IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 1 Ser. II (Jan –Feb 2020), PP 63-69
www.Iosrjournals.Org

Method Development and Validation for the Simultaneous Estimation of Nebivolol and Valsartan in Bulk and Solid Dosage Form by Rp-Hplc

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Abstract: *A simple, accurate and precise method was developed for the simultaneous estimation of the Nebivolol and Valsartan in tablet dosage form. The retention time of Nebivolol and Valsartan were found to be 2.7min and 4.7min respectively. The % RSD of the Nebivolol and Valsartan were found to be 0.95 and 1.16 respectively. The % recovery was obtained as 100.09 and 99.77 for Nebivolol and Valsartan respectively. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular quality control tests in Industries.*

Keywords- *Nebivolol, Valsartan, retention time, regression and quality control.*

Date of Submission: 21-01-2020 Date of Acceptance: 12-02-2020

I. Introduction

The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. In HPLC, reversed phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl and octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds is reversed thus the name reverse-phase chromatography [1].

Method development and optimization in liquid chromatography is still an attractive field for theoreticians and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution. Alternate analytical methods are developed for the drug product to reduce the cost and time. When alternative analytical methods are intended to replace the existing procedure, analyst should collect the literature for all types of information related to analyte and define the separation goal. Then estimate the best separation condition from trial runs. After optimizing the separation condition, validate the method for release to routine laboratory [2].

In reverse-phase chromatography, the mobile phase is more polar than the stationary phase. Mobile phase in these systems is usually mixtures of two or more individual solvents with or without additives or organic solvent modifiers. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. Separations in these systems are considered to be due to different degrees of hydrophobicity of the solutes. The simple alteration of composition of the mobile phase or of the flow rate allows the rate of the elution of the solutes to be adjusted to an optimum value and permits the separation of a wide range of the chemical types. First isocratic run followed by gradient run is preferred [3, 4].

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method [5].

II. Experimental

2.1: Materials: Nebivolol and Valsartan, Combination Nebivolol and Valsartan tablets, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, triethylamine, orthophosphoric acid.

2.2: Instruments: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells were used for measuring absorbance for Nebivolol and Valsartan solutions.

2.3: Methods:

2.3.1: Preparation of buffer: Accurately weighed 1.41gm of disodium hydrogen phosphate in a 1000ml of volumetric flask, add about 900ml of milli-Q water and degas to sonicate and finally make up the volume with water and pH adjusted to 4.8 with dil. OPA.

2.3.2: Standard Preparation: Accurately weigh and transfer 5mg of Nebivolol and 16mg of Valsartan working standards in to 50 ml and 10ml clean dry volumetric flasks, add $3/4^{th}$ ml of diluent, sonicate for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken in to a 10ml volumetric flask and made up to 10ml.

2.3.3: Sample Preparation: 5 tablets were weighed and calculate the average weight of each tablet, then the weight equivalent to 5 tablets was transferred in to a 50mL volumetric flask, 30mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out into a 10ml volumetric flask and made up to 10ml with diluent.

2.4: Method Validation:

2.4.1: Linearity: Linearity solutions are prepared such that 0.25ml, 0.5ml, 0.75ml, 1ml, 1.25ml, 1.5ml from the stock solutions of Nebivolol and Valsartan are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 2.5ppm, 5ppm, 7.5ppm, 10ppm, 12.5ppm, 15ppm of Nebivolol and 40ppm, 80ppm, 120ppm, 160ppm, 200ppm, 240ppm of Valsartan.

2.4.2: Precision:

2.4.2.1: Standard Preparation: Accurately Weigh and transfer 5mg of Nebivolol and 16mg of Valsartan working standards into 50 ml and 10ml clean dry volumetric flasks, add $3/4^{th}$ ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

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2.4.3: Accuracy:

2.4.3.1: Standard Preparation: Accurately Weigh and transfer 5mg of Nebivolol and 16mg of Valsartan working standards in to 50 ml and 10ml clean dry volumetric flasks, add $3/4^{th}$ ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

2.4.3.2: Sample preparation:

2.4.3.2.1: 50%: 5 tablets were weighed and calculate the average weight of each tablet, then powder weight of 500mg was transferred into a 50mL volumetric flask, 35mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out in to a 10 ml volumetric flask and made up to 10ml with diluent.

2.4.3.2.2: 100%: 5 tablets were weighed and calculate the average weight of each tablet, then powder weight of 1000mg was transferred into a 50mL volumetric flask, 20mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out in to a 10ml volumetric flask and made up to 10ml with diluent. 2.4.3.2.3: 150%: 5 tablets were weighed and calculate the average weight of each tablet, then powder weight of 1500mg was transferred into a 50mL volumetric flask, 20mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipetted out in to a 10ml volumetric flask and made up to 10ml with diluent.

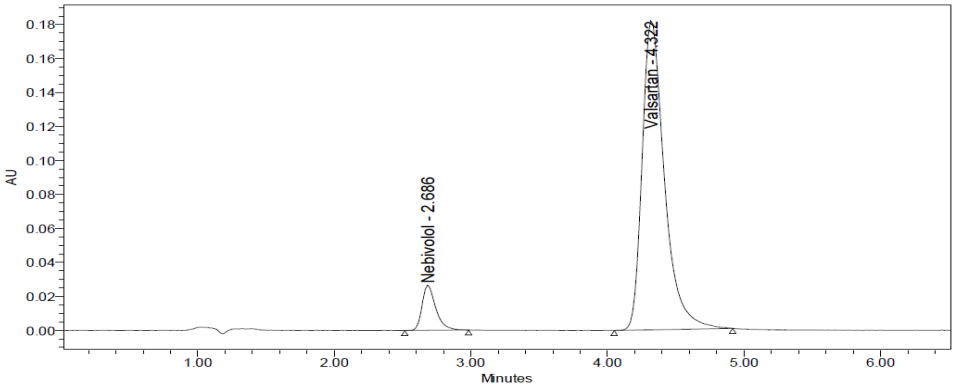
III. Results And Discussions

Optimized Method: Drugs were eluted with good retention time, resolution, all the system suitable parameters like Plate count and Tailing factor were within the limits.

Column Used	: Hypersil ODS 3V (150mm 4.6mm, 5µ)
Buffer used	: Ammonium Acetate Buffer, pH 5.2
Mobile phase	: Buffer: Acetonitrile (60:40)
Flow rate	: 1ml/min

Diluent : Methanol
Wavelength : 280
Temperature : 30°C
Injection Volume : 10µl

Figure 1: Optimized Chromatogram of Nebivolol and Valsartan

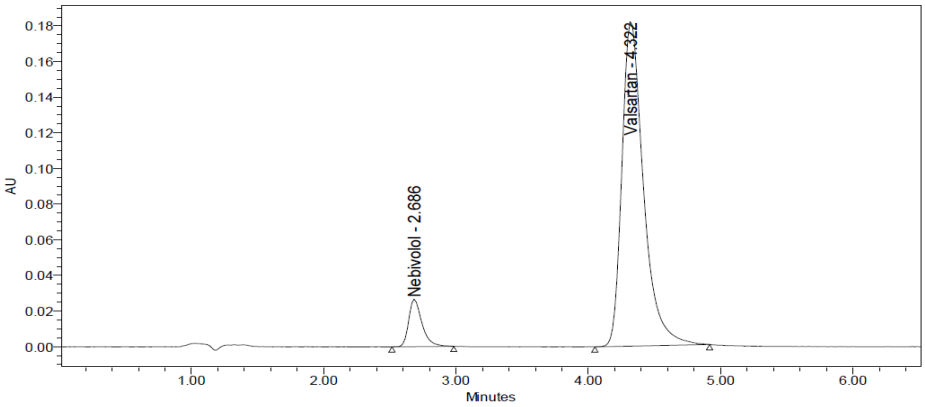


Method Validation:
System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Table 1: System suitability studies of NEBIVOLOL AND VALSARTAN

Property	Nebivolol	Valsartan
Retention time (t_R)	2.6 ± 0.3 min	4.3±0.3min
Theoretical plates (N)	3655 ± 163.48	3648± 163.48
Tailing factor (T)	1.3 ± 0.117	1.4± 0.117
Resolution		6.85

Figure 2: Typical chromatogram of Nebivolol and Valsartan



Linearity: Six Linear concentrations of Nebivolol (2.5ppm to 1.5ppm) and Valsartan (40ppm to 240ppm) are prepared and injected. Regression equation of the the Nebivolol and Valsartan are found to be, y = 17934x + 2125 and y = 13167x + 2928.
The regression co-efficient was 0.999.

Table 2 : Calibration data of Nebivolol and Valsartan

S.No.	Concentration Nebivolol (µg/ml)	Response (mV)	Concentration Valsartan	Response
1	0	0	0	0
2	2.5	46815	40	569137
3	5	92434	80	1032691
4	7.5	139416	120	1572044

5	10	183572	160	2069935
6	12.5	224648	200	2662946
7	15	269524	240	3174087

Figure 3: Calibration curve of Nebivolol

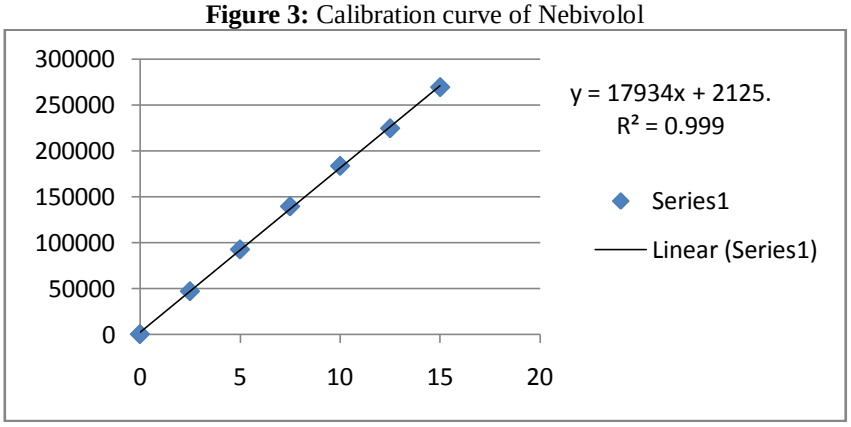
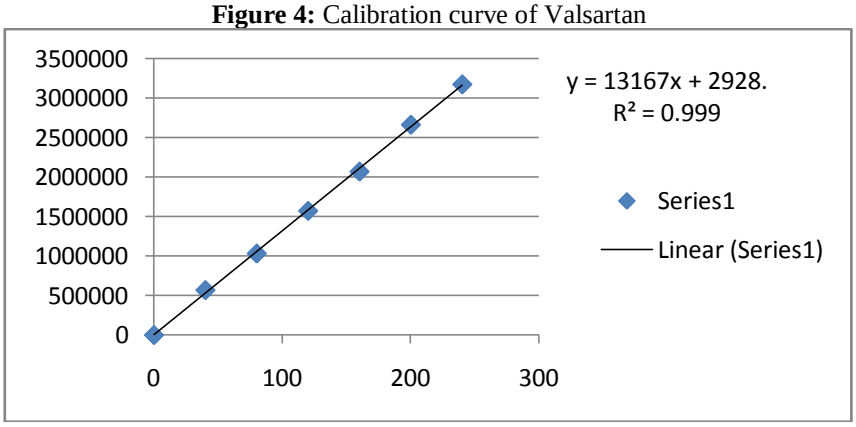


Figure 4: Calibration curve of Valsartan



Precision:

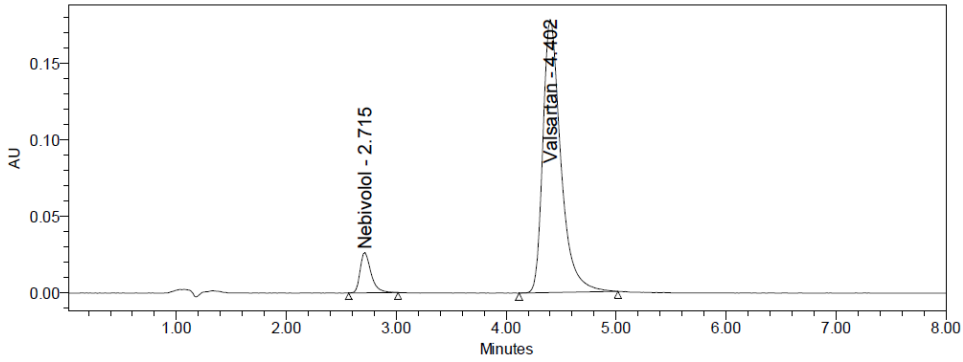
Intraday precision (Repeatability): Intraday Precision was performed and % RSD for Nebivolol and Valsartan were found to be 0.95% and 1.16% respectively.

Table 3: Repeatability results for Nebivolol and Valsartan

S.No.	Nebivolol	Valsartan
1	184889	2114307
2	189782	2060900
3	186617	2072624
4	185610	2088008
5	186430	2047709
6	188169	2058827
Mean	186916	2073729
Std. Dev.	1784.6	24147.0
%RSD	0.95	1.16

*Average of six determinations

Figure 5: Repeatability Chromatogram of Nebivolol and Valsartan

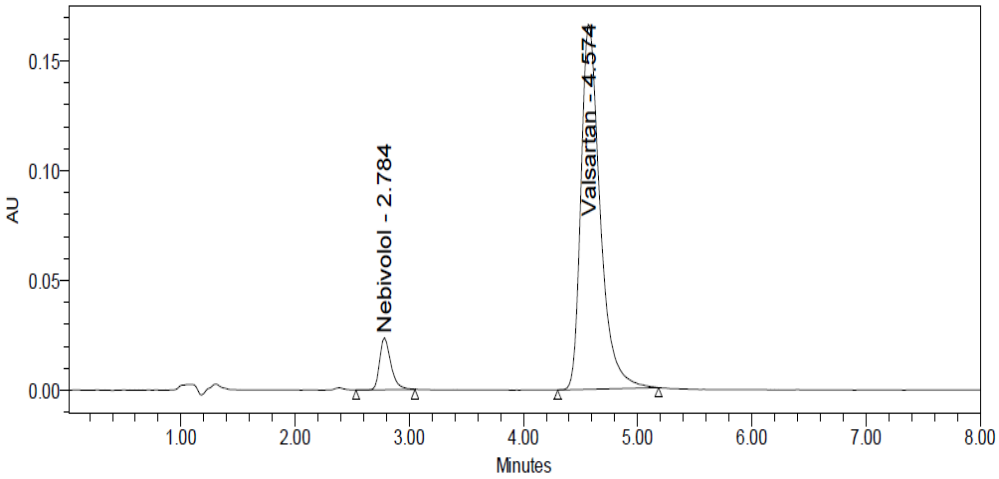


Inter day precision: Inter day precision was performed with 24 hrs time lag and the % RSD obtained for Nebivolol and Valsartan were 0.29 and 0.38.

Table 4: Inter day precision results for Nebivolol and Valsartan by RP-HPLC.

S.No.	Nebivolol	Valsartan
1	162112	1920163
2	162147	1933091
3	161563	1920511
4	162723	1934836
5	162814	1924441
6	162517	1935953
Mean	162313	1928166
Std. Dev.	467.2	7292.5
%RSD	0.29	0.38

Figure 6: Inter Day precision Chromatogram of Nebivolol and Valsartan



Accuracy: Three concentrations 50%, 100% and 150% were injected in a triplicate manner and amount recovered and % recovery were displayed in Table 5.

Table 5: Accuracy results of Nebivolol and Valsartan by RP-HPLC

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
Nebivolol	5	5.00	100.04	1.07
	10	10.03	100.31	1.18
	15	14.98	99.91	0.39

Valsartan	80	79.55	99.45	1.15
	160	160.54	100.34	0.60
	240	238.8	99.51	1.39

Figure 7: Accuracy 50% Chromatogram of Nebivolol and Valsartan

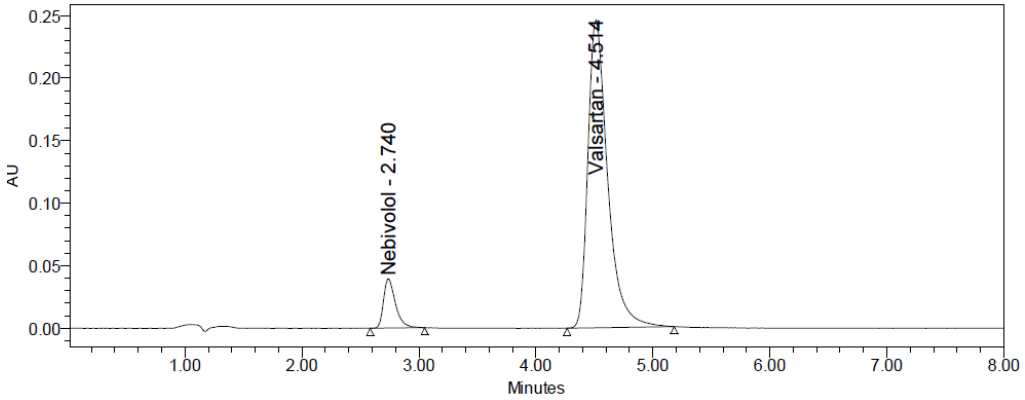


Figure 8: Accuracy 100% Chromatogram of Nebivolol and Valsartan

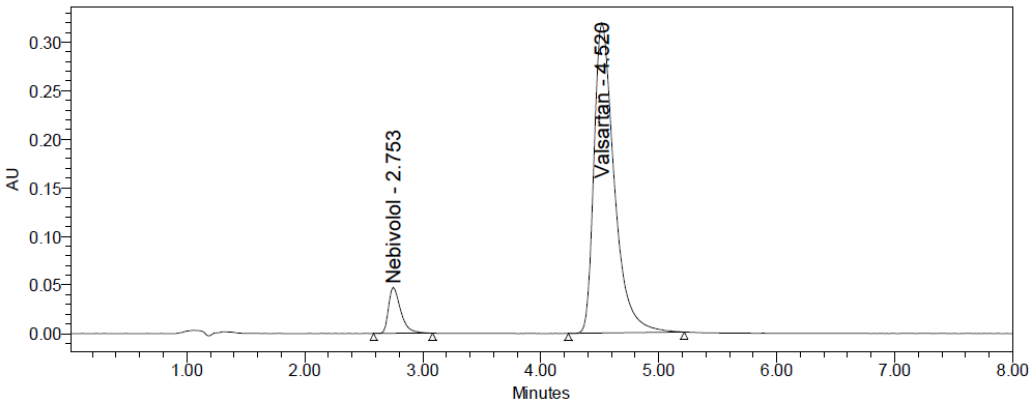
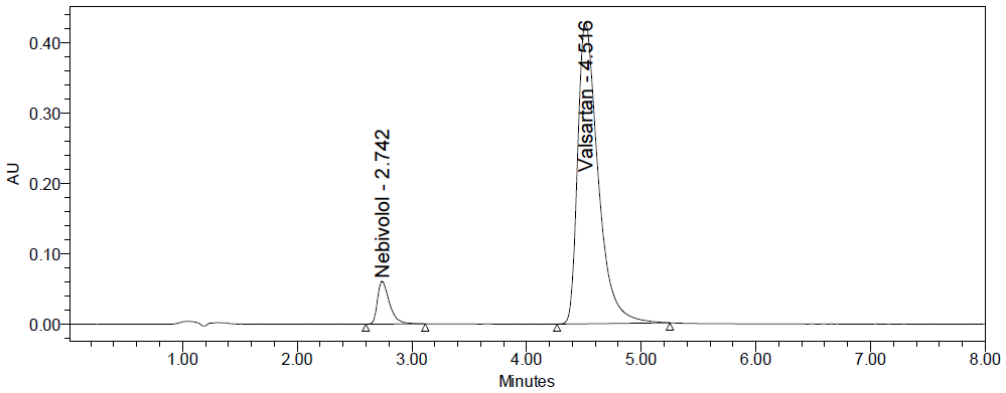


Figure 9: Accuracy 150% Chromatogram of Nebivolol and Valsartan



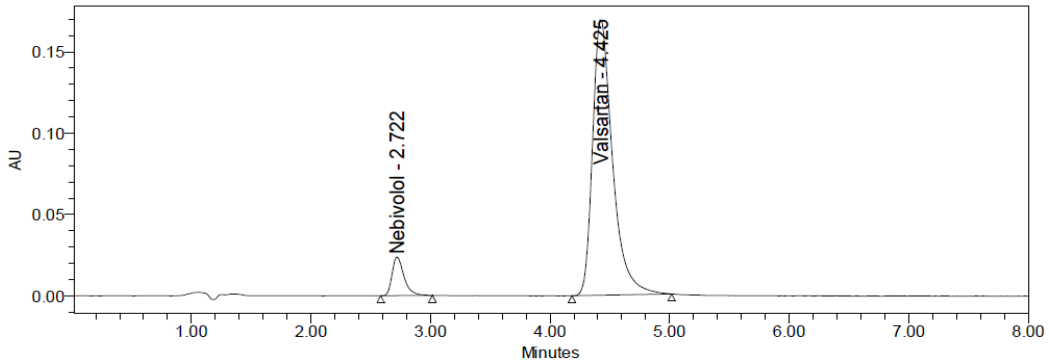
Assay of Tablet:

Table 6: Assay of Tablet

S. No.	Nebivolol %Assay	Valsartan %Assay
1	99.17	101.42
2	101.79	98.86
3	100.09	99.43
4	99.55	100.16
5	99.99	98.23

6	100.92	98.76
AVG	100.25	99.48
STDEV	0.9572	1.16
%RSD	0.95	1.16

Figure 10: Assay chromatogram of Tablet



IV. Conclusion

A simple, accurate, precise method was developed for the simultaneous estimation of the Nebivolol and Valsartan in tablet dosage form. Retention time of Nebivolol and Valsartan were found to be 2.7min and 4.7min. %RSD of the Nebivolol and Valsartan were and found to be 0.95 and 1.16 respectively. %Recovery was Obtained as 100.09 and 99.77 for Nebivolol and Valsartan respectively. LOD, LOQ values are obtained from regression equations of Nebivolol and Valsartan were 0.39ppm, 1.18ppm and 0.73ppm, 2.22ppm respectively. Regression equation of Nebivolol is y = 17934x + 2125, and of Valsartan is y = 13167x + 2928. Regression co-efficient was 0.999. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

References

[1]. Kumbhar, Chougule, A validated HPTLC Method for Simultaneous Quantification of Nebivolol and Hydrochlorothiazide in Bulk and Tablet Formulation, IJPSDR, 2011, 3(1), 62-66.
[2]. Frank A Settle, Hand book of Instrumental Techniques for Analytical Chemistry, 147-149, 484-483.
[3]. ICH, Q2A Text on validation of analytical procedures, 1994, Oct.
[4]. Validation of compendial Assays-Guidelines, United States Pharmacopoeial Convention, Rockville, MD, (1985).
[5]. Meyyanathan, Arunadevi, Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan and Nebivolol Hydrochloride and Hydrochlorothiazide in Pharmaceutical Formulations by UV Spectrophotometric Methods, Indian J. Pharm. Educ. Res, 2010, 44(2), 156-159.

Gole Rajkumar, etal. "Method Development and Validation for the Simultaneous Estimation of Nebivolol and Valsartan in Bulk and Solid Dosage Form by Rp-Hplc". *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(1), (2020): pp. 63-69.