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ORIGINAL ARTICLE

Development of stability indicating assay method for the simultaneous estimation of Nebivolol and Telmisartan in bulk and tablet dosage form

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ABSTRACT
A simple, sensitive and specific high performance liquid chromatographic with UV detection (286 nm) was developed for the simultaneous estimation of Nebivololand Telmisartan in bulk as well as tablet dosage form. The separation was achieved with Fortis C-18 (id 4.6 x100 mm) with 2.5 µm particle size column, ambient temperature with a low pressure gradient mode and mobile phase containing the acetonitrile: 0.05 % o-paraldehyde (OPA) (80: 20 v/v) pH 6.5 adjusted with 0.1% triethylamine (TEA), flow rate was 1 mL/ min and eluent was monitored at 286 nm. The selected chromatographic conditions were found to effectively separate nebivolol and telmisartan with retention time of 5.7 and 3.0 min respectively. The linearity range of nebivolol and telmisartan found in the range of 5-25 µg/mL and 40-200 µg/mL respectively. The proposed method was found to be accurate, precise, reproducible, specific, and stability indicating as no interfering peaks of degradation compounds and excipients were noticed. It can also be used for a routine quality-control analysis of these drugs in combination tablets.
Keywords: *RP-HPLC, Nebivolol, Telmisartan, Stability indicating assay method.*

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INTRODUCTION

Nebivolol Hydrochloride (NEB) (Figure1) acts as a vasodilator as it is a beta-blocker. It is used to control hypertension and in the treatment of chronic heart failure [1]. The literature review reveled that various analytical methods have been reported for the determination of nebivolol in a single dosage form involving spectrophotometry [2,3],HPLC [4], and in combination with other drugs involving spectrophotometry [5] and HPLC methods [6,7].

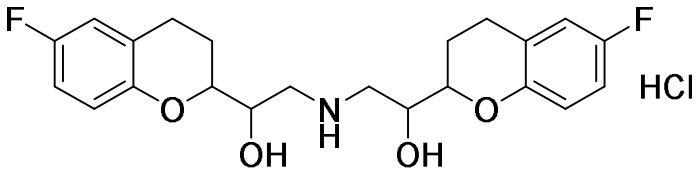

Figure 1. Structure of Nebivolol hydrochloride

Telmisartan (**Figure 2**) acts as an angiotensin II receptor antagonist (ARB) employed in the management of hypertension. According to recent works suggest that telmisartan also have PPAR-gamma agonistic properties, due to this property telmisartan could potentially confer beneficial metabolic effects [8-9]. literature survey revealed that various analytical methods have been reported for the determination of telmisartan, like UV- visible spectrophotometric[10-13], HPTLC[14-15], HPLC [16],and UPLC methods[17].

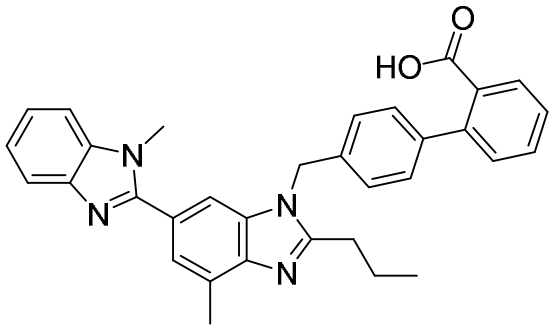

Figure 2. Structure of Telmisartan

The aim of this work to develop an accurate, specific, repeatable, and stability-indicating method for the determination of telmisartan and nebivolol hydrochloride in the presence of its degradation products as per ICH guidelines.

MATERIAL AND METHODS

Materials

Telmisartan and nebivolol hydrochloride were obtained as gift samples from Cadila Pharmaceuticals, (Ahmedabad, India). All chemicals and reagents used were of Nebivolol hydrochloride analytical-grade and purchased from Qualigen's fine chemicals (Mumbai, India). The marketed preparations were purchased from the local market Brand Name Nebicard-T which containing 300 mg of Nebivolol hydrochloride and 300 mg telmisartan manufactured by Torrent Pharma, Mumbai, and Maharashtra.

Instrumentation and Chromatographic condition

An HPLC system of Agilent (1100) Gradient System pump and with UV VWD detector was used working via Chemstation10.1software. The separation was carried on Fortis column with C18 packaging and 4.6 x100 mm dimensions,2.5µm particle size. The mobile phase consists of acetonitrile: 0.05 % OPApH6.5with 0.1% TEA in the ratio of 80:20with a flow rate of 1mL/min. The wavelength selected for the determination of telmisartan and nebivolol hydrochloride was 286.0nm according to observation.

Preparation of standard solution

The standard stock solution of 100 mg/ml each of telmisartan and nebivolol hydrochloride was prepared. 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 a final concentration of nebivolol (50 µg/ml) & telmisartan (400 µg/ml).

Validation of telmisartan and nebivolol hydrochloride *HPLC assay*

The RP-HPLC method for telmisartan and nebivolol hydrochloride assay was validated in terms of accuracy, linearity, specificity, LOD, LOQ, and robustness according to ICH Harmonized Guidelines.

System specificity

The system suitability was assessed by six replicate analyses of telmisartan and nebivolol hydrochloride at a concentration of 20 µg/ml.The acceptance criterion was ±2% for the percent relative standard deviation (% RSD) for the peak area and retention times for nebivolol hydrochloride and telmisartan.

Linearity and range

Linearity is the ability to obtain test results that are directly proportional to the concentration of the analyte. Linearity was determined by three injections of different telmisartan and nebivolol hydrochloride concentrations (40-200 µg/ml and 5-25 µg/ml). The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate the coefficient of correlation, slope, and intercept. In general, a value of correlation coefficient r2) > 0.998 is considered as the evidence of an acceptable fit for the data to the regression line.

Accuracy

The accuracy of an analytical method expresses the nearness between the expected value and the value found. In this case, to evaluate the accuracy of the developed method, successive analysis (n = 3) for three different concentrations (80%, 100 %, and 120 %) of standard different telmisartan and nebivolol hydrochloride solution was performed using the developed method. The data of the experiment were statistically analyzed using the formula [% Recovery = (Recovered conc. /Injected conc.) -100] to study the recovery and validity of the developed method. The mean recovery should be within 90–110% to be accepted.

Precision

Precision is the measure of closeness of the data values to each other for a several measurements under the same analytical conditions. The precision of the method was assessed by studying intra-day and inter-

day variation. The intraday precision was assessed by analyzing the calibration curvesof six replicates of different concentrations of both drugs within the same day. The inter-day precision was determined by analyzing six replicates of different concentrations of both drugs on three different days. The total precision of the method was expressed as the relative standard deviation (%RSD).

Limit of detection and limit of quantification

In accordance with ICH recommendations, the approach based on the standard deviation of the response and the slope of the calibration plots was used to determine detection and quantification limits. LOD is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. LOQ is the lowest concentration of analyte that can be determined with acceptable precision and accuracy. These two parameters were calculated using the formula LOD = 3.3 - S D/S and LOQ = 10 -SD/S, where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate variations inmethod parameters and provides an indication of its reliability during normal usage. The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions.

Analysis of a marketed formulation

To determine the content of telmisartan and nebivolol hydrochloride in conventional tablet (Brand name: Nebicard-T), twenty tablets were weighed, 367.6mgpowder and transferred into a 100 ml volumetric flask, about 50 ml of methanol was added, the solution n was sonicated for 15 min with intermittent shaking and diluted it up to the mark with remaining quantity of methanol and mixed well (20 µg/ml Nebivolol & Telmisartan 400 µg/ml). It was filtered through a 0.45µ nylon filter.Peak areas were measured at 286 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using the linear regression equation.

Forced degradation study:

All stress decomposition studies were performed at a drug concentration 40 µg/ml and 5 µg/ml of telmisartan and nebivolol HCl under conditions hydrolysis (acid, base), oxidation condition as mentioned in ICH guidelines Q1A (R2).Acid, base and oxidation degradation were performed by adding 1 ml of 0.1 N HCl, 1 ml of 0.1 N NaOH, and 1 ml of 3 % peroxide solution, respectively, to the sample solution, and these samples were kept on a bench for 60min and 120 min.

RESULTS AND DISCUSSION

Development of RP- HPLC method

To establish and validate an efficient method for analysis of selected drugs in pharmaceutical formulations, preliminary tests were performed with to select optimum chromatographic conditions**.** The RP-HPLC method for estimation of telmisartan and nebivolol hydrochloride chromatographic conditions were optimized to obtain good resolution and proper peak shape, show in (Figure3)**.** The effect of mobile phase composition, flow rate and pH were also studied. The best resolution with reasonable retention time was obtained with a mobile phase containing acetonitrile: 0.05 % OPApH6.5with 0.1% TEA in the ratio of 80:20 with flow rate of 1.0 ml/min**.** Detection was carried out in a U.V detector at 286.0nm to increase the sensitivity of the method displayed in (Figure4). The average retention times for telmisartan and nebivolol hydrochloride were3.0 min and 5.7min, respectively. In the determination of accuracy and precision, recovery was 100.54-101.98 % and 99.52 -99.70% for telmisartan and nebivolol hydrochloride respectively. This indicates the method is accurate, intraday and inter-day variation, as RSD, was not more than1.04% for both drugs, indicating the method is precise. In the determination of the robustness of the method, no variation of mobile phase pH, and detector wavelength had no significant effect on chromatographic resolution.

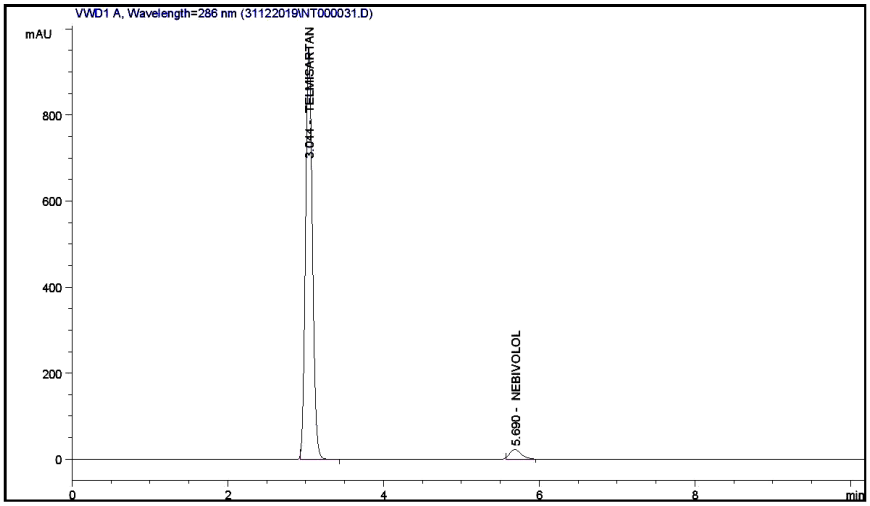

Figure3. Chromatogram of telmisartan and nebivolol hydrochloride

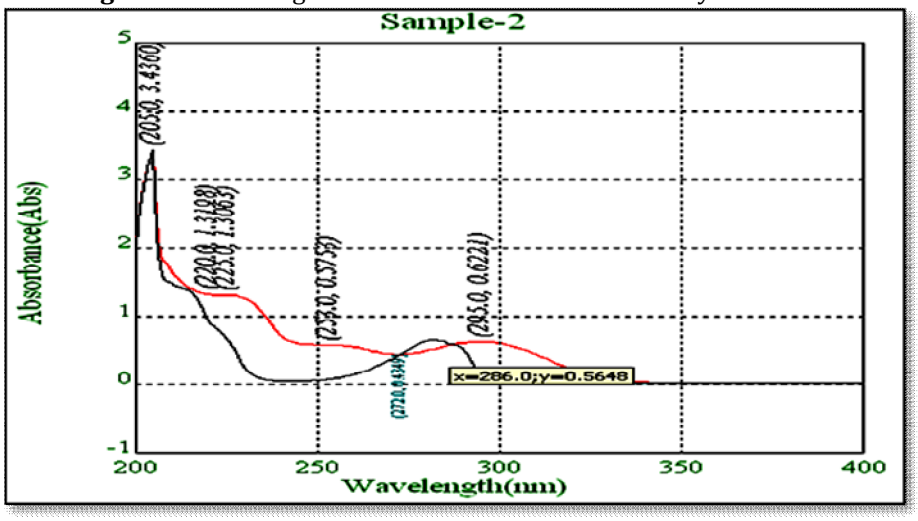

Figure.4. Absorbance spectra of telmisartan and nebivolol hydrochloride at 286nm.

System suitability

The system suitability was assessed by six replicate analyses of telmisartan and nebivolol hydrochloride at a concentration of 20 µg/ml. The RSD values of peak area and retention time fortelmisartan and nebivolol hydrochloride are within 2% indicating the suitability of the system (**Table1**). Both analytes i.e. telmisartan and nebivolol hydrochloride were continuously well resolved and retained at 3.0 and 5.7 min with RSD % less than 1 percent depicting strong reproducibility of the duplicate injections used on the integral LC system according to USP. In all chromatographic cycles, theoretical plate number still crossed over 2000 maintaining strong column efficacy across the entire separation process of investigation.

Table 1: System suitability test

Tests/parameters	Retention time (Rt)		Peak area	
Analytes	**TEL**	**NVB**	**TEL**	**NVB**
Average (n=6)	3.0	5.7	4418.76	360.15
± SD	0.02	0.03	135.54	104.78
% RSD	0.43	0.35	1.71	4.11

Linearity and range

Linearity was studied by preparing standard solutions at different concentration levels. The linearity ranges for telmisartan and nebivolol hydrochloride found to be 40-200 µg/mL and 15-25 µg/mL respectively. The linear regression equation for telmisartan was found to be 51.21x + 105.8 witha correlation coefficient 0.999,the linear regression equation for nebivolol hydrochloride was found to be 16.40x - 1.284 with correlation coefficient 0.999. The calibration table for telmisartan and nebivolol

hydrochloride was illustrated in (**Table 2**)and(**Table 3**)respectively. The calibration curves for telmisartan and nebivolol hydrochloride were displayed in (**Figure 5**)and(**Figure6**) respectively.

Table 2: Linear Regression Data for the Calibration of telmisartan

Levels	The concentration of telmisartan (µg/m	Peak area
Level 1	40	2148.59
Level 2	80	4153.98
Level 3	120	6292.13
Level 4	160	8393.11
Level 5	200	10272.85
	Average SD	**15.32**
	%RSD	**0.32**

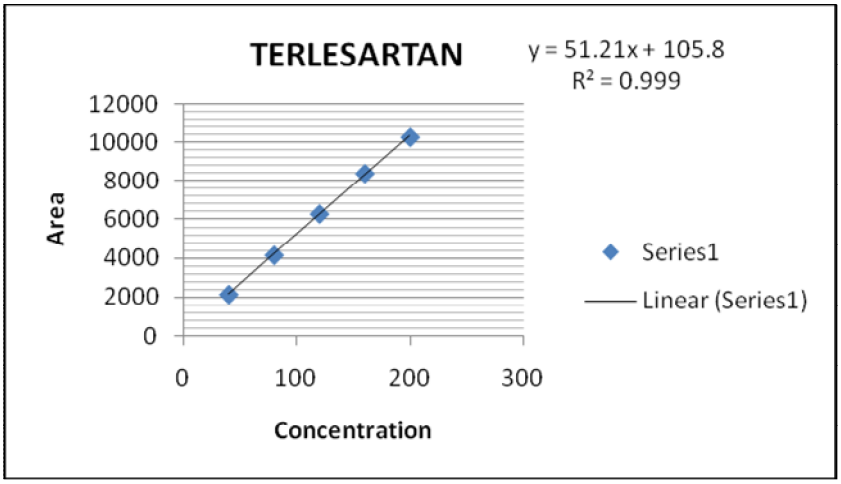

Figure 5. Calibration curves for telmisartan

Table 3: Linear Regression Data for the Calibration of nebivolol hydrochloride

Levels	Concentration of nebivolol hydrochloride (µg/mL)	Peak area
Level 1	5	79.97
Level 2	10	161.76
Level 3	15	246.87
Level 4	20	327.38
Level 5	25	407.46
	Average SD	**1.29**
	%RSD	**0.18**

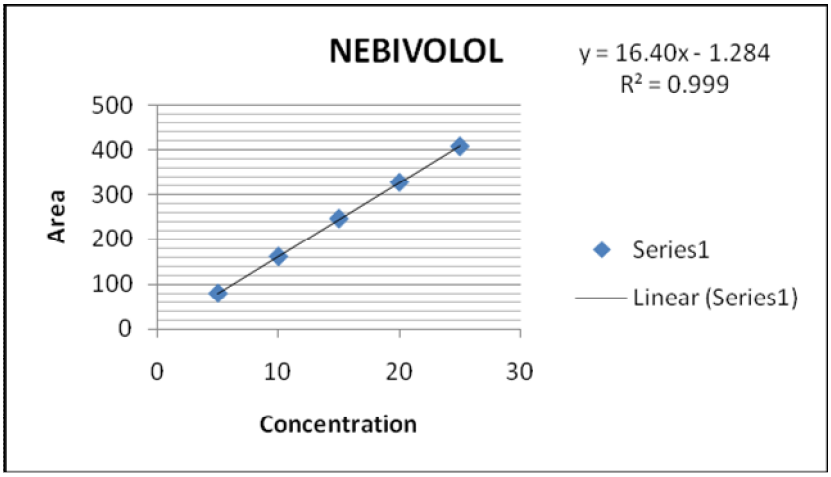

Figure6. Calibration curves for nebivolol hydrochloride

Accuracy

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out 6 times and the percentage recovery and % relative standard deviation was calculated. From the data obtained, recoveries of standard drugs were found to be accurate. The obtained result was illustrated in (**Table4**) and (**Table 5**).

Table 4: Investigation of accuracy study for telmisartan

Initial amount [µg/mL]	**Amount added** [µg/mL]	**Amount found** [µg/mL]	**% Recovery**	**% RSD**
		TEL	**TEL**	**TEL**
Level of recovery study 80 %				
40	32	71.99	99.97	
40	32	71.81	99.47	0.38
Mean ± SD 99.70±0.34				
Level of recovery study 100 %				
40	40	79.90	99.76	
40	40	79.71	99.28	0.34
Mean ± SD 99.52 ± 0.30				
Level of recovery study 120 %				
40	48	87.75	99.48	
40	48	87.80	99.60	0.09
Mean ± SD		**99.54 ± 0.17**		

***SD= standard deviation, %RSD= percent relative standard deviation**

Table 5: Investigation of accuracy study for nebivolol hydrochloride

Initial amount [µg/mL]	**Amount added** [µg/mL]	**Amount found** [µg/mL]	**% Recovery**	**% RSD**
		NVB	**NVB**	**NVB**
Level of recovery study 80 %				
5	4	8.99	99.83	
5	4	9.04	101.24	0.99
Mean ± SD 100.54±0.04				
Level of recovery study 100 %				
5	5	5.06	101.27	
5	5	5.01	100.70	0.40
Mean ± SD		**100.99 ± 0.04**		
Level of recovery study 120 %				
5	6	5.98	99.75	
5	6	6.02	100.45	0.47
Mean ± SD		**99.54 ± 0.03**		

***SD= standard deviation, %RSD= percent relative standard deviation**

Precision

The repeatability of the sample application and measurement of peak area was expressed in the terms of % RSD, and the results are depicted in (**Table 6**), which revealed intra-day and inter-day variation at three different concentration levels 80, 120, and 160 µg/mL for telmisartan and 10,15,20 µg/mL for nebivolol hydrochloride respectively.

Table 6: Precision analysis

Intra-day Precision			**Inter-day Precision**		
Concentrations	**% Amount Found**	**% RSD**	**Concentration**	**% Amount Found**	**% RSD**
For TEL					
80	99.13	0.23	80	99.14	0.24
120	100.65	0.02	120	100.62	0.03
160	101.69	0.05	160	101.11	0.06
For NVB					
10	101.46	0.23	10	100.40	0.59
15	98.85	1.48	15	98.93	0.41
20	99.60	1.44	20	100.45	0.77
***n=number of determinations, %RSD= percent relative standard deviation**					

Limit of detection and limit of quantification

The LOD was found to be 2.11 µg/mL $^{-1}$and 0.66 µg/mL $^{-1}$ for telmisartan and nebivolol hydrochloride respectively at the signal to noise ratio of 3.1. The limit of quantification was found to be 6.40 µg/mL^{-1} and 1.84 µg/mL^{-1} for telmisartan and nebivolol hydrochloride at signal to noise ratioof 10:1. The results of LOD and LOQ are summarized in (**Table 7**).

Table 7: A Sensitivity study

Name of drug	LOD	LOQ
Telmisartan	2.11	6.40
Nebivolol Hydrochloride	0.66	1.84

Robustness

The robustness of the method werestudied by deliberate changes in the method like alteration in pH of the mobile phase, percentage organic composition, changes in the wavelength. It was observed that there was no marked changes in the chromatograms demonstrate that the HPLC methods have developed are robust.

Analysis of a marketed formulation

Experimental results of the amount oftelmisartan and nebivolol hydrochloride in the selected commercial tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 100.93% and99.72 % for telmisartan and nebivolol hydrochloride, different lots of telmisartan and nebivolol hydrochloride tablets were analyzed using the proposed procedures as shown in (**Figure7**) and(**Table 8**).

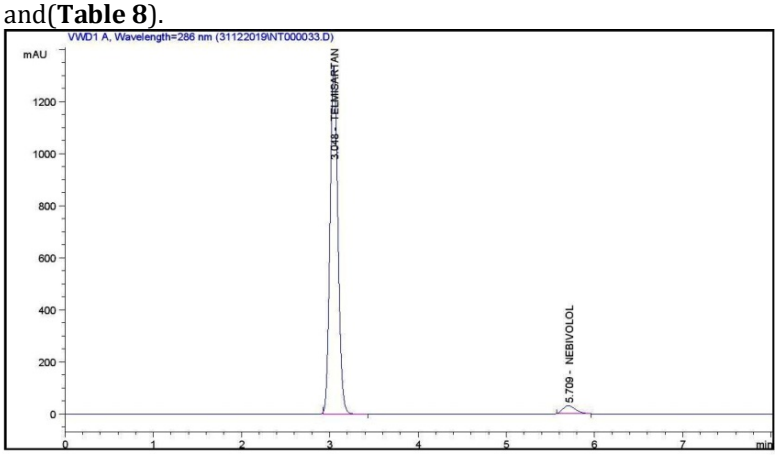

Figure 7. Chromatogram of marketer formulation of telmisartan and nebivolol hydrochloride

Table 8: Analysis of a marketed formulation

For TEL			For NVB		
Concentrations	**% Amount Found**	**% Label Claim**	**Concentration**	**% Amount Found**	**% Label Claim**
	161.40	100.88		19.89	99.46
160	161.57	100.98	20	19.99	99.98
Mean± SD	161.45**±** 0.07	100.93		19.94±0.12	99.72

Force degradation of telmisartan and nebivolol HCl

The stability of the sample was checked by forced degradation in different conditions and % of degradation was calculated. The values are given in(**Table 9**) and (**Table 10**)indicates that any other impurity is not merging with the main peak. The analyte solution was found to be stable. A method was developed for the determination of telmisartan and nebivolol hydrochloride in tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

Table 9: Force degradation data for Telmisartan

Tests	Telmisartan	
Conditions	**% Assay**	**% difference w.r. t refere**
Untreated sample	99.72	NA
Acid treated sample		
(0.1 N HCL After 1 Hrs)	97.96	1.76
(0.1 N HCL After 2 Hrs)	97.89	1.83
Base treated sample		
(0.1 N NaOH After 1 Hrs)	98.65	1.07
(0.1 N NaOH After 2 Hrs)	98.56	1.16
Peroxide treated sample		
(3% H2O2 After 1 Hrs)	99.50	0.22
(3% H2O2After 2 Hrs)	99.45	0.27

Table 10: Force degradation data Nebivolol Hydrochloride

Tests	Nebivolol Hydrochloride	
Conditions	**% Assay**	**% difference w.r. t refere**
Untreated sample	99.98	NA
Acid treated sample		
(0.1 N HCL After 1 Hrs)	99.45	0.53
(0.1 N HCL After 2 Hrs)	99.35	0.63
Base treated sample		
(0.1 N NaOH After 1 Hrs)	99.89	0.09
(0.1 N NaOH After 2 Hrs)	99.80	0.18
Peroxide-treated sample		
(3% H2O2 After 1 Hrs)	96.23	3.75
(3% H2O2After 2 Hrs)	95.63	4.35

CONCLUSION

The developed HPLC technique is precise, specific, accurate, and stability-indicating. Validation of the method proved that the method is suitable for the analysis of telmisartan and nebivolol hydrochloride in tablet formulation without any interference from common excipients or potential degradation products of telmisartan and nebivolol hydrochloride and excipients. The developed method can be used for routine analysis of telmisartan and nebivolol hydrochloride tablets for assay of telmisartan and nebivolol hydrochloride from stability batches.

CONFLICTS OF INTERESTS

All authors declared no conflict of interest.

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