Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage Form

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Abstract:

A simple, accurate, precise and rapid RP-HPLC method has been developed and validated for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk and fixed-dosage formulation. The separation was achieved on ACE 5 C18 (Length 150 mm × Diameter 4.6 mm Particle size 5 µm) column with gradient flow. The mobile phase at a flow rate of 1.5 mL/min consisted of Water: Acetonitrile: Orthophospharic acid (95:5:1 Mobile Phase A) and Water: Acetonitrile: Orthophospharic acid (5:95:1 Mobile Phase B) (Gradient ratio). The UV detection was carried out at 280 nm. The retention time of Hydrochlorothiazide and Telmisartan was found to be 4.19 and 9.12 min. respectively. The method has been validated for Specificity, Linearity, Accuracy, Precision and Robustness. The calibration curve for Telmisartan and Hydrochlorothiazide were linear from the range of 160.1-480.4 µg/mL and 25.2 - 75.7 µg/mL respectively. The mean recoveries obtained for Telmisartan and Hydrochlorothiazide were 100.1% and 99.8% respectively. The developed method was found to be Specific, accurate, Precise, Robust and rapid for the simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk Pharmaceutical Dosage Form.

Keywords:

Telmisartan, Hydrochlorothiazide, RP-HPLC, Simultaneous estimation, Method development and Validation.

INTRODUCTION:

Telmisartan (TEL) is an Angiotensin II receptor antagonist used an Antihypertensive drug [1-7]. chemically it is 4′-[[4-methyl-6-(1-methyl-2- benzimidazolyl)-2-propyl-1-benzimidazolyl]methyl]-2-biphenylcarboxylic acid (Figure.1). It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and U.S. Pharmacopoeia (USP). It is estimated by Liquid Chromatography as per IP and Potentiometric titration as per BP and USP [5- 7]. Literature review reveals that HPLC [8-12], UV [13-15] spectrophotometric and HPTLC [16-22] methods has been reported for estimation of TEL in pharmaceutical dosage forms.

Hydrochlorothiazide (HCTZ) belongs to Thiazide class of diuretics, acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. This increases the osmolarity in the lumen, causing less water to be reabsorbed by the collecting ducts. This leads to increase urinary output. It is chemically 6-chloro-1, 1-dichloro-3, 4,dihydro -2H-1, 2, 4-benzoliadiazine-7-sulphanomide1, 1-dioxide (Figure. 2).

Literature survey revealed HPLC [23], LC-MS [24], spectroflurimetric and simultaneous UVspectrophotometric methods are reported for the estimation of hydrochlorothiazide [25-29] alone or in combination with other anti-hypertensive agents. So the present study aim to develop a simple, selective and precise RP-HPLC method was developed for the simultaneous estimation of Telmisartan and hydrochlorothiazide in bulk drug and in combined dosage form.

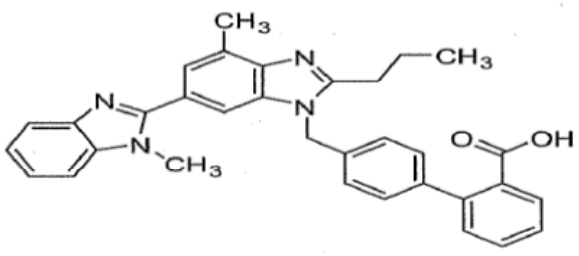

Figure. 1: Structure of Telmisartan (TEL)

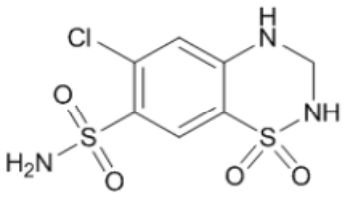

Figure. 2: Structure of Hydrochlorothiazide (HCTZ)

MATERIALS AND METHODS:

Chemicals and Reagents:

Drug substances, working standards of Telmisartan, Hydrochlorothiazide and Telmisartan and Hydrochlorothiazide Tablets kindly sponsored by Aurobindo pharma limited, Hyderabad, India. All the chemicals and reagents sodium hydroxide, hydrochloric acid, hydrogen peroxide (30 %) and Orthophosphoric acid (88%) were used of Analytical grade. HPLC grade Methanol, Acetonitrile (Merck) was used. Milli-Q water was used in mobile phase and diluents preparation.

Instruments:

Integrated HPLC system, waters 2695 separations module with 2998 PDA detector and Empower 3 Software was used for method development and method validation. This system comprised of a quaternary gradient pump, auto sampler, column oven and a photodiode array detector. PC installed Empower 3 Software was used to record and integrates the chromatograms. The analysis was carried out at ambient temperature.

Method Development and Chromatographic Conditions:

A variety of mobile phases were investigated in the development of a stability-indicating LC method for the analysis of Telmisartan and Hydrochlorothiazide Tablets. The suitability of mobile phase was decided on the basis of selectivity and sensitivity of the assay, stability studies and separation among impurities formed during forced degradation studies.

Chromatographic separations were achieved by using ACE 5 C-18, 150mm x 4.6mm, 5µ analytical column. The mobile phase is consisting of Water: Acetonitrile: Orthophospharic acid (95:5:1 Mobile Phase A) and Water: Acetonitrile: Orthophospharic acid (5:95:1 Mobile Phase B) with the gradient as mentioned in **Table 1**. A membrane filter of 0.45 µm porosity was used to filter and degas the mobile phase. The flow rate was maintained at 1.5 mL/min with injection volume of 10µl. The UV detection was made at 280 nm and all analysis were done at column temperature (25 ± 2°C). For complete extraction of actives from formulations, trials were taken and 0.005M methanolic solution of sodium hydroxide was finalized as diluent.

Table 1: Mobile Phase Gradient Programme for Chromatographic Method

Time (min)	Mobile Phase A	Mobile Phase B
0.01	95	5
8.0	65	35
12.0	50	50
14.0	5	95
14.1	95	5
20.0	95	5

Preparation of Solutions:

Preparation of Standard Solution:

Accurately 80mg of Telmisartan, 50 mg of Hydrochlorothiazide standards were weighed and taken in 50 mL and 100 mL volumetric flask respectively. Dissolved by sonication in sufficient quantity of diluent and then diluted up to the mark. Further 10 mL of the above Telmisartan standard stock and 5mL of Hydrochlorothiazide Standard stock solutions were taken in 50 mL volumetric flask and made up to mark with diluent to get a concentration of 320µg/mL and 50µg/mL for Telmisartan and Hydrochlorothiazide respectively.

Preparation of sample solution:

Weigh and finely powder not less than 10 tablets in a suitable mortar and pestle. Accurately weigh and transfer tablets powder equivalent to one tablet (equivalent 12.5 mg of Hydrochlorothiazide and 80mg of Telmisartan) into a 100 mL clean, dry volumetric flask, add about 70 mL of diluent and sonicate for about 20 minutes with intermittent shaking at room temperature. Allow the solution to cool to room temperature and

dilute the volume with diluent and mix. Centrifuge a portion of the solution at 5000 rpm for about 10 minutes to get a clear solution. Then transfer 10 mL of clear, supernatant solution into a 25 mL volumetric flask, dilute to the volume with diluent and mix. Filter the solution through a suitable 0.45 µ membrane filter.

Standard and sample solutions were injected five and two times respectively to get the chromatograms. Responses obtained were calculated with other important variables taken into consideration.

Analytical Method Validation:

The optimized chromatographic conditions were validated for assay of Telmisartan and Hydrochlorothiazide in Telmisartan and Hydrochlorothiazide Tablets by evaluating specificity, linearity, precision, accuracy, robustness and system suitability parameters in accordance with the ICH guideline Q2 (R1).

Specificity:

Specificity-Blank and Placebo interference:

To establish the interference of placebo, study was conducted. Assay was performed on placebo in duplicate equivalent to concentration of test preparation as per proposed method.

Linearity:

Linearity was studied by plotting a graph of concentration versus response and determining the correlation coefficient, slope and Y-intercept. A series of solutions of Telmisartan and Hydrochlorothiazide standard solutions were prepared in the concentration range of about 160.1µg/mL to 480.4µg/ mL for Telmisartan and in the concentration range of about 25.2 µg/mL to 75.7 µg/ mL for Hydrochlorothiazide.

Method Precision and Intermediate Precision:

The precision of the proposed method was evaluated by carrying out six independent assays of test samples. %RSD of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument.

Accuracy:

The recovery of Telmisartan and Hydrochlorothiazide from spiked placebo was conducted at three different spike levels i.e. 50%, 100% and 150 %. Samples were prepared by mixing placebo with Telmisartan and Hydrochlorothiazide drug substances equivalent to test concentration. Sample solutions were prepared in triplicate for each spike level and recovery (%); RSD (%) were calculated.

Solution Stability:

Standard and Sample Solutions were prepared as per proposed method and analyzed initially and at different time intervals by keeping the solutions at Room Temperature (~ 25°C) for 24 hours. % Difference between the areas obtained for Telmisartan and Hydrochlorothiazide at initial and different time interval should not be more than 2.0.

Robustness:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ±0.15 mL min−1), wavelength (altered by ±5 nm), variation in mobile phase composition (±0.2% absolute), and Column Oven temperature (±5°C), standard solution was prepared and injected into HPLC system. The system suitability parameters were evaluated for each deliberate variation.

System suitability:

System Suitability testing is an integral part of liquid chromatographic method validation performed to check and ensure on-going performance of a chromatographic system. The System Suitability was estimated by five replicate injections standard solution at 100% of test concentration. The column efficiency as determined from Telmisartan and Hydrochlorothiazide peaks is not less than 2000 USP plate count; the USP Tailing for the same peaks is not more than 2.0. RSD for corresponding peak areas of five replicate injections of the standard solution should not be more than 2.0%.

RESULTS AND DISCUSSION:

Analytical Method Validation:

The assay test method is validated for Specificity, Linearity, Precision, Accuracy (Recovery), Stability of Analytical Solution, Robustness and System Suitability and was found to be meeting the predetermined acceptance criteria.

Specificity:

Specificity-Blank and Placebo interference:

From the chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Telmisartan and Hydrochlorothiazide peaks. This indicates that the excipients used in the formulation do not interfere in estimation of Telmisartan and Hydrochlorothiazide in Telmisartan and Hydrochlorothiazide Tablets. The chromatogram of blank, placebo, standard and sample using the proposed method is shown in Figure 3, Figure 4, Figure 5 and Figure 6.

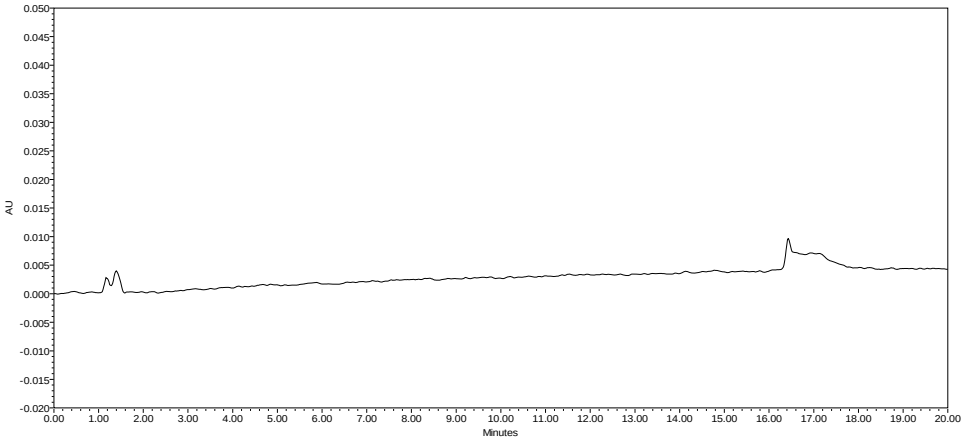

Figure 3: Diluent Chromatogram

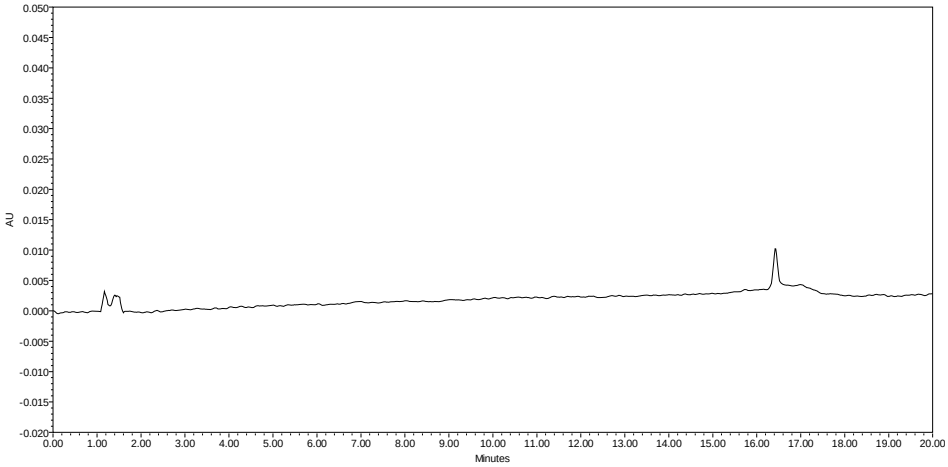

Figure 4: Placebo Chromatogram

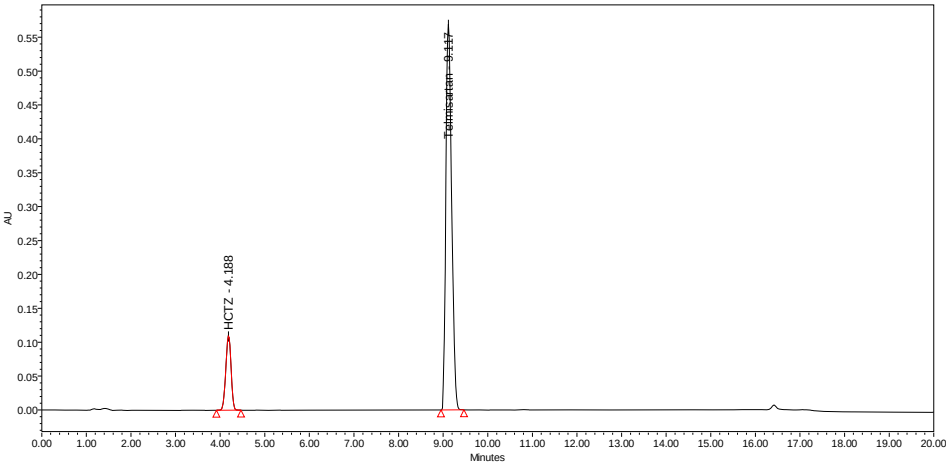

Figure 5: Standard Chromatogram

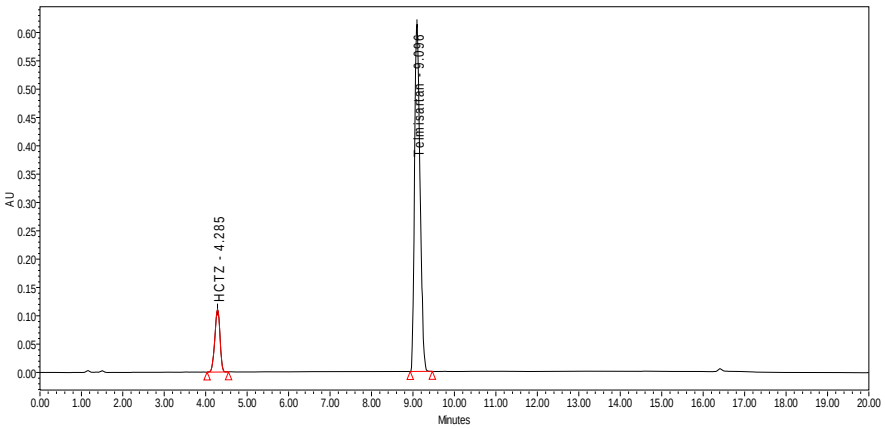

Figure 6: Sample Chromatogram

Linearity:

Calibration curve obtained by the least square regression analysis between peak area and concentration showed linear relationship with a correlation coefficient of greater than 0.999 over the calibration ranges tested for both the actives. The results show an excellent correlation obtained between peak area and concentration of Telmisartan and Hydrochlorothiazide. Linearity results obtained are presented in Table 2 and Linearity graph of Telmisartan and Hydrochlorothiazide and are shown in Figure 7 and Figure 8.

Table 2. Linearity Results for Telmisartan and Hydrochlorothiazide

% Level	**Telmisartan**		**Hydrochlorothiazide**	
	Concentration (µg/mL)	Response	Concentration (µg/mL)	Response
50% Linearity Solution	160.1	2673059	25.2	466811
80% Linearity Solution	256.2	4269095	40.4	744701
90% Linearity Solution	288.3	4817534	45.4	837654
100% Linearity Solution	320.3	5334516	50.5	928898
110% Linearity Solution	352.3	5891151	55.5	1025335
120% Linearity Solution	384.4	6416665	60.6	1115183
150% Linearity Solution	480.4	8059382	75.7	1396974
Statistical Analysis				
Slope	16806		18414	
Intercept	-312327		1418	
%Y-Intercept	-0.6		0.2	
Correlation Coefficient	0.9999		0.9999	

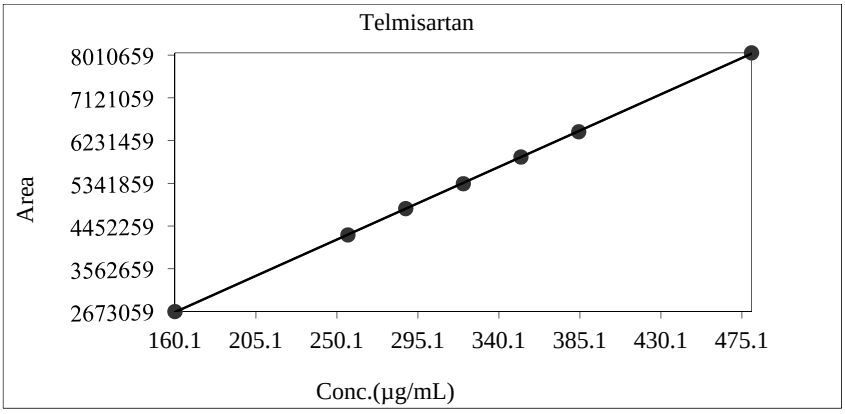

Figure 7: Linearity Graph of Telmisartan

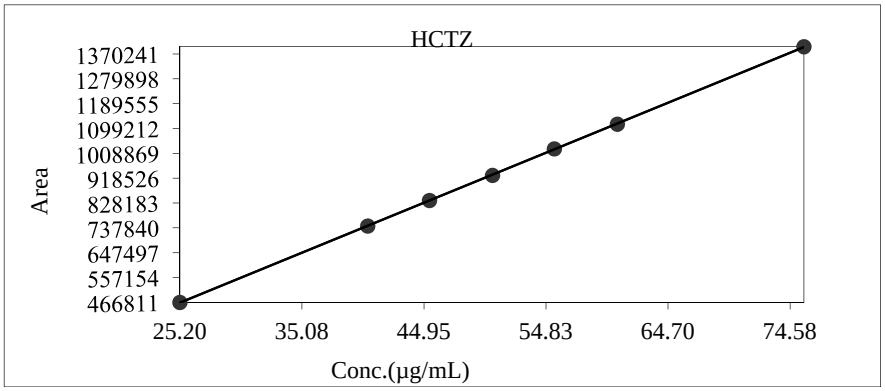

Figure 8: Linearity Graph of Hydrochlorothiazide

Method Precision and Intermediate Precision:

The average % assays of Telmisartan and Hydrochlorothiazide in tablets were found to be 100.1 and 99.8 respectively. The %RSD found to be 0.4 and 0.4 respectively. The average results between method precision and intermediate precision has also shown less than 1.0% RSD. The results were given in Table 3.

Table 3. Method Precision and Intermediate Precision Results for Telmisartan and Hydrochlorothiazide

Sr. No	**% Assay - Telmisartan**		**%Assay - Hydrochlorothiazide**	
	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
1	99.6	100.5	99.3	100.7
2	99.9	100.3	99.9	100.5
3	100.2	99.9	99.5	100.2
4	100.5	100.5	99.9	99.9
5	100.7	99.8	100.1	100.2
6	99.9	99.9	100.2	99.9
Statistical Analysis				
Mean	100.1	100.2	99.8	100.2
SD	0.41	0.32	0.35	0.32
%RSD	0.4	0.3	0.4	0.3
95% Confidence Interval (±)	0.4	0.3	0.4	0.3
Overall Statistical Analysis for Method Precision and Intermediate Precision				
Mean	100.1		100.0	
SD	0.35		0.39	
%RSD	0.3		0.4	
95% Confidence Interval (±)	0.2		0.2	

Accuracy:

Accuracy was assessed at three different levels including 50%, 100% and 150% of the test concentration level for both components. The observed recovery results were found in the range between 98 to 102%. The recovery results indicated that the test method has an acceptable level of accuracy for the assay of Telmisartan and Hydrochlorothiazide in Telmisartan and Hydrochlorothiazide Tablets from 50% to 150% test concentration. The results were given in Table 4.

Table 4: Recovery on Synthetic Mixture of Both Drug Substances

Concentration / Sample ID	**Telmisartan**			**Hydrochlorothiazide**		
	Amount Added (mg)	Amount Found (mg)	% Recovery	Amount Added (mg)	Amount Found (mg)	% Recovery
50% Level Sample-1	40.52	40.43	100.2	6.28	6.25	100.5
50% Level Sample-2	40.75	40.62	100.3	6.26	6.26	100.0
50% Level Sample-3	40.82	40.55	100.7	6.25	6.25	100.0
100% Level Sample-1	80.12	80.33	99.7	12.66	12.65	100.1
100% Level Sample-2	80.53	80.56	100.0	12.35	12.39	99.7
100% Level Sample-3	80.66	80.43	100.3	12.56	12.65	99.3
150% Level Sample-1	120.42	120.63	99.8	18.35	18.56	98.9
150% Level Sample-2	120.62	120.35	100.2	18.25	18.35	99.5
150% Level Sample-3	120.83	120.75	100.1	18.66	18.65	100.1
Overall Mean			100.1	Overall Mean		99.8
Overall SD			0.28	Overall SD		0.49
Overall %RSD			0.3	Overall %RSD		0.5
95% Confidence Interval (±)			0.2	95% Confidence Interval (±)		0.4

Solution Stability:

No significant changes are observed in the area of Telmisartan and Hydrochlorothiazide during solution stability experiment. From the results it can be concluded that the Standard and Sample Solutions are stable upto 24 hours at room temperature (~ 25°C). The results were given in Table 5.

Table 5: Results for Solution Stability

Time	**Standard Solution**				**Sample Solution**			
	Telmisartan		**Hydrochlorothiazide**		**Telmisartan**		**Hydrochlorothiazide**	
	Area	% Difference	Area	% Difference	Area	% Difference	Area	% Difference
Initial	5205209	-	904525	-	5105363	-	892536	-
After 1 hour	5212533	0.1	905636	0.1	5094263	0.2	891253	0.1
After 5 hours	5242363	0.7	908526	0.4	5093656	0.2	896355	0.4
After 10 hours	5205693	0.0	910563	0.7	5125366	0.4	893625	0.1
After 15 hours	5201253	0.1	908563	0.4	5142563	0.7	900036	0.8
After 20 hours	5236363	0.6	902536	0.2	5098563	0.1	899563	0.8
After 24 hours	5245636	0.8	908456	0.4	5125633	0.4	900053	0.8

Robustness

Based on the obtained results from the method robustness, in all the cases, the %RSD obtained was less than 1.0. From the above study the proposed method was found to be robust. The results were given in Table 6.

Table 6: Results for Robustness

Parameter	Variation	Telmisartan			Hydrochlorothiazide		
		USP Plate Count	USP Tailing	%RSD	USP Plate Count	USP Tailing	%RSD
STP	-	22349	1.3	0.2	5722	1.0	0.3
Flow Rate	-10%	18236	1.5	0.6	4685	1.1	0.3
	+10%	23563	1.2	0.7	5812	1.2	0.4
Wavelength	-5 nm	22456	1.3	0.4	5623	1.0	0.5
	+5 nm	22356	1.3	0.6	5714	1.0	0.3
Organic in Mobile Phase	-2% absolute	16258	1.4	0.9	4825	1.2	0.3
	+2% absolute	23456	1.2	0.4	5625	1.0	0.5
Column Oven Temperature	-5°C	19563	1.6	0.5	5625	1.0	0.6
	+5°C	24563	1.4	0.6	5914	1.3	0.8

System suitability:

The results of System Suitability test, USP Plate Count, USP Tailing and %RSD were found within the acceptable range indicating that the system was suitable for the intended analysis. The results were given in Table 7.

Table 7: Results for System Suitability

Injection ID	Area	
	Telmisartan	Hydrochlorothiazide
1	5105209	901138
2	5107373	904105
3	5128396	901080
4	5106897	901235
5	5097931	898394
Statistical Analysis		
Mean	5109221	902833
SD	11391	2468
%RSD	0.2	0.3
USP Plate Count	22349	5722
USP Tailing	1.3	1.0

CONCLUSION

The RP-HPLC method was developed and validated for simultaneous estimation of Telmisartan and Hydrochlorothiazide in bulk and tablets dosage forms. The method was found to be simple, specific, Precise and Robust and can be applied for the routine and stability analysis for commercially available formulation.

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