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Research Article

NOVEL REVERSE-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TELMISARTAN AND NEBIVOLOL HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: A simple, accurate, precise, robust reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of telmisartan and nebivolol hydrochloride (HCl) simultaneously in its combined dosage form.

Methods: The compounds were well resolved in an isocratic method using the mobile phase composition of acetonitrile: Buffer (potassium dihydrogen orthophosphate pH adjusted 3.1 with orthophosphoric acid) in a ratio of 40:60 v/v at a flow rate of 1.2 ml/min using C18 Shim-pack (150 mm × 4.6 mm, 5 µ) column. The detection was carried out at 280 nm.

Results: The retention time of telmisartan and nebivolol HCl was 4.8 min and 6.5 min, respectively. The developed method was validated by evaluating various validation parameters such as linearity, precision, accuracy, robustness, specificity, limit of detection, and limit of quantification according to the international council for harmonization guidelines. The standard calibration curve was obtained in the concentration range of 24–56 µg/ml for telmisartan and 3–7 µg/ml for nebivolol HCl. The overall average % recovery was found out to be 100.35 for telmisartan and 98.84 for nebivolol HCl.

Conclusion: Statistical analysis of the data showed that the method is reproducible and selective for the estimation of telmisartan and nebivolol HCl. The proposed method could be used for analysis of telmisartan and nebivolol HCl in their dosage form.

Keywords: Reversed-phase high-performance liquid chromatography, Telmisartan, Nebivolol hydrochloride, Simultaneous estimation.

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INTRODUCTION

Telmisartan is chemically 1 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid [1,2] (Fig. 1a). Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension [3,4]. Nebivolol hydrochloride (HCl) is (±) [2R* R* R* (S *)] α,α [iminobis(methylene)] bis-[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] (Fig. 1b), HCl is an antihypertensive drug, and it is a racemate of two enantiomers with four chiral centers. The SRRR-enantiomer (nebivolol) is a potent and cardio selective B_1-adrenergic blocker [5-7]. The combination of telmisartan and nebivolol HCl is used for lowering high blood pressure in patients suffering from hypertension. This combination is also used to relieve the symptoms of heart failure by improving the blood flow to the heart. The tablet dosage form in the combination containing telmisartan (40 mg) and nebivolol HCl (5 mg) is available in the market. To the best of our knowledge, no studies have been reported for the simultaneous estimation of telmisartan and nebivolol HCl in pharmaceutical formulation by high-performance liquid chromatography (HPLC) method, even there is no reported forced degradation studies to demonstrate the stability indicating the nature of the method [8]. Therefore, an attempt has been taken to develop a novel reverse phase (RP)-HPLC method for simultaneous estimation of telmisartan and nebivolol HCl in pharmaceutical dosage form and validate the developed method in accordance with international council for harmonization (ICH) guideline and to perform the force degradation studies using developed method.

METHODS

Raw materials and marketed formulations

All reagents used in this assay were of HPLC or analytical grade. All dilutions were performed in standard class A volumetric glassware. For the estimation of commercial formulation, Nebicard T (telmisartan - 40 mg and nebivolol HCl - 5 mg) manufactured by Torrent Pharmaceuticals Ltd was procured from the local market of Mumbai, Maharashtra, India.

Instrumentation and analytical conditions

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system LC-2030 with pump p-5000, ultraviolet (UV) detector, and a fixed injector equipped 20 µl loop was used for the chromatographic separation. The chromatograms were recorded, and the peaks were quantified by means of "LabSolutions" software. Chromatographic separation was carried out at flow rate 1.2 ml/min using a C18 column (Shim-pack GIST, 150 mm × 4.6 mm, 5 µ). The detection was carried out at 280 nm. The mobile phase consists of acetonitrile and buffer (potassium dihydrogen orthophosphate, pH adjusted to 3.1 with orthophosphoric acid) in a ratio of 40:60 v/v. The mobile phase was filtered through a Whatman filter. Ultrasonic bath was used for degassing mixing of the mobile phase.

Selection of wavelength

A UV spectrum of telmisartan and nebivolol HCl in acetonitrile was noted by scanning the solution in a range of 200–400 nm. Telmisartan and nebivolol HCl were showing significant absorption at 280 nm. Thus, 280 nm was selected as wavelength for analysis (Fig. 2).

Preparation of 0.04 M phosphate buffer (pH 3.1)

About 5.44 g of potassium dihydrogen phosphate was accurately weighed and dissolved in 950 ml of water. The pH was adjusted to 3.1 with orthophosphoric acid, and the volume was made up to 1000 ml in a volumetric flask. The solution was filtered using Whatman filter.

Preparation of standard solution

The standard stock solution of 100 mg/ml each of telmisartan and nebivolol HCl was prepared. Further, solution with concentration of

Table 1: Forced degradation data for telmisartan and nebivolol HCl

Conditions	Telmisartan		Nebivolol HCl	
	% Assay	% Difference w.r.t control	% Assay	% Difference w.r.t control
Untreated sample	99.09	NA	99.48	NA
Acid-treated sample	97.38	1.74	99.56	0.08
Base-treated sample	98.60	0.46	99.67	0.19
Peroxide-treated sample	98.87	0.22	84.94	14.54
Thermal-treated sample	99.84	0.75	99.28	3.32
UV-treated sample	98.79	0.3	99.09	0.2
Humidity-treated sample	89.26	9.83	88.52	10.96

HCL: Hydrochloride, UV: Ultraviolet

Table 2: System suitability parameters for telmisartan and nebivolol HCl by proposed method

Drugs	Retention time (min)	Peak area	Theoretical plate	Tailing factor
Telmisartan	4.899	11080622	4287	1.79
Nebivolol HCl	6.597	616323	8525	1.38

HCl: Hydrochloride

Table 3: System precision of telmisartan and nebivolol HCl

Replicate (n=6)	Peak area of telmisartan (40 μg/ml)	Peak area of nebivolol HCl (5 μg/ml)
1	1108062	616323
2	1108062	616323
3	1118026	616332
4	1108062	616332
5	1108062	616323
6	1108062	616324
Mean±SD	1109722±40677.9	617992.8±4085.342
±SEM	16610.0	1668.16
%RSD	0.366	0.661

n: Number of injection, SD: Standard deviation, SEM: Standard error of mean, %RSD: % Relative standard deviation. HCl: Hydrochloride

Table 4: Method precision data for telmisartan and nebivolol HCl in tablet

Replicate (n=6)	% Assay of telmisartan	% Assay of nebivolol HCl
1	102.49	100.51
2	101.71	100.86
3	101.72	100.98
4	101.52	100.51
5	100.52	102.67
6	98.57	100.98
Mean±SD	101.08±1.385	101.08±0.805
±SEM	0.5655	0.3287
%RSD	0.0137	0.007

n: Number of injection, SD: Standard deviation, SEM: standard error of mean, %RSD: % Relative standard deviation. HCl: Hydrochloride

40 μg/ml of telmisartan and 5 μg/ml of nebivolol HCl was prepared by diluting the stock solution with the mobile phase.

Preparation of sample solution

Ten tablets were weighed, and the equivalent weight of telmisartan and nebivolol HCl was transferred to 100 ml of volumetric flask and dissolve in the mobile phase. The solution was filtered through Whatman filter. This was further diluted with mobile phase to get the final concentration of 40 μg/ml of telmisartan and 5 μg/ml of nebivolol HCl.

HPLC method development

Optimized chromatographic conditions

The optimized mobile phase was acetonitrile: Buffer (potassium dihydrogen orthophosphate, pH adjusted to 3.1 with orthophosphoric acid) in a ratio of 40:60 v/v and flow rate was kept 1.2 ml/min and column temperature was set at 25°C. The retention time of telmisartan and nebivolol HCl for this mobile phase was found to be 4.8 min and 6.5 min, respectively (Figs. 3 and 4).

HPLC method validation

The optimized chromatographic method was validated by evaluating, system suitability, linearity, precision, robustness, limit of detection (LOD), limit of quantification (LOQ), and accuracy.

Forced degradation studies in different stress conditions were conducted away in conformity with the ICH guideline Q2 (R1).

Forced degradation of telmisartan and nebivolol HCl

All stress decomposition studies were performed at a drug concentration 40 μg/ml and 5 μg/ml of telmisartan and nebivolol HCl under conditions of dry heat (thermal studies), hydrolysis (acid, base), oxidation, photolysis, and humidity, as mentioned in ICH guidelines Q1A (R2) [9-11]. Acid, base, and oxidation degradation were performed by adding 1 ml of 1 N HCl, 1 ml of 1 N NaOH, and 1 ml of 30% peroxide solution, respectively, to the sample solution, and these samples was kept on bench for 30 min. Test sample and placebo were subjected to thermal degradation by keeping the sample at 105°C for 1 h. A photolytic degradation was carried out by placing the test sample and placebo were exposed in photostability chamber for 24 h. Humidity degradation was carried out by keeping test sample and placebo were exposed at 25°C temperature and 80% relative humidity for 24 h. The results of forced degradation are summarized in Table 1.

RESULT AND DISCUSSION

System suitability

To know reproducibility of the method, the system suitability test was done to establish the parameter such as retention time, tailing factor, theoretical plate, and peak area. This was performed by injecting the standard mixture. The results obtained for system suitability are summarized in Table 2.

Linearity

The linearity of an analyte procedure is its ability (within a given range) to obtained test results which are directly proportional to the concentration of analyte in the sample. Linearity was evaluated by analyzing the plot of area as a function of concentration of analyte. The result was evaluated by calculating of regression coefficient (r^2).

The standard calibration curve was obtained in the concentration range of 24–56 μg/ml for telmisartan and 3–7 μg/ml for nebivolol HCl with a correlation coefficient (r^2) of 0.9999 and 0.9994,

Table 5: Intermediate precision data for telmisartan and nebivolol HCl

Replicate (n=6)	% Assay of telmisartan		% Assay of nebivolol HCl	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2
1	100.52	98.45	100.52	98.45
2	100.63	99.54	100.63	99.54
3	101.72	99.54	101.72	99.54
4	102.49	101.38	102.49	101.38
5	99.54	98.63	99.54	98.63
6	101.71	100.45	101.71	100.45
Mean±SD	99.66±1.107	101.10±1.065	100.09±1.017	99.88±0.871
±SEM	0.4520	0.4348	0.4152	0.3556
%RSD	1.111	1.053	1.016	0.872

n: Number of injection, SD: Standard deviation, SEM: standard error of mean, %RSD, % Relative standard deviation. HCl: Hydrochloride

Table 6: Recovery data for telmisartan and nebivolol HCl

Drug	Level of recovery (%) n=3	Sample amount (μg/ml)	Standard amount (μg/ml)	Total amount of drug (μg/ml)	Amount found (μg/ml)	% Recovery
Telmisartan	80	20	16	36	36.70	101.94
	100	20	20	40	39.76	99.40
	120	20	24	44	43.88	99.72
Nebivolol HCl	80	2.5	2.0	4.5	4.22	98.22
	100	2.5	2.5	5	4.97	99.4
	120	2.5	3.0	5.5	5.36	98.9

HCl: Hydrochloride

Table 7: LOD and LOQ for telmisartan and nebivolol HCl

Drugs	LOD	LOQ
Telmisartan	0.163 μg/ml	0.494 μg/ml
Nebivolol HCl	0.107 μg/ml	0.035 μg/ml

LOD: Limit of detection, LOQ: Limit of quantification. HCl: Hydrochloride

Table 8: Robustness of method

Parameter	% Assay	
	Telmisartan	Nebivolol HCl
Minus flow rate [1 ml/min]	99.65	98.90
Plus flow rate [1.4 ml/min]	98.75	99.80
Minus temperature [24ºC]	99.87	99.94
Plus temperature [26ºC]	99.85	99.64
Minus wavelength [279 nm]	99.21	99.89
Plus wavelength [281 nm]	99.52	100.45

HCl: Hydrochloride

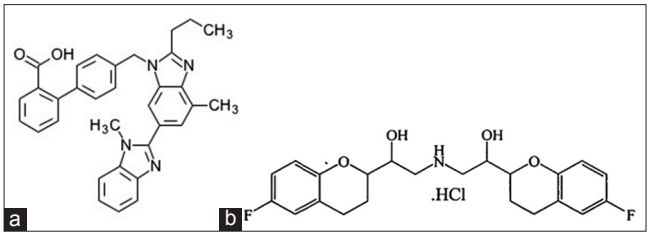

Fig. 1: Chemical structures of (a) telmisartan, (b) nebivolol hydrochloride

respectively. Three independent determinations were performed at each concentration (Figs. 5 and 6). The linear regression equation was obtained y = 67503x + 951191 for telmisartan and y = 502601x + 266100 for nebivolol HCl. The results obtained for linearity are summarized in Table 3.

Precision

The precision of an analytical method is the closeness of replicating result obtained from analysis of the same homogeneous sample. Precision was considered at different levels, i.e., system precision and method precision.

System precision

System precision was carried out with 6 replicates (n=6) of standard at working concentration of 40 μg/ml of telmisartan and 5 μg/ml of nebivolol HCl. The repeatability of sample applications and measurement of peak area were expressed in term of % relative standard deviation (%RSD).

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0%, and hence, the developed method was found to be precise. Data obtained from precision experiments for repeatability studies are shown as below Table 3.

Method precision

The method precision of the proposed method was determined by injecting six replicates (n=6) of sample from the same homogeneous mixture. The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0 %, and hence, the developed method was found to be precise. Data obtained from method precision are summarized in Table 4.

Intermediate precision or ruggedness

The ruggedness of the method was verified by analyzing six samples of the same batch used for method precision as per proposed method by different analyst.

The repeatability of sample applications and measurement of peak area were expressed in term of %RSD since their %RSD is <2.0 %, and hence, the developed method was found to be precise. Data obtained from intermediate are summarized in Table 5.

Accuracy

The accuracy of an analytical method is the closeness of the results obtained by that method to the true value of the sample. It is expressed as recovery (%), which is determined by the standard addition method. The accuracy was evaluated by the recovery of telmisartan and nebivolol HCl at three different levels (80%, 100%, and 120%).

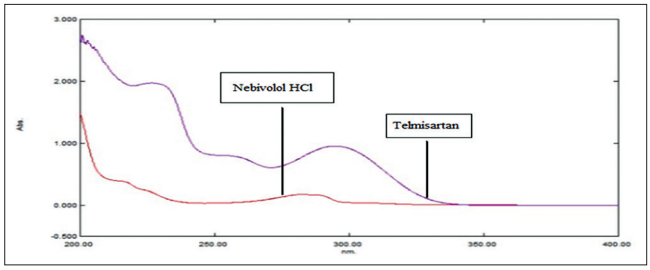

Fig. 2: Ultraviolet spectrum of telmisartan and nebivolol hydrochloride

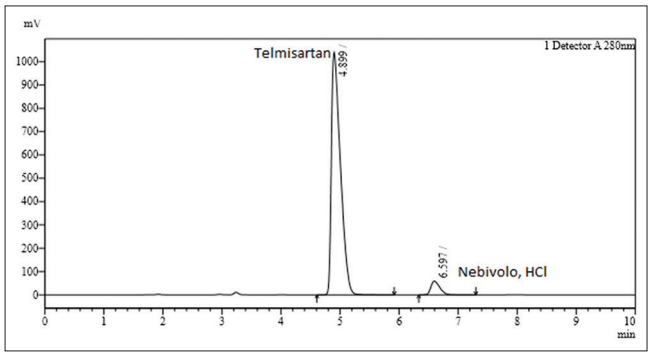

Fig. 3: A typical high-performance liquid chromatography chromatogram showing the peak of telmisartan and nebivolol HCl

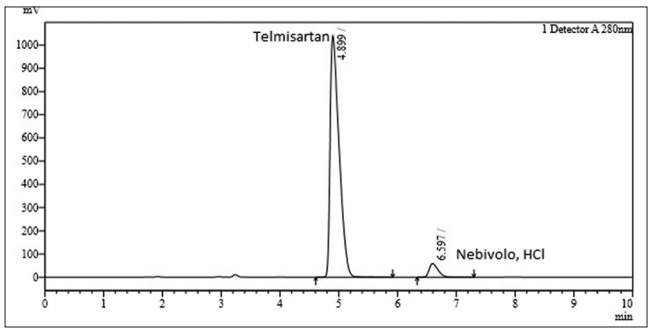

Fig. 4: Chromatogram shows a peak of telmisartan and nebivolol hydrochloride

The % recovery was found to be 99.92 for telmisartan and 99.33 for nebivolol HCl. %RSD was found to be <2, and hence, the method is said to be accurate. The results of accuracy studies are shown in Table 6.

LOD and LOQ

The LOD of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

The LOQ of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

The sensitivity of measurement of telmisartan and nebivolol HCl by use of the proposed method estimated in term of the LOQ and LOD. The results of LOD and LOQ are summarized in Table 7.

Robustness

The robustness of the method we determined by assessing the ability of the developed method to remain unaffected by the small changes in the parameters such as Flow rate (±1 nm), oven temperature (±1°C), detection wavelength (±0.2 ml/min).

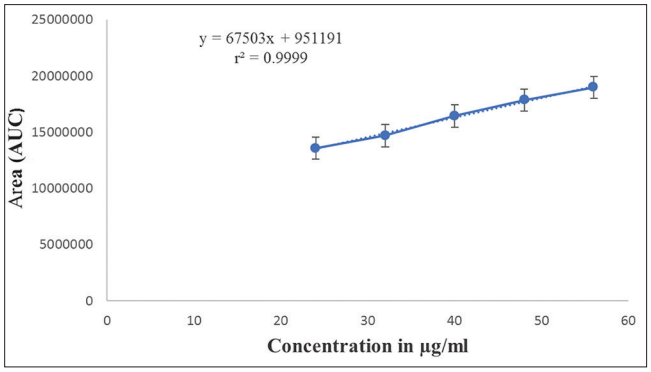

Fig. 5: Graph representing calibration curve of telmisartan. Error bars represent standard deviation (SD) of the mean (±SD)

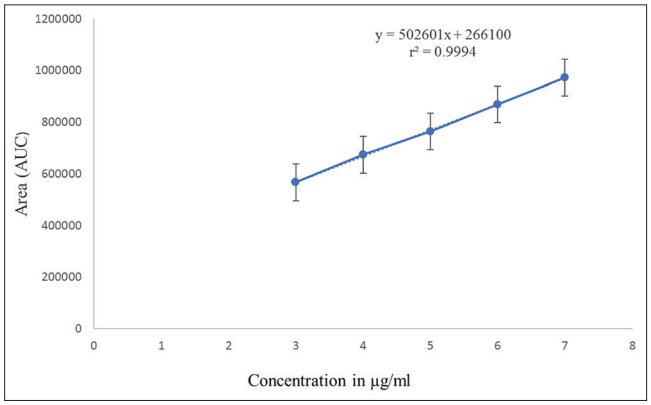

Fig. 6: Graph representing calibration curve of nebivolol HCl. Error bars represent standard deviation (SD) of the mean (±SD)

The % assay was within the acceptance criteria in the condition described in this report, and hence, the method is robust. The results are summarized in Table 8.

Force degradation studies

In literature survey, there exist many reports where stability-indicating assay has been established by carrying out stress test directly on pharmaceutical formulations. In the present study, the stress testing was carried out on telmisartan and nebivolol HCl containing pharmaceutical formulation, and degradation was observed when the analyte was subjected to acid, base, oxidation, photolytic, thermal, and humidity stress conditions.

CONCLUSION

A novel validated isocratic RP-HPLC method has been developed for the determination of telmisartan and nebivolol HCl in dosage forms. The developed method is validated according to the guidelines provided in ICH Q2 (R1) in term of linearity, precision, LOD, LOQ, accuracy, and robustness. Its chromatographic run time is 10 min which allows the analysis of a great number of samples in a little stop of time, and hence, developed method can be employed for everyday analysis of telmisartan and nebivolol HCl in tablet dosage form.

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AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

All authors declared no conflict of interest.

REFERENCES

1. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. 4th ed., Vol. 11. Daryaganj, New Delhi: Bloomsbury Publishing PLC; 1997. p. 278.
2. The Indian Pharmacopoeia Commission. Indian Pharmacopoeia. 6th ed., Vol. 3. Ghaziabad: The Indian Pharmacopoeia Commission; 2010. p. 2186.
3. Mahesh M, Kumanan R, Jayaveer KN. Isocratic RP-HPLC UV method development and validation for the simultaneous estimation of hydrochlorothiazide and ramipril in tablet dosage form and bulk drug. Int J Curr Pharm Res 2011;3:119-23.
4. Shah DA, Bhatt KK, Mehta RS, Baldania SL. Determination of nebivolol hydrochloride and hydrochlorothiazide in tablets by first-order derivative spectrophotometry and liquid chromatography. J AOAC 2008;5:1075-82.
5. Nekkala K, Shanmukha V, Kumar J, Ramchandran D, Ganji R, Ganta S. Method development and validation of stability indicating RP-HPLC method for simultaneous estimation nebivolol HCl and valsartan in bulk and its pharmaceutical formulation. Am J Adv Drug Deliv 2014;2:624-37.
6. ICH. Q2 (R1), Harmonized Tripartite Guideline, Validation of Analytical Procedures Text and Methodology. Geneva: International Conference on Harmonization (ICH); 2005.
7. Surekha LM, Kumara GS, Ashwini GL. Development and validation RP-HPLC method for the estimation of telmisartan in bulk and tablet dosage form. Int J Drug Dev Res 2012;4:200-5.
8. Shinde R, Bohir SI, Pawar NS, Yadav SB, Ghumatkar AS, Bhagwat AM. Simultaneous determination of valsartan and nebivolol HCl in tablet dosage form by RP-HPLC. Asian J Research in Chem 2009;4:519-22.
9. Patel ND, Mehta R, Captain AD, Karkhanis VV, Patel PD, Chavda AA. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of nebivolol hydrochloride and cilnidipine in tablet dosage form. J Pharm Sci Bioscientific Res 2017;7:140-7.
10. Hafez H, Abdullah AE, Abdelaziz LM. Stability indicating HPLC method for simultaneous determination of several angiotensin- II-receptor antagonist in their dosage forms. Pharmaceutica Analytica Acta 2012;8:1-11.
11. Marineni B, Reddy TS. Development and validation of stability-indicating RP-HPLC assay method for azacytidine and its bulk drug. Int J Pharm Pharm Sci 2014;6:240-4.