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STABILITY INDICATING ASSAY METHOD DEVELOPMENT AND VALIDATION FOR NEBIVOLOL AND TELMISARTAN IN ITS COMBINED PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A new simple, rapid and sensitive stability indicating RP-HPLC method has been developed for the determination of Nebivolol and Telmisartan in its combined pharmaceutical dosage form. The method employs Agilent C18 (250 x 4.6mm; 3µm particle size) column for the chromatographic separation and Acetonitrile: 0.05 M (pH 6.5) Disodiumhydrogen (NA_2HPO_4) buffer was used as a mobile phase. Separation was completed within 10 min with a flow rate of 1 ml/min and detection was at 235 nm. The retention time of Nebivolol and Telmisartan was found to be 2.920 min & 8.093 min respectively. The proposed method was found to have the linearity in the concentration range of range of 25-75 µg/ml for Nebivolol and 100-300 µg/ml for Telmisartan. Linearty regression coefficient was not less than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98.04 – 101.11 % of Nebivolol and 99.92-100.37 % for Telmisartan. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH Guidelines. The method was found to have suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: Nebivolol, Telmisartan, RP-HPLC Estimation, Analytical Method Validation.

1. INTRODUCTION

Nebivolol is chemically known as α, α- [iminobis (methylene)] bis [6-flouro-3,4-dihydro-2H-1-benzopyran-2-methanol] (Figure 1). It is a highly selective β1-blocker with nitric oxide-

mediated vasodilatory actions and beneficial effects on vascular endothelial function. Nebivolol is used in the management of hypertension. It is given by mouth as the hydrochloride although doses are expressed in terms of base. The usual dose is 5 mg daily. An initial dose of 2.5 mg daily is employed in the elderly and in patients with renal impairment.[1-2] Telmisartan is in the drug class of angiotensin receptor blockers (ARBs) and is prescribed for the treatment of high blood pressure, reducing the risk of heart attack, stroke, or death from cardiovascular causes, Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Recent studies suggest that telmisartan may also have PPAR-gamma agonistic properties that could potentially confer beneficial metabolic effects. [4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]1,1‘biphenyl]–2 carboxylic acid.[3-4] (Figure 2).Many methods have been described in the literature for the determination of Nebivolol hydrochloride and Telmisartan individually and in combination with other drugs either individually or in combination with other drugs. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms.[5-11] The aim of this work was to develop an RP-HPLC method with determination of Nebivolol hydrochloride and Telmisartan in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines.[12,14]

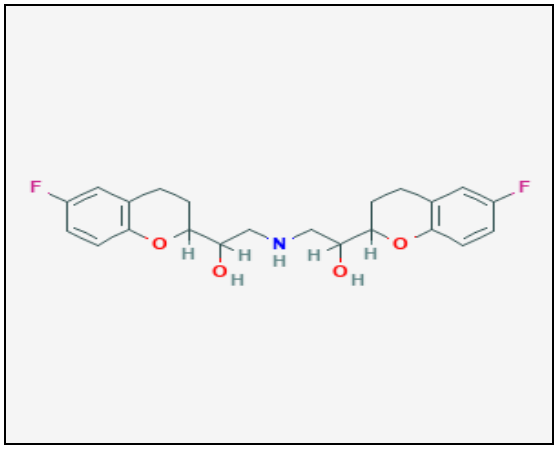

Figure 1: Structure of Nebivolol.

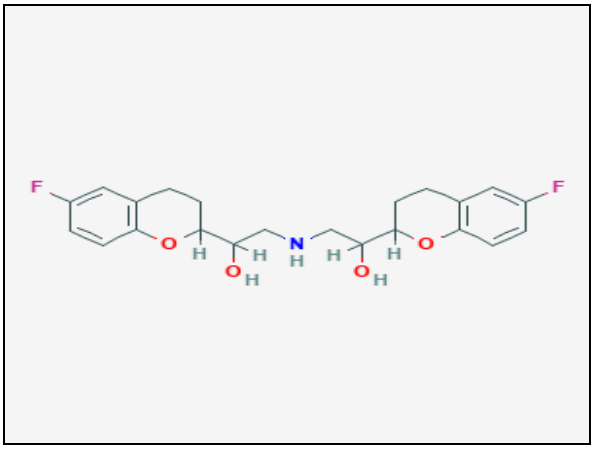

Figure: 2: Structure of Telmisartan.

2. MATERIAL AND METHOD

Instruments and Apparatus

HPLC An Agilent’s HPLC 1260 with photodiode array detector. software used was EZ Chrome, Analytical weight balance, Mettler Toledo, Schwerzenbach, Switzerland; Water

bath, Metalab scientifi industries Ltd; Oven, Lab line, India; pH meter, Lab line, India; 0.45 micron nylon filters; Glasswares and Syringe.

Chemical, Material & Reagents

Standard Nebivolol and Telmisartan was procured from Zydus Pharmaceuticals Ltd., Changodar, Ahmedabad; marketed formulation named Neb Card T manufactured by Torrent Pharmaceuticals Pvt. Ltd. with label claim 5mg Nebivolol and 40 mg Telmisartan was procured from local market. Methanol & water (Milli-Q) of HPLC grade; Acetonitrile, Triethanolamine, Disodium hydrogen phosphate, Hydrochloric Acid, Hydrogen Peroxide, Sodium hydroxide of AR grade (Merck) were used during the whole experimental work.

Preparation of standard stock solution of Nebivolol (1000µg/ml)

Weighed accurately 100mg of Nebivolol and transferred in to 100 ml volumetric flask (V.F.), about 10ml of methanol was added and sonicated to dissolve. Cooled to RT, and Volume was made up with methanol up to the mark and mixed.

Preparation of standard stock solution of Telmisartan (1000µg/ml)

Weighed accurately 100mg of Telmisartan and transferred in to 100 ml volumetric flask (V.F.), about 10ml of methanol was added and sonicated to dissolve. Cooled to RT, and Volume was made up with methanol up to the mark and mixed.

Preparation of combined standard solution of Nebivolol (50µg/ml) & Telmisartan (400µg/ml)

Take 1 ml from the Nebivolol stock solution and 8 ml from Telmisartan stock solution and transferred to 20 ml volumetric flask and volume made up to the mark by mobile phase, which gave final concentration of Nebivolol (50 µg/ml) & Telmisartan (400 µg/ml).

Sample preparation of Nebivolol Hydrochloride (50 µg/ml) & Telmisartan (400 µg/ml) in tablets

About 10 tablets (Label claim = Nebivolol 5mg & Telmisartan 40mg) were accurately weighed and transferred into a 100 ml volumetric flask, about 50 ml of mobile phase was added, the solution was sonicated for 15 min with intermittent shaking and diluted it up to the mark with mobile phase and mixed well (50 µg/ml Nebivolol & Telmisartan 400 µg/ml). It was filtered through 0.45µ nylon filter.

Method Development

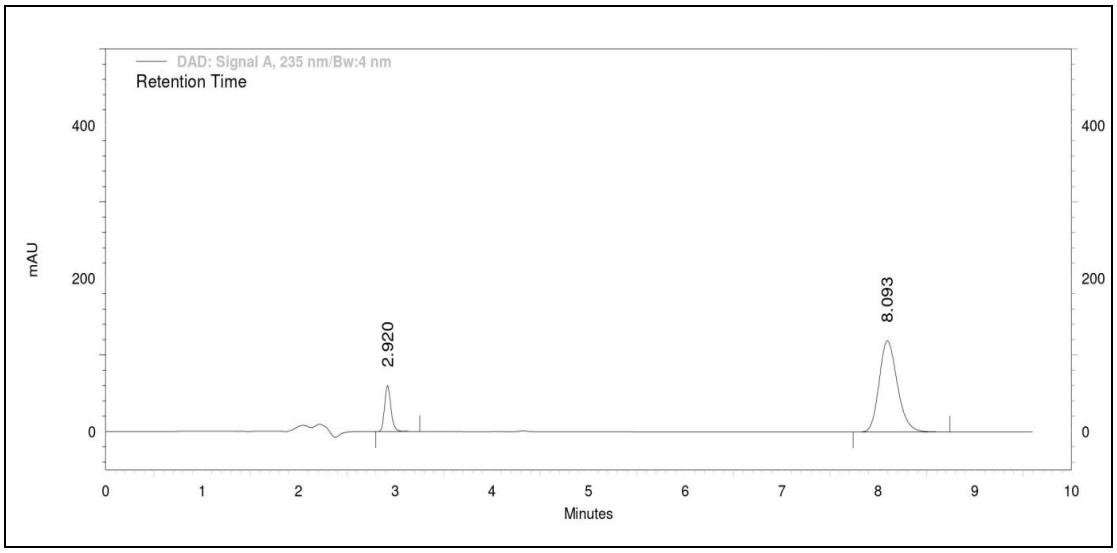

Figure 3: Chromatogram showing separated peaks of Nebivolol and Telmisartan.

Method Validation: The method was validated according to the International Conference on Harmonization guidelines for validation of analytical procedures Q2 (R1) (ICH, 1996).

Specificity

Specificity of the method was determined by checking the interference from blank and by performing force degradation in acid, base, peroxide and thermal condition.

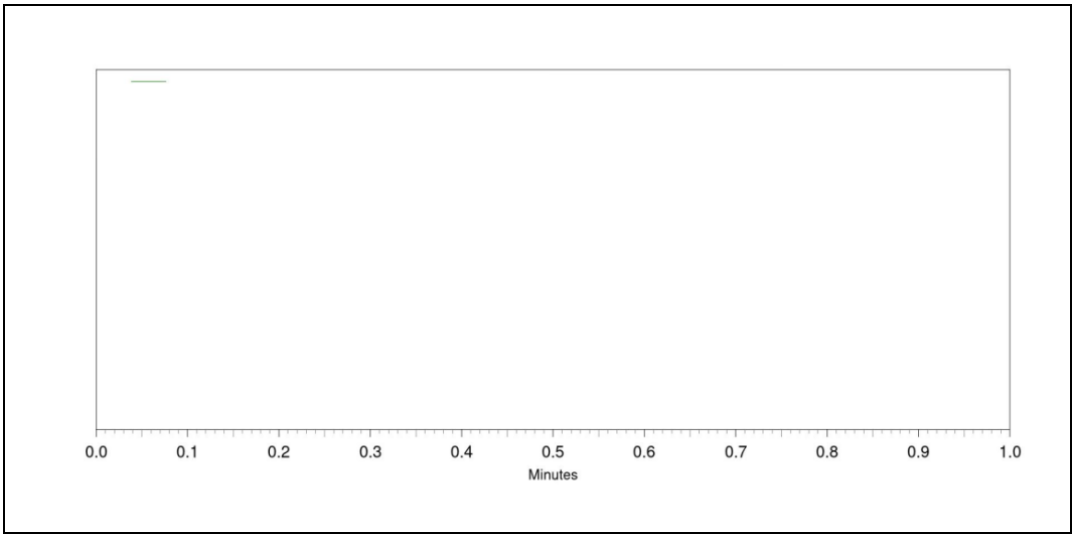

Fig. 3: Chromatogram of Blank.

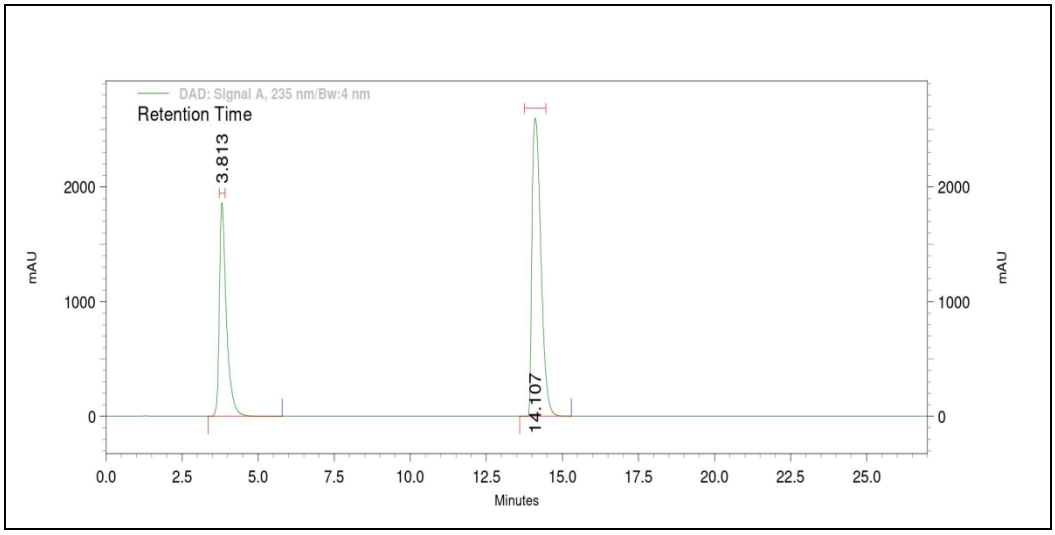

Fig. 4: Chromatogram of Nebivolol and Telmisartan Standard at 235nm.

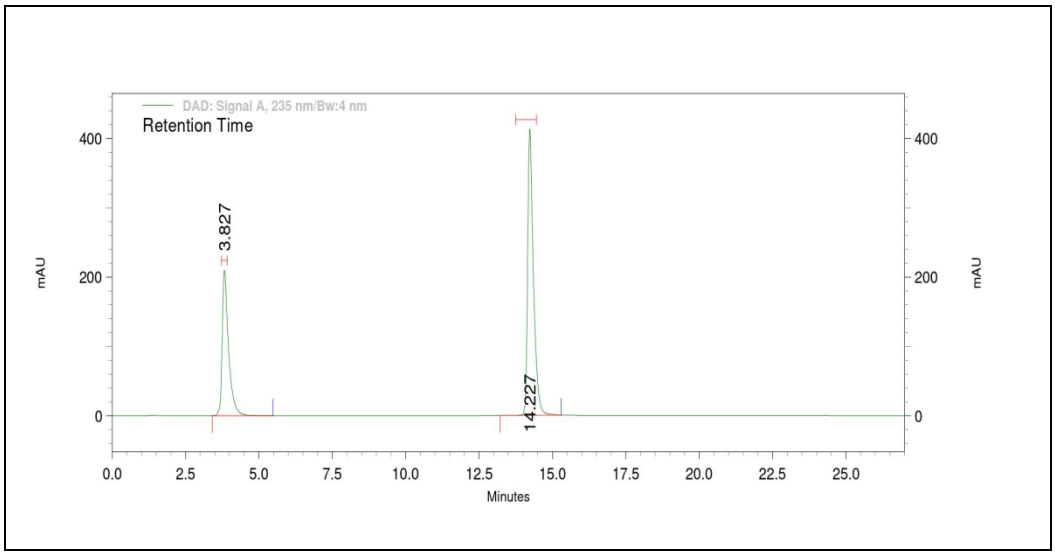

Fig. 5: Chromatogram of Nebivolol and Telisartan Sample at 235nm.

Table 1: Degradation profile of Nebivolol and Telmisartan.

Stress condition	% Degradation of Nebivolol	% Degradation of Telmisartan
Acidic	10.48	98.8662485
Alkaline	82.05	90.84160325
Oxidative	25.52	97.44008144
Thermal	99.06	98.85250892

Linearity and Range (n=5)

It was found that Lambert-Beer's law was followed in the concentration ranges of 25-75µg/ml (25, 40, 50, 60, 75 µg/ml) for Nebivolol and 100-300 µg/ml (100, 160, 200, 240, 300 µg/ml) for Telmisartan. The straight line equations and correlation coefficient for Nebivolol and Telmisartan are shown in below tables.

Table 2: Data of regression analysis of Nebivolol.

Drug	Straight line equation of calibration curve	R^2	Correlation coefficient (r)
Nebivolol	y=13035x+11469	0.999	0.9995

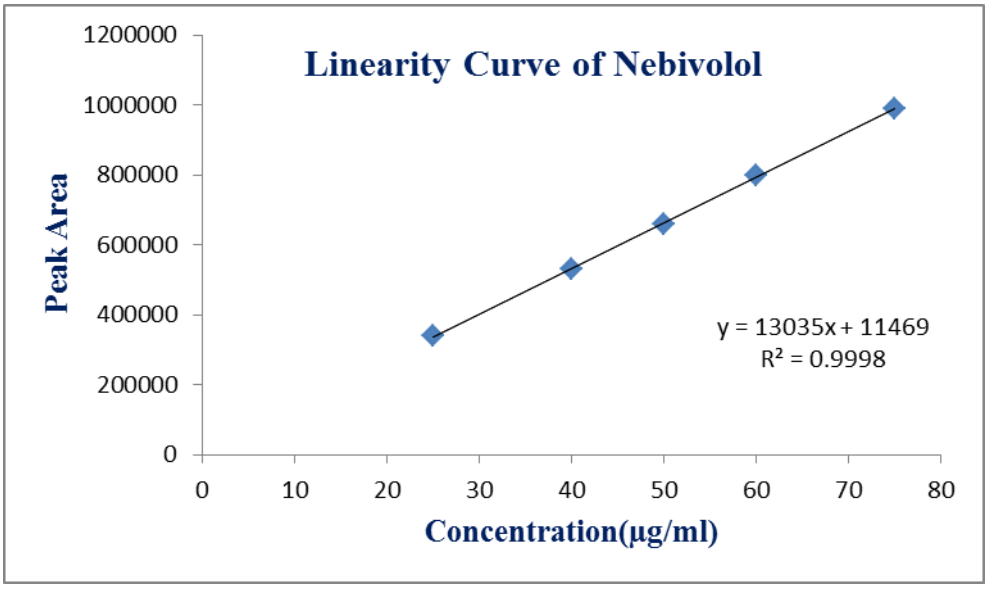

Fig. 6: Linearity graph of Nebivolol.

Table 3: Data of regression analysis of Telmisartan.

Drug	Straight line equation of calibration curve	R^2	Correlation coefficient (r)
Nebivolol	y= 58623x-0.469	1	1

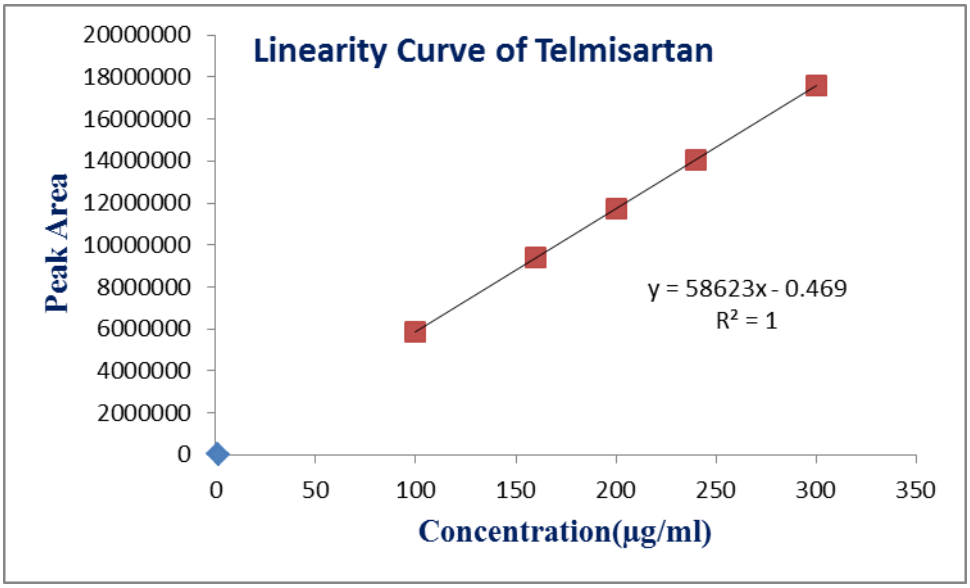

Fig. 7: Linearity graph of Telmisartan.

Accuracy (% Recovery, n = 3)

The accuracy of the method was determined by calculating recovery of Nebivolol by using standard addition method at 80, 100, 120 % level. Percentage recovery was calculated by injecting of each solution three times and % recovery was calculated with help of regression equation. Recovery for Nebivolol was obtained in the range of 98.04 – 101.11 % which indicates accuracy of the method, as shown in table 4.

Table 4: Accuracy Study Data for Nebivolol.

Sample Conc. (µg/mL)	% Level	Conc. Added (µg/mL)	Total Amount (µg/mL)	Total Amount Recovered (µg/mL)	Mean Area ± SD	% Recovery	% CV
50	80	20	70	69.39267	915883.333±5915.6	99.13239	0.645892
50	100	25	75	75.83469	999835.667±134.10	101.1129	0.013412
50	120	50	100	98.04885	1289330.667±178.12	98.04885	0.013815

The accuracy of the method was determined by calculating recovery of Telmisartan by using standard addition method at 80, 100, 120 % level. Percentage recovery was calculated by injecting of each solution three times and % recovery was calculated with help of regression equation. Recovery for Telmisartan was obtained in the range of 99.68 – 100.37 % which indicates accuracy of the method, as shown in table 5.

Table 5: Accuracy Study Data for Telmisartan.

Sample Conc. (µg/mL)	% Level	Conc. Added (µg/mL)	Total Amount (µg/mL)	Total Amount Recovered (µg/mL)	Mean Area ± SD	% Recovery	% CV
200	80	80	280	281.0507	16476035.000±33715.06	100.3753	0.204631
200	100	100	300	299.0489	17531142.667±80887.87	99.68296	0.461395
200	120	120	320	319.7653	18745603.333±551.16	99.92667	0.00294

Precision

The repeatability of method was checked by analyzing (n = 6) for Nebivolol and Telmisartan and response was recorded.

The intra-day (3 times on the same day) and inter-day (3 different days over a period of 1 week) a precision of the proposed method was checked by measuring the responses for 3 different concentration of Nebivolol and Telmisartan. The result of precision for Nebivolol and Telmisartan is as shown in Table 5.

The % CV for repeatability of Nebivolol and Telmisartan was found to be 0.00097 and 0.1484 respectively. The % CV of Nebivolol and Telmisartan for intra-day precision was found to be in the range of 0.0015-0.0085 and 0.05-0.4 respectively. while inter-day precision of Nebivolol and Telmisartan was found to be in the range of 0.004-.035 and 0.33-1.29 respectively., which indicates the method is precise.

Table 6: Precision.

	Parameters	Concentration (µg/ml)	Result For Nebivolol	Concentration (µg/ml)	Result For Telmisartan
Precision (% CV)	Repeatability (n = 6)	50	0.00097	200	0.14846
	Interday (n = 3)	25	0.035904466	100	0.338796856
		50	0.00416073	200	1.298645661
		75	0.005411538	300	0.420245571
	Intraday (n = 3)	25	0.008521872	100	0.051713515
		50	0.008123739	200	0.138319507
		75	0.001537424	300	0.40234233

LOD and LOQ

The Limit of detection (LOD) and Limit of quantitation (LOQ) were calculated by the equation,

LOD = 3.3 x (SD/Slope)

LOQ = 10 x (SD/Slope)

Where,

SD = Standard deviation of the Y- intercepts of the calibration curve.

Slope = Mean slope of the calibration curve.

The limit of detection (LOD) & limit of quantification (LOQ) was calculated by using its equation and it is shown below, which indicate that the method is sensitive.

Table 7: LOD & LOQ Data for Nebivolol and Temisartan.

Drug	LOD	LOQ
	Concentration (µg/ml)	
Nebivolol	0.0020489	0.006208828
Telmisartan	0.1557507	0.471971959

Robustness

Results of robustness studies of RP – HPLC method are shown in table 6. The method was found to be in term of variation in composition and flow rate, pH of the mobile phase and column temperature. As % CV for all parameter was found to be less than 2%, which indicates robustness of method.

Table 8: Robustness Data for Nebivolol (n = 3)

Chromatographic condition		Nebivolol		Telmisartan	
		Mean peak Area ± SD	% CV	Mean peak Area ± SD	% CV
Flow rate ± 0.2	0.8	658698.00±49.42	0.007637	11757945.67±32147.72	0.273413
	1.0 (optimum)	658696.67±49.66	0.007673	11748237.67±33491.65	0.285078
	1.2	658568.33±98.04	0.015151	11814665.33±5989.75	0.050698
pH ± 0.2	6.0	658689.00±58.50	0.00904	11767026.00±16276.09241	0.13832
	6.5 (optimum)	658642.00±50.68	0.007832	11788237.67±18584.41972	0.157652
	7.0	659045.00±567.25	0.087596	11788237.67±18584.41972	0.157652

System suitability parameters

The results of system suitability test parameters are listed in table 7 and % CV for all parameter was found to be less than 2%.

Table 9: System suitability parameters Data for Nebivolol & Temisartan (n = 6).

Sr. No.	Retention time, min		Tailing Factor		Theoretical Plates	
	Nebivolol	Telmisartan	Nebivolol	Telmisartan	Nebivolol	Telmisartan
1	2.8	7.900	1.196	1.187	9310	8895.000
2	2.82	8.000	1.188	1.188	9414	8756.000
3	2.84	8.012	1.197	1.199	9426	8937.000
4	2.85	8.013	1.246	1.246	9436	8999.000
5	2.867	8.056	1.205	1.205	9613	9020.000
6	2.920	8.093	1.194	1.21753	9834	9057.000
Mean	2.8495	8.012333333	1.2043333	1.206911667	9505.5	8944
SD	0.04168333	0.065092754	0.0211656	0.022308065	188.2612546	108.9623788
% CV	1.462829619	0.812406968	1.7574615	1.848359404	1.980550783	1.218273466

Summary of Validation and System Suitability Parameters

All the validation and system suitability parameters are shown in Table 10.

Table 10: Summary of validation parameters & System suitability Parameters.

Validation parameters			
		Nebivolol	Telmisartan
Linearity (n = 5)		25-75 µg/ml	100-300 µg/ml
Accuracy (% Recovery) (n = 3)		98.04-101.11	99.92-100.37
Precision (% CV)	Repeatability (n = 6)	0.00097	0.14846
	Interday (n = 3)	0.0015-0.0085	0.33-1.29
	Intraday (n = 3)	004-0.03	0.05-0.4
LOD		0.0020489	0.155750746
LOQ		0.006208828	0.471971959
Robustness	pH	Complies	Complies
	Temperature	Complies	Complies
	Flow rate	Complies	Complies
System suitability Parameters			
Retention time (min)		2.8495	8.012333333

Theoretical plates	9505.5	8944
Tailing factor	1.2043	1.206911667

Estimation of Nebivolol and Telmisartan in the tablet formulation by the proposed RP-HPLC method

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation Nebcard T. The assay data are shown in the Table 11. The assay results were comparable to labeled value of each drug in tablet dosage form. It can be used in the routine quality control of dosage form in industries.

Table 11: Data of sample preparation for assay (n=5).

Sr. No.	Area of Nebivolol	Area of Telmisartan
1	658652	11724667
2	658667	11725664
3	658970	11725962
4	658666	11725970
5	658672	11725965
Mean Area ± SD	658665.40±7.86	11725645.60±562.44
% CV	0.001194	0.0047
% Mean Assay	98.85-102.64	100.00-100.35

3. CONCLUSION

Based on the results of the above studies, it is concluded that the method for determination of assay of Nebivolol and Telmisartan is precise, linear over the concentration range, stability indicating, and robust. The method is specific for the quantization of assay of Nebivolol and Telmisartan in pharmaceutical formulation. So the developed method can be easily applied for routine analysis of Nebivolol and Telmisartan in its combined dosage form.

The method was found to be simple accurate economical and rapid and it can be applied for routine analysis in laboratories and suitable for the quality control of bulk and pharmaceutical formulations.

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