# Simultaneous High-Performance Liquid Chromatographic Determination of Telmisartan and Hydrochlorothiazide in Pharmaceutical Preparation

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## Abstract

**A simple, rapid, and precise method was developed for the quantitative simultaneous determination of telmisartan and hydrochlorothiazide in combined pharmaceutical dosage form. Chromatographic separation of two drugs was achieved on an ACE 5 $C_{18}$ 25-cm analytical column using buffer–acetonitrile (60:40, v/v) of pH 5.5, adjusted with acetic acid. The buffer used in mobile phase contains 50mM ammonium acetate in double distilled water. The instrumental settings were: flow rate, 1 mL/min; column temperature, 30°C; and detector wavelength, 260 nm. The internal standard method was used for the quantitation of the ingredients of this combination. Methyl paraben was used as an internal standard. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification, and robustness. The calibration curve shows excellent linearity over the concentration range for telmisartan and hydrochlorothiazide were 10–150 and 5–75 µg/mL, respectively. The correlation coefficient for telmisartan and hydrochlorothiazide were 0.9999. The relative standard deviation for six replicate measurements in two sets of each drug in tablets are always less than 2%. The proposed method was found to be suitable and accurate for quantitative determination of telmisartan and hydrochlorothiazide in pharmaceutical preparation and it can be used for the quality control of formulation products.**

## Introduction

Telmisartan, (Figure 1) a nonpeptide molecule, is chemically 4'-[(1,4-dimethyl-2'-propyl [2,6'-1H-benzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid, and hydrochlorothiazide is chemically 6-Chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7sulfonamide1,1-dioxide (1). Telmisartan is an angiotensin II receptor antagonist that is highly selective for type 1 angiotensin II receptor for the treatment of essential hypertension usually given in combination with hydrochlorothiazide. Angiotensin II is the principle pressor agent of the renninangiotensin system, with effects that include vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation, and renal reabsorbtion of sodium. Hydrochlorothiazide is a thiazide diuretic. Thiazide affects the renal tubular mechanisms of electrolytes reabsorbtion, directly increasing excretion of sodium salt and chloride in approximately equivalent amount. The combination is useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors (2). A combination of 40 mg of telmisartan and 12.5 mg of hydrochlorothiazide is available commercially as tablets.

The literature survey reveals that several methods were reported for the individual estimation of telmisartan and hydrochlorothiazide. The methods (3–9) for telmisartan in combination with other drugs in plasma, serum, and in tablets by high-performance liquid chromatography (HPLC) and (10–16) is for the estimation of hydrochlorothiazide in combination with other drugs in plasma, serum, and in tablets by HPLC. However, recently two methods were published for the simultaneous determination of the telmisartan and hydrochlorothiazide in combined pharmaceutical-dosage form by high-performance thin-layer chromatography (HPTLC) and HPLC (17,18).

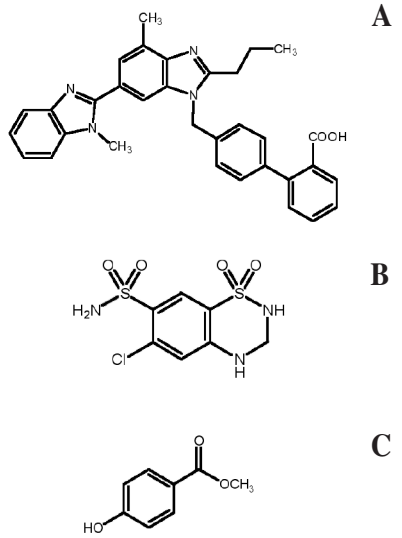


**Figure 1.** The structures of telmisartan (A), hydrochlorothiazide (B), and methylparaben IS (C).

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In the present research study, attempts were made to develop a rapid, economical, precise, and accurate method for the simultaneous estimation of the ingredients of this combination. An internal standards method was used for the quantitation of telmisartan and hydrchlorothiazide. Methylparaben was used as an internal standard. A good separation of the analytes of this combination was achieved by using a mobile phase containing ammonium acetate and acetonitrile as mobile phase. The proposed method is rapid, less expensive, and is successfully applied for the simultaneous determination of telmisartan and hydrochlorothiazide in combined-dosage form (tablets) available in the commercial market. It can be used for the quality control of formulation products.

## Experimental

### Materials and reagents

Telmisartan and hydrochlorothiazide standards were obtained from Lupin Pharmaceutical Ltd. (Mumbai, India); ammonium acetate, acetic acid, and acetonitrile (HPLC grade) were obtained from Qualigens Fine Chemicals (Mumbai, India). Methyl paraben was obtained from Merck Laboratories Ltd. (Mumbai, India). The 0.45-Pump nylon filter was obtained from Advanced Micro devices Pvt. Ltd. (Ambala Cantt, India). The (Telista) tablets of the combination of telmisartan and hydrochlorothiazide were purchased from Lupin pharmaceutical Ltd. (Mumbai, India). Double -distilled water was used throughout the experiment. Other chemicals used were analytical or HPLC grade

### Chromatographic conditions

A chromatographic system (Shimadzu, Japan) consisting of quaternary solvent delivery pump, a degasser, an auto-injector, column oven, and photodiode array detector, 10A-VP series with LC-10 software. ACE 5 $C_{18}$ (4.6 × 250 mm, 5 µm, Advance separation technology) column was used. The instrumental settings were a flow of 1 mL/min. The injection volume was 10 µL. The detection wavelength was 260 nm for all three analytes. The UV spectra of hydrochlorothiazide, telmisartan, and methylparaben (IS) in methanol are shown in Figure 2. The peak purity was checked with the photodiode array detector from 10A-VP series model with LC-10 software.

### Mobile phase

The mobile phase consisted of buffer and Acetonitrile in the ratio of 60:40 (v/v). The pH of the mobile phase was adjusted to 5.5 with acetic acid. The buffer used in the mobile phase contained 50mM of ammonium acetate in double-distilled water. The mobile phase was premixed and filtered through a 0.45-µm nylon filter and degassed.

### Standard stock solutions

Standard solutions were prepared by dissolving the drugs in the diluents and diluting them to the desired concentration. Diluents used for the standards and sample preparation was prepared as follows: diluent A was composed of methanol and diluents B was composed of water–actonitrile in the ratio of 60:40 (v/v).

*Telmisartan*

A 25-mg sample of telmisartan (99.78%) was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with the diluent A.

*Hydrchlorothiazide*

A 12.5-mg sample of hydrochlorothiazide (99.85%) was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with diluent A.

*Methylparaben*

A 5-mg sample of methylparaben was accurately weighed, transferred in a 25-mL volumetric flask, and dissolved with diluent A.

*Mixed standard solution*

A mixed standard solution was prepared from these stock solutions by transferring 5 mL of telmisartan standard solutions, 5 mL of hydrochlorothiazide by transferring standard solution, and 5 mL of methylparaben standard solution in a 50 mL volumetric flask and diluted with diluent B. This solution contained 100 µg/mL of telmisartan, 50 µg/mL of hydrochlorothiazide, and 20 µg/mL of methylparaben.

*Calibration curve solutions*

The calibration curve solutions containing 10–150 µg/mL of telmisartan, 5–75 µg/mL of hydrochlorothiazide, and 20 µg/mL of methylparaben in each calibration level were prepared.

### Preparation of sample

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 40 mg of telmisartan and 12.5 mg of hyrdochlorothiazide was transferred in a 100-mL volumetric flask. To this flask, 50 mL of diluent A was added, and the solution was sonicated for 10 min with intermittent shaking. An accurately measured volume of 10 mL acetonitrile was added to the flask and mixed well. Further sonication was performed for another 25 min with intermittent shaking. The solution was cooled to ambient temperature. An accurately measured volume of 20 mL methanol was added to the flask, and centrifuged at

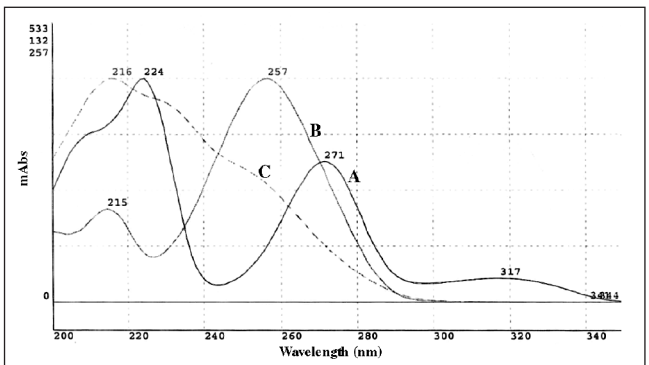


**Figure 2.** UV spectra of hydrochlorothiazide (A), methylparaben (IS) (B), and telmisartan (C) all in methanol.

10,000 rpm for 10 min. From the centrifuged solution, 5 mL of clear solution was transferred into a 50-mL volumetric flask, and 5 mL of internal standard solution was added into it and diluted to volume with diluent B.

## Results and Discussion

### Optimization of the chromatographic conditions

The primary target in developing this LC method is to achieve simultaneous determination of telmisartan and hydrochlorothiazide in combined pharmaceutical dosage form, under common conditions that are applicable for the routine quality control of this product in ordinary laboratories. Our objective of the chromatographic method development was to achieve a peak tailing factor < 2, retention time in between 3 to 15 min, along with resolution between telmisartan, hydrochlorothiazide, and internal standard (methylparaben) > 2.

The chromatographic separation was achieved using end capped C18 (ACE 5 C18, 25-cm) column. The chromatographic method was optimized by changing the composition of the mobile phase and pH of the mobile phase. Mobile phase was selected in terms of its components and proportions.

From the development studies, finally a mobile phase consisting of mixture of 50mM ammonium acetate in water and acetonitrile in the ration of 60:40 (v/v) at pH 5.5 was adopted, which produces good resolution and reasonable retention and acceptable for both drugs and internal standard methyl paraben and the chromatographic analysis was less than 10 min. A typical chromatogram for a tablet sample is shown in Figure 3. The retention time is 3.75 for hydrochlorothiazide, 6.17 for methylparaben, and 8.23 for telmisartan, respectively. The run time is less than 10 min.

## Validation of the Method

### Specificity

The specificity of the method was checked by a peak purity test of the sample preparation performed by a photodiode array detector. The peak purity for the peaks of telmisartan, hydrochlorothiazide, and internal standard (methylparaben) was observed to be 999, and 998 at wavelength 260 nm, which shows that the peaks of analyte were pure and also that formulation excipients were not interfering with the analyte peaks.

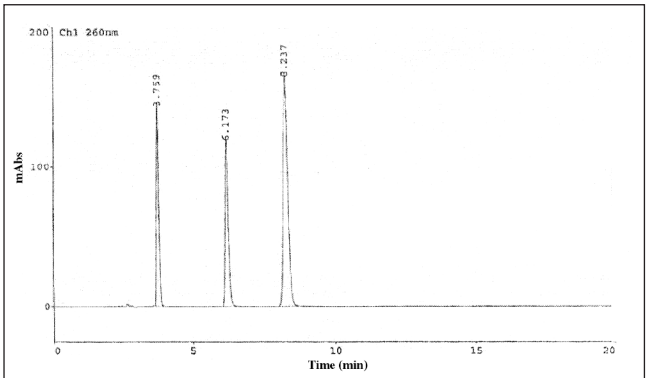


**Figure 3.** A typical chromatogram of the tablet: HCTZ (3.75), methylparaben (6.17), and telmisartan (8.23).

### Calibration and linearity

An internal standard method was used for quantitative determinations. Linearity of the method was tested from 10% to 150% of the targeted level of the assay concentration (telmisartan: 100 µg/mL and hydrochlorothiazide: 50 µg/mL) for both the analytes. Mixed standard solutions containing 10–150 µg/mL of telmisartan, 5–75 µg/mL of hydrochlorothiazide, and 20 µg/mL of methylparaben in each linearity level were prepared. Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The equations of the calibration curves for telmisartan and hydrochlorothiazide obtained were $y = 18967x - 9710.7$ and $y = 8240.1x - 386.09$, respectively. In the simultaneous determination, the calibration graphs were found to be linear in the aforementioned concentrations (the slopes and correlation coefficients are shown in Table I).

### Precision (repeatability)

The precision of the method was studied by determining the concentrations of each drug in the tablets six times. The results of the precision study (Table I) indicate that the method is reliable (RSD% < 2).

### Accuracy (recovery test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 80%, 100%, and 120% of the label claim of the tablet (40 mg of telmisartan and 12.5 mg of hydrochlorothiazide). Placebo equivalent to one tablet was transferred into a 200-mL volumetric flask, and the amounts of telmisartan and of hydrochlorothiazide at 80%, 100%, and 120% of the label claim of the tablet were added to it. The recovery samples were prepared as per the procedure mentioned, and then 5 mL of each of the solutions were transferred into a 50-mL volumetric flask; 5 mL of internal standard solution was added to it and diluted to volume with diluent B. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for telmisartan and hydrochlorothiazide ranged from 100.96% to 101.63% and 99.31–100.42%, respectively (Table II). The average recovery of

**Table I. Results of the Linearity Study and Precision ($n$ = 6)**

| Ingredient | Precision (% RSD) | Linearity (µg/mL) | Slopes* ($n$ = 3) | Coefficients of correlations |
|---|---|---|---|---|
| Telmisartan | 1.2 | 10–150 | 1896.1 (0.25) | 0.9999 |
| HCTZ† | 1.15 | 5–75 | 8240.1 (0.31) | 0.9999 |

* Standard deviation shown in parentheses.
† HCTZ = hydrochlorothiazide.

three levels (nine determinations) for telmisartan and hydrochlorothiazide were 101.04% and 99.95%, respectively.

**Intermediate precision (reproducibility)**

Intermediate precision of the method was determined by analyzing the samples six times on different days, by different chemists, by using different analytical columns of the same make and different HPLC systems. The percentage assay was calculated using calibration curves. The assay results are shown in Table III.

**Robustness**

The robustness of a method is the ability of method to remain unaffected by small changes in parameters. To determine the robustness of the method, experimental conditions were purposely altered and chromatographic resolution between telmisartan and hydrochlorothiazide were evaluated.

The flow rate of the mobile phase was 1.0 mL/min. To study the effect of flow rate on resolution of telmisartan and hydrochlorothiazide, it was changed 0.2 units from 1.0 to 1.2 mL/min. The effect of change in percent acetonitrile on resolution was studied by varying from –1 to +1% while the other mobile phase components were held constant. The effect of column temperature on resolution was studied at 20°C and 25°C instead of 30°C while other mobile phase components were held constant. The robustness results are shown in Table IV.

**Determination of limit of quantitation and detection**

For determining the limit of detection (LOD) and quantitation (LOQ), the method based on the residual standard deviation of a regression line and slope was adopted (19). To determine the LOD and LOQ, a specific calibration curve was constructed using samples containing the analytes in the range of LOD and LOQ. The LOD and LOQ for telmisartan and hydrochlorothiazide were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively. The LODs for telmisartan and hydrochlorothiazide were 0.018 and 0.022 µg/mL, and the LOQs were 0.052 and 0.068 µg/mL, respectively.

LOD and LOQ were calculated by using following equations.

$$LOD = Cd \times Syx/b$$

$$LOQ = Cq \times Syx/b$$

Where Cd/Cq is coefficient for LOD/LOQ; Syx is residual variance due to regression; b is slope.

**Solution stability**

The stability of the standard solutions and the sample solutions was tested at intervals of 24, 48, and 72 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The RSD for the assay results determined up to 75 h for telmisartan, hydrochlorothiazide, and the methylparaben internal standard were 0.55%, 0.74%, and 0.59%, respectively. The assay values were within 2% after 72 h. The results indicate that the solutions were stable for 72 h at ambient temperature.

Standard solutions were used, and the RSD of peak area ratio, column efficiency, resolution, and tailing factor of the peaks were calculated. The results are shown in Table V.

**Table II. Results of the Recovery Tests for the Drugs**

| Level of addition (%) | Ingredient | Amount added ($n$ = 3) (mg) | % Recovery* | % Average recovery† |
|---|---|---|---|---|
| 80 | Telmisartan | 32.0 | 101.55 (0.45) | |
| | HCTZ | 10.0 | 100.42 (0.37) | |
| 100 | Telmisartan | 40.0 | 100.79 (0.24) | 101.15 (0.39) |
| | HCTZ | 12.5 | 100.87 (0.55) | 100.55 (0.56) |
| 120 | Telmisartan | 48.0 | 101.32 (0.49) | |
| | HCTZ | 15.0 | 100.53 (0.31) | |

* RSD shown in parenthesis.
† Average recovery = the average of three levels, nine determinations.

**Table III. Assay Results of Active Ingredients in Tablets**

| Set | Ingredient | Label value (mg) | Found (mg)* | % Label Claim |
|---|---|---|---|---|
| Repeatability | Telmisartan | 40.0 | 40.15 | 100.375 |
| | HCTZ | 12.5 | 12.35 | 98.8 |
| Reproducibility | Telmisartan | 40.0 | 40.42 | 101.20 |
| | HCTZ | 12.5 | 12.47 | 99.76 |

* Average of six analysis.

**Table IV. Robustness of the Method**

| Parameter | USP Resolution between Hydrochlorothiazide and Telmisartan |
|---|---|
| *Flow rate (mL/min)* | |
| 0.8 | 19.2 |
| 1.0 | 17.5 |
| 1.2 | 15.2 |
| *Column temperature (°C)* | |
| 20 | 19.1 |
| 25 | 18.3 |
| 30 | 17.5 |
| *Acetonitrile percentage in mobile phase* | |
| 39 | 17.7 |
| 40 | 17.5 |
| 41 | 17.2 |

**Table V. System Suitability Parameters**

| Parameter | HCTZ | Methylparaben | Telmisartan |
|---|---|---|---|
| Theoretical plates (per column length) | 8047 | 12879 | 11319 |
| Resolution | | 12.54 | 7.83 |
| Tailing factor | 1.29 | 1.24 | 1.19 |
| % RSD | | 0.35 | 0.28 |

**Determination of active ingredients in tablets**

The contents of two drugs in tablets were determined by the proposed method using the calibration curve. The determinations were done in two sets, one for precision and the second for ruggedness, and six samples were prepared for each set. The results are shown in Table III. The chromatogram of the tablet sample is shown in Figure 3.

## Conclusion

The proposed LC method is rapid and accurate for the simultaneous determination of telmisartan and hydrochlorothiazide in the pharmaceutical-dosage form. It can be used in the quality control departments for the assay of tablets of the combined pharmaceutical-dosage forms containing telmisartan and hydrochlorothiazide.

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