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

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Subject: Analytical chemistry

Abstract
This paper deals with a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the simultaneous determination of Telmisartan and Hydrochlorothiazide in bulk and in pharmaceutical formulation. The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV- Visible dual absorbance detector (PDA), using Inertsil 250 x 4.6 mm 5-µm packing L11 column. The mobile phase consisting of buffer and mixture, acetonitrile and methanol in the ratio of (50:50) [adjust pH to 3.0 with ortho phosphoric acid] and it was flowed at 1.2 ml/min. The chromatographic detection was made at 298 nm for telmisartan and 270 nm for hydrochlorothiazide. The proposed method was validated by parameters such as suitability, specificity, linearity, accuracy and precision over a linearity range 50–150 µg/ml and stability according to the ICH guidelines (r > 0.9990). The retention times of telmisartan and hydrochlorothiazide were found to be 18.43 min and 8.11 min respectively. Hence, the method could be successfully applied for routine analysis of telmisartan and hydrochlorothiazide in pharmaceutical formulation.

Keywords*: Telmisartan, Hydrochlorothiazide, Pharmaceutical formulation, RP-HPLC, Validation.*

Introduction
Telmisartan (Fig.1) chemically[1], 4'-[(1, 4-dimethyl-2'-propyl [2, 6'-1H-benzimidazol]-1'-yl) methyl]-[1, 1'-biphenyl]-2-carboxylic acid, is a non peptide molecule under the class of angiotensin II receptor antagonist. It is used for the treatment of essential hypertension as alone or in combination with other agents [2]. Hydrochlorothiazide (Fig.2) chemically [3], 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide, is a widely used thiazide diuretic. The combination of telmisartan and hydrochlorothiazide is useful mainly in the treatment of mild to moderate hypertension [4].

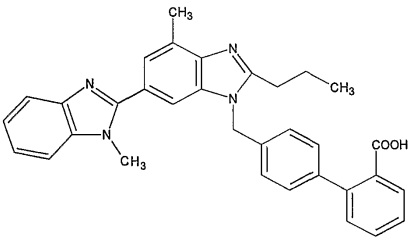

Figure 1: Structure of Telmisartan

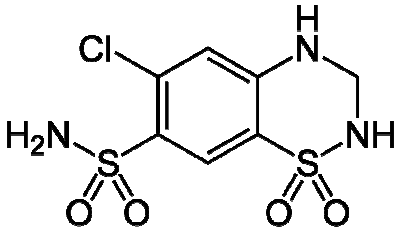

Figure 2: Structure of Hydrochlorothiazide

Literature survey revealed that few analytical methods such as HPTLC [5], LC-MS [6], spectrofluorimetry [7], capillary electrophoresis [8], spectrophotometry [9] and HPLC [10], have been reported for telmisartan as alone and in combination with hydrochlorothiazide. In this present work, an attempt was made to develop a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of Telmisartan and Hydrochlorothiazide in bulk drug and in pharmaceutical dosage forms. The proposed method was validated according to ICH guidelines.

Materials and Methods

Experimental

Chemicals and reagents

Acetonitrile of HPLC grade and methanol were purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Telmisartan and Hydrochlorothiazide were a gift sample by The Madras Pharmaceuticals, Chennai – 600 096, Tamil Nadu, India. The commercially available tablets containing Telmisartan and Hydrochlorothiazide were procured from the local market.

Instrumentation and chromatographic conditions

The chromatography was carried out by using HPLC system (Shimadzu LC2010HT) with UV-Visible dual absorbance detector (PDA), using Inertsil 250 x 4.6 mm 5-µm packing L11 column. The mobile phase consisting of acetonitrile and methanol in the ratio of (50:50) [adjust pH to 3.0 with ortho phosphoric acid] and filtered through 0.45µ membrane filter before use, degassed and were pumped into the column at a flow rate of 1.2 ml/min. The chromatographic detection was made at 298 nm for telmisartan and 270 nm for hydrochlorothiazide. The volume of injection loop was 20 µl prior to the injection of the drug solution; the column was equilibrated for at least 15 min. with the mobile phase following through the system.

Preparation of standard stock solution:

Accurately weighed about 320 mg of telmisartan WS and 100 mg of hydrochlorothiazide WS in to a clean 100 ml volumetric flask. 20 ml of standard stock solution (12.50mg of Benzothiadiazine related compound A USP in 500ml of diluents) was added. The volume was finally made up with diluents. 10 ml of above resulting solution was taken in 100 ml volumetric flask and make up the volume with a 1:1 solution of buffer and solution contains acetonitrile : methanol (1:1). (Telmisartan 0.32mg/ml, Hydrochlorothiazide 0.1mg/ml and Benzothiadiazine related compound A 0.5 µg/ml)

Sample Preparation

Twenty tablets were randomly selected and transferred into 500 ml volumetric flask. 25ml of 0.1N sodium hydroxide solution was added and shake until the tablets have completely disintegrated. Methanol (80% of the total volume of the flask) was added, Sonicated for 10 minutes and stirred vigorously for 30 minutes. Allowed to cool to room temperature, diluted with methanol to volume and mixed well. The concentration of the sample stock solution is about 1.6mg/ml of Telmisartan and 0.5 mg/ml of Hydrochlorothiazide. A portion of the solution was centrifuged at 4000 RPM. 10 ml of above solution was taken in 50 ml volumetric flask and make up the volume with 1:1 solution of buffer and solution contains acetonitrile: methanol (1:1). (320 µg/ml for Telmisartan and 100 µg/ml for Hydrochlorothiazide)

Calculation

The amount of telmisartan and hydrochlorothiazide present in each tablet formulation was calculated by using the formula:

$$\frac{\text{Spl. area}}{\text{Std. area}} \times \frac{\text{Std.dil}}{\text{Spl.dil}} \times \frac{\text{Purity}}{100} \times \frac{\text{Avg.wt}}{\text{L.C}} \times 100$$

The proposed method was validated by parameters such as suitability, specificity, linearity, accuracy and precision (repeatability & reproducibility) and stability according to the ICH guidelines.

Results and Discussion

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [11].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, Telmisartan and Hydrochlorothiazide were given separately to the system. The mean area, standard deviation and %RSD were calculated for the standard drug solutions and mentioned in Table 1and 2. It was observed that all the values are with in the limits.

Table 1: System suitability for Telmisartan

S.No.	Standard	System suitability parameters		
		Peak area response	Number of theoretical plates	Retention time (min)
1.	Standard -1	16068395	85986	18.606
2.	Standard -2	15994039	80611	18.602
3.	Standard -3	16129546	80639	18.603
4.	Standard -4	16034289	78931	18.617
5.	Standard -5	16069044	88691	18.612
			Mean	**18.608**
			Standard deviation	**0.0063**
			RSD in %	**0.03**

Table 2: System suitability for Hydrochlorothiazide

S.No.	Standard	System suitability parameters		
		Peak area response	Number of theoretical plates	Retention time (min)
1.	Standard -1	6289389	6247	8.666
2.	Standard -2	6293528	6464	8.700
3.	Standard -3	6309963	6416	8.653
4.	Standard -4	6319474	6454	8.654
5.	Standard -5	6278727	6608	8.664
			Mean	**8.667**
			Standard deviation	**0.0191**
			RSD in %	**0.22**

Specificity

The specificity of the HPLC method is illustrated in Fig. 3, where a complete separation of Telmisartan and Hydrochlorothiazide were noticed in presence of other inactive excipients used in tablets. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 3 and 4. It was observed that all the values are within the limits.

Table 3: Specificity for Telmisartan

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	16129546
4.	Sample	1	16129550

Table 4: Specificity for Hydrochlorothiazide

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	6289389
4.	Sample	1	6289392

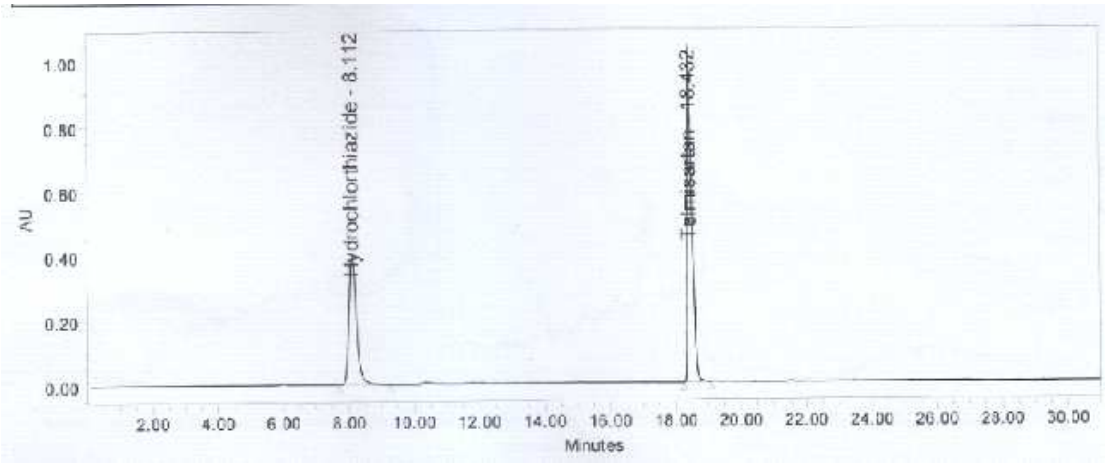

Figure 3 : Typical HPLC Chromatogram of Sample Tablets

(Telmisartan and Hydrochlorothiazide)

Linearity

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for Telmisartan and Hydrochlorothiazide and it was shown in Table 5 and 6 . The plots of peak area of each sample against respective concentrations of Telmisartan and Hydrochlorothiazide were found to be linear (Figure 4 and 5) in the range of 50%– 150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 46366x+1E+06 and Y= 61940x+57848, for Telmisartan and Hydrochlorothiazide respectively and correlation coefficient of the standard curves were found to be 0.9995 and 1.000 for Telmisartan and Hydrochlorothiazide respectively. It observed that correlation coefficient and regression analysis are within the limits.

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Table 5: Linearity of response for Telmisartan

S.No	Linearity Level	Target Level (%	Concentration (µg/ml)	Area
1.	Linearity -1	50	160	8398067
2.	Linearity -2	75	240	12167558
3.	Linearity -3	100*	320	16034230
4.	Linearity -4	125	400	19730215
5.	Linearity -5	150	480	23163318

Table 6: Linearity of response for Hydrochlorothiazide

S.No	Linearity Level	Target Level (%	Concentration (µg/ml)	Area
1.	Linearity -1	50	25	1614164
2.	Linearity -2	75	37.5	2370008
3.	Linearity -3	100*	50	3156705
4.	Linearity -4	125	62.5	3925706
5.	Linearity -5	150	75	4707534

***** Operating concentration

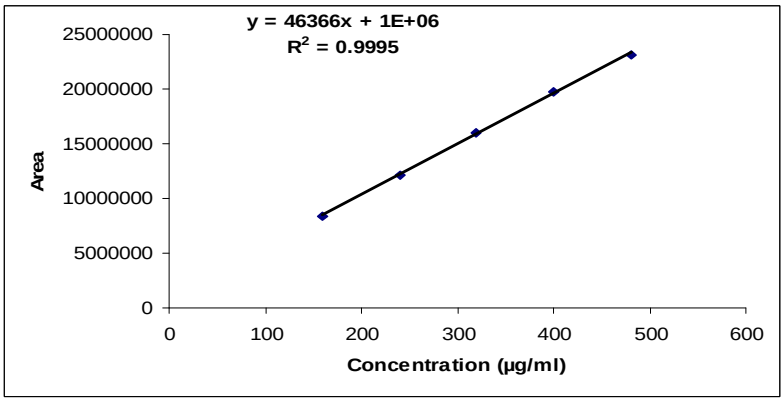

Figure 4: Linearity of response for Telmisartan

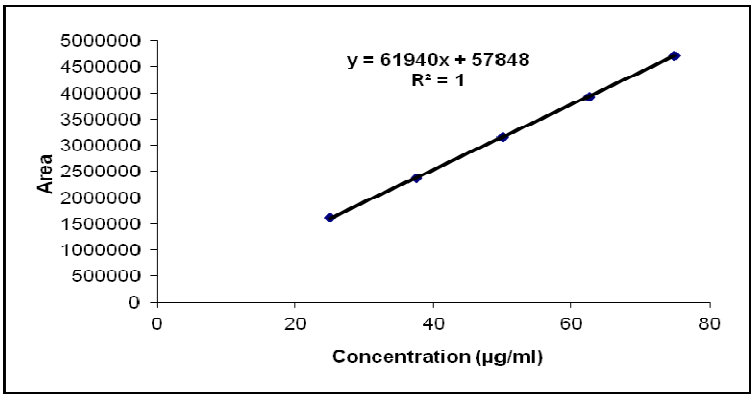

Figure 5: Linearity of response for Hydrochlorothiazide

Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, Telmisartan and Hydrochlorothiazide were added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 7 and 8. It was observed that the mean percentage recoveries were found to be for Telmisartan and Hydrochlorothiazide which demonstrated that the method was highly accurate.

Table 7: Accuracy for Telmisartan

S.No.	Target level	Area	Drug Recovery (%)
1.	80%	13694177	101.59
2.	80%	13751455	101.81
3.	80%	13729102	101.35
4.	100%	16881622	100.19
5.	100%	17236505	101.71
6.	100%	16804965	99.35
7.	120%	19734447	98.58
8.	120%	19856807	98.57
9.	120%	21008516	101.66
	Mean		**100.53**
	Standard deviation		**1.380**
	RSD %		**1.37**

Table 8: Accuracy for Hydrochlorothiazide

S.No.	Target level	Area	Drug Recovery (%)
1.	80%	4898396	99.34
2.	80%	4884222	98.75
3.	80%	4884780	99.51
4.	100%	6349232	100.87
5.	100%	6472426	100.50
6.	100%	6301825	100.51
7.	120%	7370832	99.32
8.	120%	7464171	100.68
9.	120%	7768496	101.28
	Mean		**100.08**
	Standard deviation		**0.8663**
	RSD %		**0.87**

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for Telmisartan and Hydrochlorothiazide were shown in Table 9 and 10. This indicated that method was highly precise.

Table 9: Precision - Reproducibility for Telmisartan

S.No.	Sample Name	Area	Drug Recovery (%)
1.	Sample -1	15771133	99.26
2.	Sample -2	15883503	99.96
3.	Sample -3	16017056	100.80
4.	Sample -4	15588983	100.00
5.	Sample -5	15756517	99.16
6.	Sample -6	15910440	100.13
	Mean		**99.88**
	Standard deviation		**0.6053**
	RSD %		**0.61**

Table 10: Precision - Reproducibility for Hydrochlorothiazide

S.No.	Sample Name	Area	Drug Recovery (%)
1.	Sample -1	5938671	98.58
2.	Sample -2	5927322	98.39
3.	Sample -3	5960768	98.95
4.	Sample -4	5925739	98.37
5.	Sample -5	5952645	98.81
6.	Sample -6	5910516	98.11
	Mean		**98.535**
	Standard deviation		**0.3094**
	RSD %		**0.31**

Stability

Standard and sample solutions to be used in the analytical method were scrutinized for their solution's stability. This study was performed by injecting standard and sample solution for the period of 24 hours and results were presented in the Table 11 and 12. It was found that there were no remarked changes in the system suitability parameters.

Table 11: Stability results obtained for Telmisartan standard solution

Condition	Average standa response	Response	Average Response	%Recovery	% Difference from initial
Initial	16059062	15739257 15803009	15771133	99.26	-
12th hr	16469413	16300189 16315144	16307666	99.65	0.39
24th hr	15850204	15834255 15815221	15824738	100.05	0.79

Table 12: Stability results obtained for Hydrochlorothiazide standard solution

Condition	Average standa response	Response	Average Response	%Recovery	% Difference from initial
Initial	6298216	5938578 5938765	5938671	98.58	-
12th hr	6533690	6031753 6015389	6023571	98.96	0.38
24th hr	6271969	5862660 5860338	5861499	98.08	0.50

Conclusion

The Proposed study describes a simple, feasible and sensitive reverse-phase high-performance liquid chromatographic method for the quantitative determination of Telmisartan and Hydrochlorothiazide in bulk and in pharmaceutical formulations. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore the proposed method can be successfully used for the routine analysis of Telmisartan and Hydrochlorothiazide in solid dosage form without interference.

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