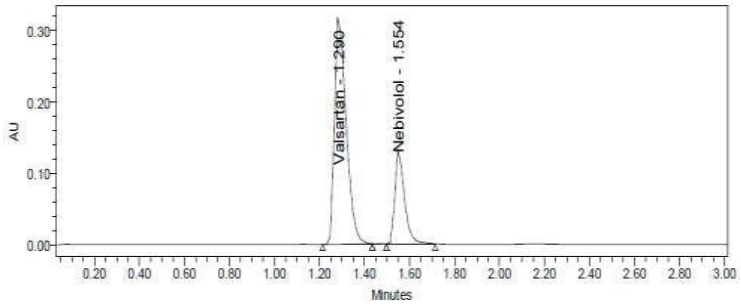

Fig. No. 4: Chromatogram showing optimized condition

Table 1: Linearity results for Valsartan and Nebivolol

Valsartan		Nebivolol	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
20	307154	1.25	84322
40	606823	2.5	163474
60	916767	3.75	250512
80	1187605	5	334450
100	1470423	6.25	414407
120	1793708	7.5	494358

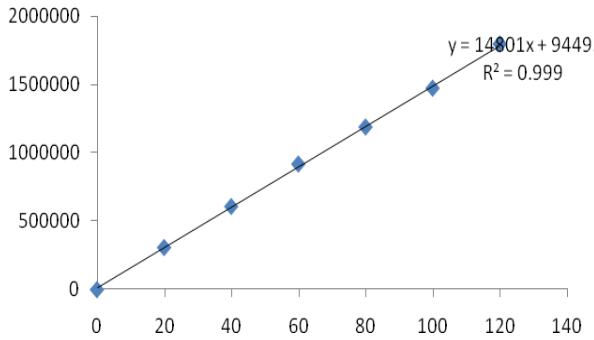

Fig: 5. Calibration curve of Valsartan

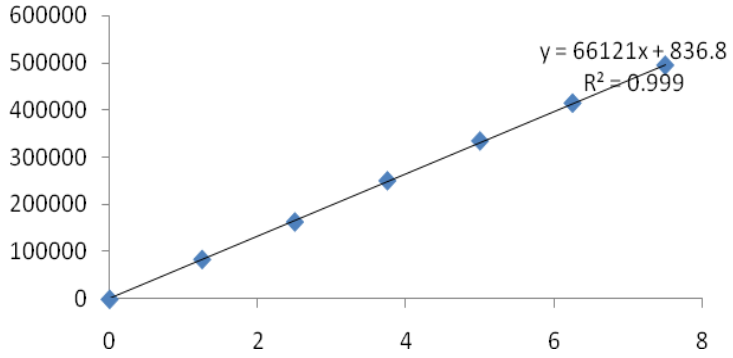

Fig 6- Calibration curve of Nebivolol

Table 2: Accuracy results for Valsartan

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	40	39.692	99.23	100.27%
100%	80	80.468	100.59	
150%	120	121.190	100.99	

Table 3: Accuracy results for Nebivolol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	2.5	2.502	100.09	99.62%
100%	5	4.953	99.06	
150%	7.5	7.479	99.72	

Table 4: System precision results for Valsartan and Nebivolol

S. No	Area of Valsartan	Area of Nebivolol
1.	1133316	335637
2.	1141839	335147
3.	1153770	332294
4.	1156435	334385
5.	1145430	330292
6.	1132148	337278
Mean	1145924	334172
S.D	10111.6	2504.8
%RSD	0.9	0.7

Table 5: Intermediate precision results for Valsartan and Nebivolol

S. No	Area of Valsartan	Area of Nebivolol
1.	995355	329775
2.	998902	326866
3.	994511	329328
4.	995345	329760
5.	994522	332000
6.	999451	330997
Mean	996348	329788
S.D	2229.5	1738.1
%RSD	0.2	0.5

Table 6: LOD & LOQ results for Valsartan and Nebivolol

Molecule	LOD	LOQ
Valsartan	0.25	0.77
Nebivolol	0.02	0.07

Table 7: Robustness data for Valsartan and Nebivolol

S.no	Condition	%RSD of Valsartan	%RSD of Nebivolol
1	Flow rate (-) 0.0.2ml/min	0.7	1.2
2	Flow rate (+) 0.4ml/min	0.3	0.9
3	Mobile phase (-) 70B:30A	0.3	0.3
4	Mobile phase (+) 60B:40A	0.4	0.2
5	Temperature (-) 25°C	1.1	0.5
6	Temperature (+) 35°C	0.2	0.3

Specificity: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. the specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak

Linearity: To establish the linearity of the method, serial dilution were prepared to obtain the mixture of Valsartan and Nebivolol ranging from 20ppm to 120ppm and 1.25ppm to 7.5ppm level all the solutions were filtered through a 0.45µm Millipore filters.the final solution were injected in duplicate manner keeping the injection volume 10µl.Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of Valsartan and Nebivolol was shown in figure no. 5&6. The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear .The were tabulated in table 1.

Accuracy: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 &3.

Precision: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration Valsartan and Nebivolol was 80µg/ml and 5µg/ml at two levels intra and inter day precision. The %RSD was calculated and results were reported and table no. 4&5.

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

The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1.

The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6.

Robustness : The small deliberate changes in method like flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.2ml/min), flow plus (0.4ml/min), mobile phase minus, mobile phase plus, temperature minus (25ºc), temperature plus (35ºc) was maintain and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no. 7.

CONCLUSION:

The proposed stability indicating RP-UPLC method was found to be simple, accurate, precise, robust, and rapid. This method gives good resolution between two compounds with a short analysis time. Hence this method can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Valsartan and Nebivolol.

Acknowledgement: The author expresses sincere thanks to Spectrum research solution .Hyderabad to provide gift samples of Nebivolol and Valsaton drug samples

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