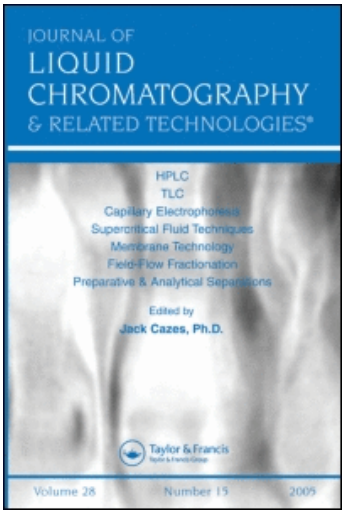

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Validated RP-HPLC Method for Simultaneous Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Formulation

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Abstract: A simple, selective, and precise reverse phase high performance liquid chromatographic method has been developed for the simultaneous determination of Telmisartan and hydrochlorothiazide from pharmaceutical formulation. The mobile phase consisted of methanol and acetonitrile (70:30 v/v) at a flow rate of 1 mL/min and the wavelength of detection was 270 nm. Rabeprazole was used as an internal standard. The retention times of Telmisartan, hydrochlorothiazide and rabeprazole were 1.79 ± 0.01, 2.80 ± 0.01, and 3.19 ± 0.01 minutes, respectively. The developed method was validated according to ICH guidelines. The proposed method can be used for determination of these drugs in combined dosage forms.

Keywords: Telmisartan, Hydrochlorothiazide, Rabeprazole, RP-HPLC

INTRODUCTION

Telmisartan (TEL) is chemically designated as 4′-[(1,4′-dimethyl-2′-propyl [2,6′-bi-1H-benzimidazol]-1′-yl) methyl] [1,1′-biphenyl]-2-carboxylic acid. Telmisartan is an Angiotensin II Type I blocker and is used as an antihypertensive along with hydrochlorothiazide (HCT), which is a diuretic. Telmisartan is not official in IP, BP, and USP. Hydrochlorothiazide is chemically 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide-1, 1-dioxide. It is

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official in IP'96,[1] BP 2004,[2] and USP 2006.[3] A detailed survey of literature of Telmisartan revealed several methods based on different techniques, viz., HPLC,[4–8] and electrochemical[9,10] methods for the determination in plasma, urine, and pharmaceutical formulation. Similarly, a survey of literature of hydrochlorothiazide revealed several methods based on HPLC[11] and electrochemical[12] methods in human plasma, urine, and pharmaceutical formulation. UV spectrophotometric, TLC, and spectrofluorimetric methods were found to be reported for the determination of Telmisartan and hydrochlorothiazide in a combined dosage form.[13] The fixed dose combination containing Telmisartan 40 mg and hydrochlorothiazide 12.5 mg is the tablet available in the market. The present work describes the development of a validated reverse phase high performance liquid chromatographic (RP-HPLC) method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated following the ICH guidelines.

EXPERIMENTAL

Reagent and Chemicals

Acetonitrile and methanol of HPLC grade were procured from Merck specialties Pvt. Ltd., Mumbai. A working standard of Telmisartan was provided by Glenmark Pharmaceuticals Ltd. and Hydrochlorothiazide was by Torrent pharmaceuticals Ltd. Rabeprazole (RAB) was received from Medley Pharmaceuticals Ltd, Dadra and Nagar Haveli.

Method Development

Different mobile phases containing methanol, water, and acetonitrile in different proportions were tried, and finally, methanol: acetonitrile (70:30 v/v), was selected as an appropriate mobile phase, which gave good resolution and acceptable peak parameters for both Telmisartan and hydrochlorothiazide as well as the internal standard.

Equipment

Chromatographic separation was performed on a Jasco chromatographic system equipped with a Jasco PU-2080 plus HPLC pump and Jasco UV-2075 plus UV/VIS detector and Rheodyne injector with 20 μL loop volume.

Chromatographic Conditions

HiQ Sil C8 (250 mm × 4.6 mm) was used for the separation; mobile phase consisting of a mixture of methanol and acetonitrile (70:30 v/v) was

delivered at a flow rate of 1 mL/min with detection at 270 nm. The mobile phase was filtered through a 0.45 μ membrane filter and sonicated for 15 minutes. The injection volume was 20 μL. Analysis was performed at an ambient temperature.

Preparation of Working Standard Solutions

Working Standard stock solutions of 1.0 mg/mL Telmisartan, hydrochlorothiazide and rabeprazole were prepared separately using methanol. From the standard stock solution of each drug, an appropriate amount of respective solutions were mixed to get the required (3.2:1 for Telmisartan and hydrochlorothiazide, respectively) ratio of both drugs and diluted with the mobile phase. The final concentration of the solution was 100 μg/mL of Telmisartan and 31.2 μg/mL of hydrochlorothiazide. Rabeprazole was used as an internal standard at 5 μg/mL concentration.

System Suitability Studies

The resolution, number of theoretical plates, and peak asymmetry were calculated for the working standard solutions and are as shown in Table 1. The values obtained demonstrated the suitability of the system for the analysis of these drugs in combination. The typical chromatogram of the standard solution is as shown in Fig. 1.

Calibration Curve

Calibration curves were obtained by plotting the response factor (area of drug peak divided by area of internal standard peak) versus concentration of drug. Regression equations were calculated. Accurately measured volumes of the working standard solution of Telmisartan and hydrochlorothiazide were transferred into a series of 10 mL volumetric flasks and diluted appropriately with mobile phase. In each flask, rabeprazole solution was added to get 5 μg/mL concentration in final solutions. The calibration curves were plotted over a

Table 1. System suitability studies

S. No.	Parameters	Telmisartan	Hydrochlorothiazide	Rabeprazole
1	Theoretical plates/ meter	2267.93	4689.59	5061.25
2	Resolution	6.71	6.55	—
3	Asymmetry factor	1.09	1.24	1.21
4	LOD (μg/mL)	0.0694	0.0185	NA
5	LOQ (μg/mL)	0.2104	0.0563	NA

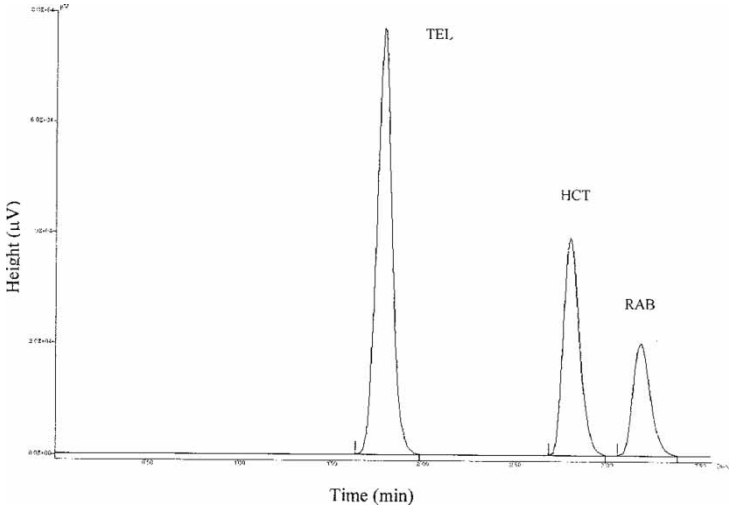

Figure 1. Chromatogram of Telmisartan (t_R1.79 min), hydrochlorothiazide (t_R 2.80 min) and rabeprazole (t_R 3.19 min).

concentration range 2 μg/mL to 10 μg/mL for Telmisartan and 0.62 μg/mL to 3.12 μg/mL for hydrochlorothiazide. Of each solution, 20 μL was injected under the operating chromatographic conditions described above.

Assay Method

Preparation of Sample Solutions

Twenty tablets, each containing 40 mg Telmisartan and 12.5 mg hydrochlorothiazide were weighed and finely powdered. A quantity of powder equivalent to 25 mg Telmisartan and 7.812 mg of hydrochlorothiazide was weighed and transferred to a 50 mL volumetric flask. Methanol was added to the same flask and shaken for 5 minutes. The volume was made up to 50 mL with methanol. The solution was filtered using Whatman filter paper No. 41. From the filtrate, appropriate dilution was done to get a solution of 500 μg/mL of Telmisartan and 156 μg/mL of hydrochlorothiazide. From this solution, appropriate dilutions were made in mobile phase to obtain a concentration in the Beer's Law range for both the drugs. Before making up the volume, the internal standard was added to get 5 μg/mL concentration in final solutions and this solution was used for the estimation.

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed working standard solution was injected, and the chromatogram was recorded. The retention times of Telmisartan, hydrochlorothiazide, and rabeprazole were found to be 1.79, 2.80, and 3.19 minutes $\pm$ 0.01,

respectively. The proposed method was found to be specific and no interference from common tablet excipients like lactose, starch, etc., was observed. The response factors of the standard solutions and sample solutions were calculated. The assay was calculated from the equation of the regression line for each drug. The assay procedures were repeated 3 times; the percentage of individual drug in formulation was calculated and presented in Table 2. The results of the analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations. Three brands, Brand 1, Brand 2, and Brand 3 were assayed by this method for comparison.

Method Validation

As per the ICH guidelines, the method validation parameters checked were linearity, precision, accuracy, limit of detection, limit of quantitation, and robustness.

Linearity and Range

The linearity of Telmisartan and hydrochlorothiazide were determined at five concentration levels, ranging from 2 μg/mL to 10 μg/mL for Telmisartan and 0.62 μg/mL to 3.12 μg/mL for hydrochlorothiazide, using working standards. The linear regression equations of the lines are:

$$\text{Telmisartan } y = 0.3593x + 0.0223, \quad (r^2 = 0.9992)$$

$$\text{hydrochlorothiazide } y = 0.5238x - 0.0006, \quad (r^2 = 0.9993)$$

An excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Table 2. Results of assay of formulation and recovery studies

				Recovery studies		
Drug	Labeled (mg/tab)	Amount found (mg/tab)	Label claim (%)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
Telmisartan	40	39.20	98.44	32.0	72.14	100.19
	40	39.63	99.08	40.0	80.13	100.16
	40	40.00	100.00	48.0	88.04	100.04
Hydrochlorothiazide	12.5	12.27	98.18	10.0	22.55	100.22
	12.5	12.39	99.17	12.5	24.88	99.52
	12.5	12.65	101.22	15.0	27.64	100.50

Precision and Accuracy

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, solutions of standard and sample were repeated thrice in a day and percentage relative standard deviation (%RSD) was calculated. The intra day percentage RSD of Telmisartan and hydrochlorothiazide were found to be 0.3754% and 0.4587%, respectively. In the inter day variation studies, injections of standard and sample solutions were made on 3 consecutive days and percentage RSD were calculated. The percentage RSD for Telmisartan and hydrochlorothiazide were found to be 0.4732% and 0.5264%, respectively. From the data obtained, the developed RP-HPLC method was found to be precise.

The accuracy of the method was determined by recovery experiments. The recovery studies were performed by the standard addition method, at 80%, 100%, 120% level, and the percentage recovery was calculated and presented in Table 2. Recovery was within the range of $100 \pm 2\%$, which indicates accuracy of the method. A known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovered was calculated by comparing the area before and after the addition of the working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. The percent recovery indicates the accuracy of the developed method. The results of recovery studies were found to be satisfactory.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

$$\text{LOD} = \frac{3.3 \times \text{standard deviation}}{\text{Slope of calibration curve}}.$$

The LOD for Telmisartan and hydrochlorothiazide was found to be 0.0694 μg/mL and 0.0185 μg/mL, respectively. The LOQ is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula:

$$\text{LOQ} = \frac{10 \times \text{standard deviation}}{\text{Slope of calibration curve}}.$$

The LOQ was 0.2104 μg/mL and 0.0563 μg/mL for Telmisartan and hydrochlorothiazide, respectively, as given in Table 4.

Robustness

Robustness of the method was determined by making slight deliberate changes in chromatographic conditions, such as 2% change in ratio of the mobile phase constituents, room temperature, and wavelength of detection $\pm$ 1 nm. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Solution Stability

In order to demonstrate the stability of both standard and sample solutions, the solutions were analyzed over a period of 4 hours at room temperature. The results show that for both solutions, the retention time and peak area of Telmisartan and hydrochlorothiazide remained almost unchanged (%RSD less than 1.5) and no significant degradation within the indicated period was observed, this indicates that both solutions were stable for at least 4 hours, which was sufficient to complete the whole analytical process.

RESULTS AND DISCUSSION

Three marketed combinations were analyzed by the same method and "One Way-Analysis of Variance" (ANOVA) was applied to the data obtained. The ANOVA analyses for all samples were performed at a significance level of 0.05. Calculated F values are less than Critical F values. For both drugs, variation among brands tested was not statistically significant. Details are given in Table 3.

Table 3. Calculated ANOVA table for 3 formulations of Telmisartan and hydrochlorothiazide

Sources of variation	SS[a]	df[b]	MS[c]	F	F critical
For Telmisartan					
Treatment (between column)	3.316	2	1.658	2.265	3.8853
Residual (within column)	8.782	12	0.7319		
Total	12.098	14			
For hydrochlorothiazide					
Treatment (between column)	3.415	2	1.7080	2.051	3.8853
Residual (within column)	9.989	12	0.8324		
Total	13.404	14			

[a]SS: Sum of square values.
[b]df: Degrees of freedom.
[c]MS: Mean square values.

Table 4. Summary of validation parameters of proposed HPLC method

	HPLC method	
Parameters	Telmisartan	Hydrochlorothiazide
Linearity range (μg/mL)	2–10	0.62–3.12
Correlation co-efficient	0.99960	0.99963
LOD[a] (μg/mL)	0.0694	0.0185
LOQ[b] (μg/mL)	0.2104	0.0563
Accuracy (%Recovery)	100.04–100.19	99.52–100.50
Precision (%RSD)[c]		
Intra day (n[d] = 3)	0.35–0.39	0.43–0.47
Inter day (n = 3)	0.44–0.50	0.49–0.55

[a]LOD = Limit of detection.
[b]LOQ = Limit of quantitation.
[c]RSD = Relative standard deviation.
[d]n = Number of determination.

The proposed method was found to be simple and sensitive with linearity in the concentration range of 2 to 10 μg/mL for Telmisartan and 0.62 to 3.12 μg/mL hydrochlorothiazide. The method was found to be accurate and precise, as indicated by recovery studies and %RSD not more than 1.5. Moreover, LOD and LOQ for Telmisartan were found to be 0.0694 μg/mL and 0.2104 μg/mL, respectively, and for hydrochlorothiazide were 0.0185 μg/mL and 0.0563 μg/mL, respectively. Thus, the method is specific and sensitive. The summary of validation parameters of proposed HPLC method is given in Table 4.

CONCLUSION

The proposed RP-HPLC method for the simultaneous estimation of Telmisartan and hydrochlorothiazide in combined dosage forms is simple, accurate, precise, robust, and rapid. Hence, the present RP-HPLC method is suitable for quality control of the raw materials and formulations.

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