



**FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC AND HPTLC METHOD
FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND NEBIVOLOL IN
THEIR COMBINED DOSAGE FORM**

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ABSTRACT

The present article portrays simple, sensitive, accurate, precise and cost effective First order derivative Spectrophotometric method and HPTLC method for the simultaneous estimation of Telmisartan and Nebivolol in bulk and combined dosage form. In The first order derivative method absorption at 283 nm (zero crossing point for Nebivolol) was used for Telmisartan and 278 nm (zero crossing point for Telmisartan) was used for Nebivolol. The linearity was taken in the concentration range of 10-50 µg/ml and 5-25 µg/ml for Telmisartan and Nebivolol respectively. In the HPTLC method, the chromatograms were developed using a mobile phase of Toluene: Ethyl Acetate: Methanol: 25% Ammonia (6: 3: 3: 0.2 v/v/v/v) on precoated plate of 200-µm layers of silica gel 60 F₂₅₄S and quantified by densitometric absorbance mode at 290 nm. The R_f of Telmisartan and Nebivolol were 0.46 and 0.57 respectively. Proposed technique has been validated as per ICH Guideline and can be used in routine pharmaceutical analysis.

Keywords: Telmisartan, Nebivolol, HPTLC, Spectrophotometric

INTRODUCTION

Telmisartan, 4- [(1,4-dimethyl-2-propyl [2,6-bi-1H benzimidazole]-1-yl) methyl]- [1,1-biphenyl] 2-carboxylic acid, is an antihypertensive agent [1]. Telmisartan blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of

angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland [2, 3]. Telmisartan has been estimated by various methods like spectrophotometric [4], HPLC [5, 6], HPTLC [7–10], and LC-MS [11, 12] in pharmaceutical formulations and biological fluids.

Nebivolol, 1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol, is a cardioselective beta blocker used in the management of hypertension, angina pectoris, cardiac arrhythmias, myocardial infarction, and heart failure [13]. Nebivolol has been estimated by spectrophotometric [14], HPLC [15, 16], and HPTLC [17, 18] methods.

Together they allow the blood to flow more smoothly and heart to pump more efficiently, liquid chromatography method for the analysis of Telmisartan and Nebivolol in combined dosage form has been reported [19], there have been no reports concerning the simultaneous determination of Telmisartan and Nebivolol by spectroscopic and HPTLC method.

In the present work, an effort has been made to estimate Telmisartan and Nebivolol in bulk and in combined tablet dosage form by UV and HPTLC Method and validated according to ICH guidelines.

MATERIALS AND METHODS

Reagents

Telmisartan and Nebivolol were provided as a gift sample by Zim laboratories ltd (Nagpur) and Pure chem Pvt Ltd (Ankleshwar) respectively. All other reagents required for experimentation were of analytical reagent (AR) grade.

Apparatus and Equipment

UV spectral measurement recorded in JASCO V-630 UV spectrophotometer with Spectra Manager Software and 1 cm UV matched quartz cells were used. Chromatography was performed on HPTLC system [Semi automated spotting device (Camaglinomat iv), Scanner (Camag scanner iii), UV cabinet (Camaglinomat iv), Syringe Hamilton syringe (100 µL), Software (Camag cats4 software)].

Chromatographic Condition

10 cm×10 cm aluminium-backed HPTLC plates coated with 200-µm layers of silica gel 60 F₂₅₄S were used. The plates were washed with methanol before chromatographic measurements and activated at 60 °C for 5min. The samples were applied as 6mm wide bands with the help of sample applicator fitted with a 100-µL sample syringe (Hamilton, Bonaduz, Switzerland). The plate was developed in a presaturated Camag twin trough glass chamber (10 cm × 10 cm).

Preparation of Stock and Standard solution

Two stock solutions were prepared by dissolving 100 mg of Telmisartan and

Nebivolol in a 100 mL of methanol respectively. This was used as standard for both the methods Spectrophotometric and HPTLC.

Preparation of Standard solution for HPTLC: Aliquots of Nebivolol HCl 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mL and Telmisartan 1.6, 3.2, 4.8, 6.4, 8.0, 9.6 mL from the stock solution were pipetted out in the series and transferred into 10 mL volumetric flask and made up to the mark with methanol (NEB HCl: 20-120 $\mu\text{g}/\text{mL}$ and TEL: 160-960 $\mu\text{g}/\text{mL}$).

Preparation of Standard solution for Spectroscopic method: Pipetted out 5 ml from the stock solution and transferred into two different 50 ml volumetric flask and diluted with Methanol to obtained 100 $\mu\text{g}/\text{ml}$ Nebivolol HCl and Telmisartan.

Preparation of test solution for Assay

Twenty tablets were weighed accurately and powdered. An aliquot of powder equivalent to the average weight of 50 mg of Telmisartan was weighed directly into a 50 ml volumetric flask and dissolved in methanol and the volume was made up to the mark with methanol (1000 $\mu\text{g}/\text{mL}$ Telmisartan and 125 $\mu\text{g}/\text{mL}$ Nebivolol HCl).

Aliquots of 3.2 mL from the above solution were pipetted out and transferred to 10 mL volumetric flasks in triplicate and made up to the mark with methanol. This solution was used in HPTLC method. 5 μL was

spotted along with the standard solutions of different concentrations. The content of Nebivolol HCl and Telmisartan was calculated from the linear regression equation on the basis of peak area, SD and % assay was calculated.

Aliquots of 1 mL from the above solution were pipetted out and transferred to 10 mL volumetric flasks in triplicate and made up to the mark with methanol. This solution was used in spectroscopy method. The content of Nebivolol HCl and Telmisartan was calculated from the linear regression equation on the basis of Absorbance, SD and % assay was calculated.

Procedure of Selection of Wavelength

For UV spectroscopy Working solutions were prepared from further dilution of the stock solutions in different concentration ranging from 10-50 $\mu\text{g}/\text{mL}$ and 5-25 $\mu\text{g}/\text{mL}$ for Telmisartan and Nebivolol respectively. All the working solutions were scanned in the range of 200-400 nm. Zero Order spectra were converted into First Order spectra. From the overlain of first order derivative spectra, Nebivolol HCl shows ZCP (Zero Crossing Point) at 283 nm and Telmisartan show ZCP at 278 nm. Hence, these Wavelengths 283 nm and 278 nm were selected as analytical wavelength for Telmisartan and Nebivolol respectively.

Method Validation

Linearity

A calibration curve was constructed by replicate (n=5) analysis of five standards 10-50 µg/mL Telmisartan and 5-25 µg/mL Nebivolol for spectroscopic method and 100-600 ng/spot Telmisartan and 800-4800 ng/spot Nebivolol for HPTLC method. Linearity of both the drugs were checked in term of slope, intercept and correlation coefficient. Calibration curve were obtained.

Precision

Repeatability performed by analysis of replicate sample (n=6) i.e. 30 µg/mL Telmisartan and 15 µg/mL for Nebivolol. Intraday precision was established by following analysis of replicate samples (n=3) at three concentrations indicative of low, medium and high levels within the linear range viz., 5, 15, 25 µg/mL for Telmisartan and 10, 30, 50 µg/mL for Nebivolol. Analysis was performed over a short period of time on the same day. Inter-day precision or reproducibility was assessed at low, medium and high concentration on three consecutive days and the percent relative standard deviation (% RSD) was used to assess Repeatability, intra and inter-day precision.

Accuracy

Accuracy of the developed method was confirmed by doing recovery study by addition of standard drug to the prequantified sample preparation at three different concentration levels 80 %, 100 %

and 120 %, taking in to consideration percentage purity of added drug sample. The amounts of Telmisartan and Nebivolol were estimated by applying obtained values to the respective regression line equations. Each concentration was analysed 3 times and average recoveries were measured.

Limits of detection (LOD) and quantitation (LOQ)

In the present study, the LOD and LOQ were based on the third approach and were calculated according to the $3.3 \times (SD/Slope)$ and $10 \times (SD/Slope)$ criteria, respectively; where SD is the standard deviation of y-intercept of regression line and S is the slop of the calibration curve.

Specificity

The specificity of an analytical method is defined as the ability of a method to ensure that the peak(s) of interest elute as distinct responses in the presence of excipients, impurities or degradation compounds.

RESULT AND DISCUSSION

A Simple, Precise and Accurate First Order Derivative Spectrophotometric Method and HPTLC method have been developed for simultaneous estimation of Telmisartan and Nebivolol in Bulk and Pharmaceutical dosage form.

Overlay spectra shown in **Figure 1**. The results of linearity study of both the methods summarized in **Table 1, 2** having $R^2 < 0.995$ reveals that the method is linear. Spectra of linearity shown in **Figure 2** and

Chromatogram of linearity shown in **Figure 3**. The results of precision study (repeatability, Intra-Inter day) of both the methods were all within the 2% limit and summarized in **Table 3, 4**. To ensure the reliability and accuracy of the method recovery studies were carried out in triplicate at 80%, 100% and 120% of target concentration. Results of accuracy study are within the range of 98% to 102% and $RSD < 2\%$ in both the methods. Recovery results of UV method and HPTLC method are shown in **Table 5 and Table 6** respectively. For spectroscopic method LOD for Telmisartan and Nebivolol were found out to be 0.34, 0.24 $\mu\text{g/ml}$ respectively, and LOQ for Telmisartan and Nebivolol were 1.04, 0.74 $\mu\text{g/ml}$ respectively. For HPTLC method LOD for Telmisartan and Nebivolol were found out

to be 157.18, 5.29 ng/ml respectively, and LOQ for Telmisartan and Nebivolol were 476.32, 16.03 ng/ml respectively. The purity of Telmisartan and Nebivolol was ascertained by peak purity spectrum at three levels peak start, peak apex and peak end. It shows correlation coefficient of 0.999522 and 0.996085 (M, E) for Telmisartan and Nebivolol respectively. Overlay chromatograms, indicating the specificity are shown in **Figure 4**. Both the developed and validated methods were successfully applied for the estimation of Telmisartan and Nebivolol in tablet dosage form. The assay results were within the range. The results of assay indicate that the method is specific for the analysis of Telmisartan and Nebivolol without interference from the excipients used to prepare and formulate these tablets.

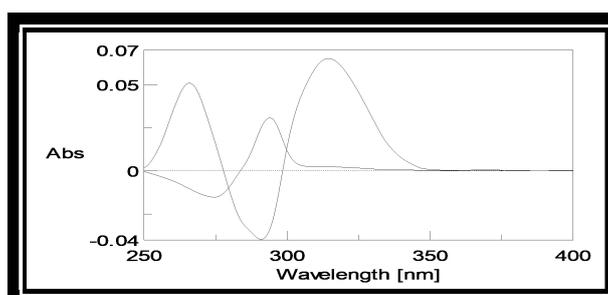


Figure 1: First order overlay spectra of Nebivolol HCl (20 $\mu\text{g/mL}$) and Telmisartan (40 $\mu\text{g/mL}$)

Table 1: Calibration curve data for Telmisartan at 283 nm (ZCP of NEB) Nebivolol HCl at 278 nm (ZCP of TEL)

Telmisartan			Nebivolol		
Concentration ($\mu\text{g/mL}$)	Average mean (n=5) (Mean \pm S.D)	% RSD	Concentration ($\mu\text{g/mL}$)	Average mean (n=5) (Mean \pm S.D)	% RSD
10	-0.00662 ± 0.0001005	1.51	5	-0.00349 ± 0.000055	1.60
20	-0.01356 ± 0.0001588	1.17	10	-0.0067 ± 0.0001011	1.50
30	-0.02074 ± 0.0002170	1.04	15	-0.01018 ± 0.0001343	1.31
40	-0.02768 ± 0.0002578	0.93	20	-0.01267 ± 0.0001525	1.20
50	-0.03347 ± 0.0002991	0.89	25	-0.01536 ± 0.0001814	1.18

Table 2: Calibration curve data for Telmisartan and Nebivolol by HPTLC method

Telmisartan			Nebivolol		
Concentration (ng/spot)	Average Area (n=5) (Mean±S.D)	% RSD	Concentration (ng/spot)	Average Area (n=5) (Mean±S.D)	% RSD
800	8443.12±101.74	1.20	100	611.72±7.82	1.27
1600	10883.16±122.81	1.12	200	1071.1±12.35	1.15
2400	12744.62±127.75	1.00	300	1588.1±16.1	1.01
3200	14738.78±139.11	0.94	400	2069.1±20.5	0.99
4000	16571.86±142.63	0.86	500	2450.7±22.14	0.90
4800	18133.68±137.37	0.75	600	2874.76±24.14	0.83

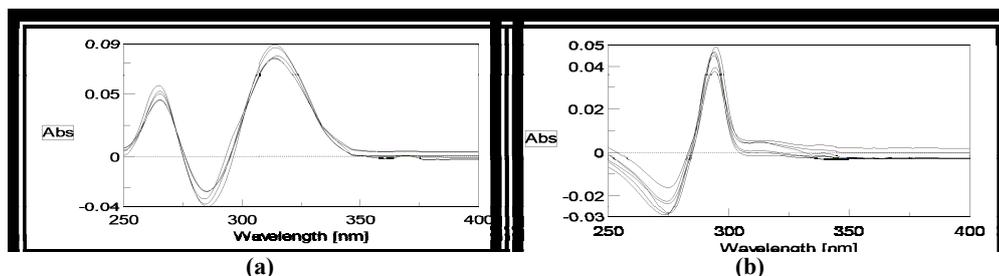


Figure 2: First order linearity spectra of (a) Telmisartan (10-50µg/mL) (b) Nebivolol (5-25µg/mL)

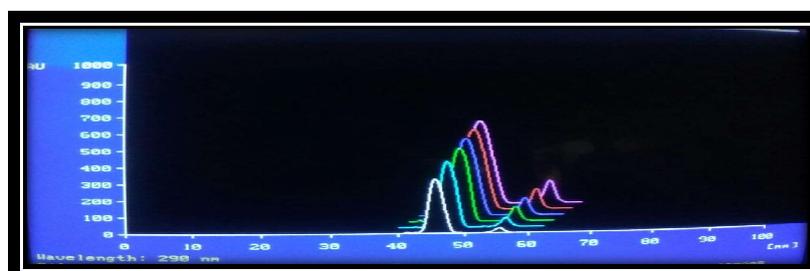


Figure 3: Calibration curve track integration of Nebivolol HCl and Telmisartan (NEB HCl – 100-600 ng/spot and TEL – 800-4800 ng/spot)

Table 3: Precision data of Nebivolol HCl and Telmisartan by UV method

Telmisartan			Nebivolol		
Intraday Precision					
Concentration (µg/mL)	Area (n=3) ±SD	%RSD	Concentration (µg/mL)	Area (n=3) ±SD	%RSD
10	0.00708 ± 0.0000854	1.19	5	0.00431 ± 0.00005	1.16
30	-0.02111 ± 0.000199	0.93	15	0.01111 ± 0.000162	1.45
50	-0.03408 ± 0.00025	0.72	25	-0.01644 ± 0.00023	1.39
Interday Precision					
10	-0.00729 ± 0.000085	1.15	5	-0.00452 ± 0.0000603	1.33
30	-0.02146 ± 0.000206	0.95	15	-0.01117 ± 0.000166	1.48
50	-0.03435 ± 0.00027	0.78	25	-0.01656 ± 0.000203	1.22
Repeatability					
30	-0.01127 ± 0.000104	0.92	15	-0.02130 ± 0.0001774	0.83

Table 4: Precision data of Nebivolol HCl and Telmisartan by HPTLC method

Telmisartan			Nebivolol		
Intraday Precision					
Concentration (ng/inj.)	Area (n=3) ±SD	%RSD	Concentration (ng/inj.)	Area (n=3) ±SD	%RSD
800	8878.9±96.71	1.10	100	825.63±8.97	1.08
1600	12884.8±128.28	1.00	200	1250.46±12.36	1.06
2400	14790.4±154.39	1.04	300	1782.46±16.45	0.92
Interday Precision					
800	8629.93±99.02	1.14	100	866.73±10.55	1.08
1600	12738.73±141.81	1.11	200	1264.36±13.99	1.06
2400	14651.43±134.76	0.91	300	1769.9±17.06	0.92
Repeatability					
1600	11837.52±22.96	0.19	200	1221.3±5.23	0.42

Table 5: Accuracy of Nebivolol HCl and Telmisartan by UV method

% Level	Amount of Test (mg)	Amount of standard spike (mg)	Amount of standard Recovered(mg±SD)	% Recovered±SD	%Avg. Recovered
Nebivolol HCl					
0	5	-	4.947±0.035	98.9±0.70	99.4
80	5	4	3.964±0.028	99.1±0.7	
100	5	5	4.996±0.027	99.9±0.55	
120	5	6	5.597±0.037	99.3±0.62	
Telmisartan					
0	40	-	39.69±0.24	99.2±0.60	99.4
80	40	32	31.722±0.22	99.1±0.70	
100	40	40	39.850±0.42	99.6±1.00	
120	40	48	47.76±0.43	99.5±0.9	

Table 6: Accuracy data of Nebivolol HCl and Telmisartan by HPTLC method

% Level	Amount of Test (mg)	Amount of std. spike (mg)	Amount of std. recovered (mg±SD)	% recovered ± SD	%Avg recovered
Nebivolol HCl					
0	5	-	5.03±0.04	100.6±0.96	100.2
50	5	2.5	2.51±0.03	100.7±1.41	
100	5	5	5.01±0.048	100.3±0.95	
150	5	7.5	7.48±0.034	99.7±0.45	
Telmisartan					
0	5	-	39.72±0.14	99.2±0.40	99.4
50	40	20	19.96±0.094	99.7±0.50	
100	40	40	39.62±0.087	99.0±0.2	
150	40	60	59.74±0.15	99.5±0.25	

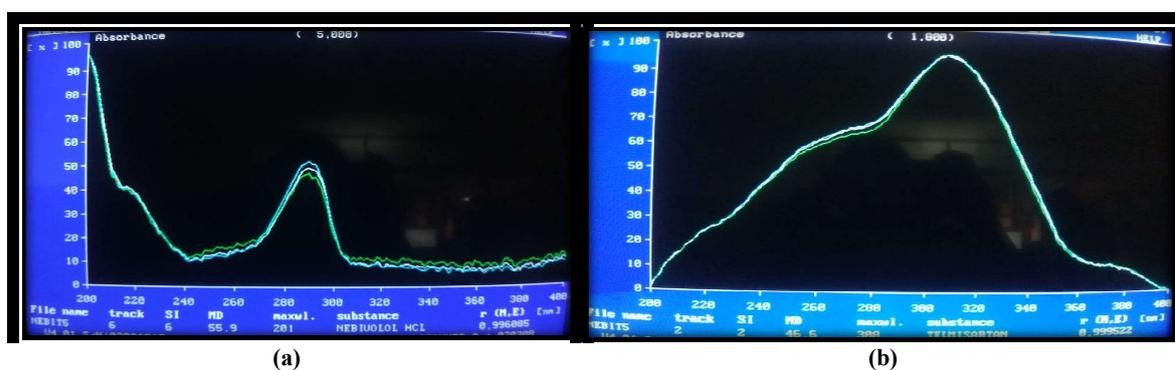


Figure 4: Peak purity spectra of (a) Nebivolol HCl (b) Telmisartan

CONCLUSION

The above-developed methods are suitable for determination of Telmisartan and Nebivolol in solid dosage form. Both methods were found to be highly sensitive, precise, accurate for determination of Telmisartan and Nebivolol in pharmaceutical dosage form. These methods are quite useful and reasonably

satisfy all the standards. Therefore, these methods can be used for routine analysis of Telmisartan and Nebivolol in pharmaceutical dosage form.

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