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RESEARCH ARTICLE

VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF TELMISARTAN AND HYDROCHLOROTHIAZIDE CONTENT IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, accurate, precise and rapid stability indicating reverse phase High performance chromatography method was used for estimation of Telmisartan and Hydrochlorothiazide in bulk and fixed-dose combination solid oral dosage form. The proposed analytical method has been validated for specificity, Linearity, Accuracy, Precision and Robustness. The chromatography was achieved in a GL science, Inertsil C8 (Length 125x Diameter 4.0mm Particle size 5µm) column with gradient flow. The optimal chromatographic condition consisted of mobile phase pH 3.0 at a flow rate of 1.2mL/min, with a column temperature of 40°C, run time 14 minutes and detector wavelength of 270nm.

Introduction:-

Telmisartan and Hydrochlorothiazide, a fixed-dose combination is used to treat high blood pressure (hypertension). Telmisartan is chemically 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. Its empirical formula is $C_{33}H_{30}N_4O_2$, its molecular weight is 514.63, and its structural formula is presented in Figure 1. It is an angiotensin receptor blocker (ARB) that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action, and has the longest half-life of any ARB.

Hydrochlorothiazide is chemically 6-chloro-1,1- dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7- sulfonamide. Its empirical formula is $C_7H_8ClN_3O_4S_2$, and its structural formula is presented in Figure 2. It belongs to the thiazide class of diuretics and acts on kidneys to reduce sodium reabsorption in the distal convoluted tubule. This increases the osmolarity in the lumen causing less water to be reabsorbed from the collecting ducts, finally increasing urinary output. It is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. [1]

Various HPLC estimations have been reported in the literature for the determination of telmisartan present in pharmaceutical dosage forms.[2,3] Only few methods were reported for the simultaneous estimation of telmisartan and hydrochlorothiazide by spectrophotometry[4,5], capillary electrophoresis[6], HPLC[7], HPTLC[8]and LC-MS[9]. Hence, in the current study we made an attempt to develop a simple, selective and precise RP-HPLC method for the simultaneous estimation of telmisartan and hydrochlorothiazide in bulk and finished dosage forms.

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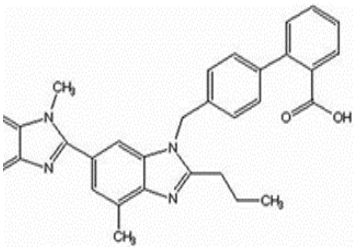


Figure. 1:- Structure of Telmisartan.

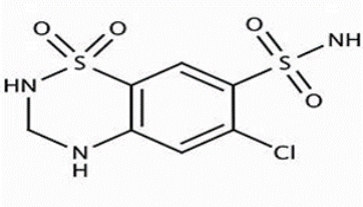


Figure. 2:- Structure of Hydrochlorothiazide.

Materials and Methods:-

Telmisartan and Hydrochlorothiazidedrug substance, working standard, and finished dosage forms were manufactured by Changzhou Pharmaceutical Factory, China. Other chemicals such as ammonium dihydrogen phosphate, sodium hydroxide, orthophosphoric acid, all reagents used were of analytical grade. Methanol (Tedia), Acetonitrile (Tedia) and Milli-Q water was used for the mobile phase and diluent preparation.

Instrument Details:

HPLC shimadzu LC20, with PDA detector and Empower 3 software was used for the purpose of method validation. This HPLC is comprised of quaternary pump. Analytical balance (Mettler Toledo) and pH Meter (Thermo).

Method development and cinematographic conditions:

Various mobile phase types 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.

Finally good separations were achieved in GL Science, Inertsil C8(125x4.0mm,5µm) analytical column. The mobile phase with a flow rate of 1.2 mL/min consisted of Mobile phase A: 2g/L ammonium dihydrogen phosphate monohydrate (Adjust the pH3.0±0.2 with phosphoric acid): Acetonitrile (85:15) and Mobile phase B: 2g/L ammonium dihydrogen phosphate monohydrate (Adjust the pH3.0±0.2 with phosphoric acid): Acetonitrile (55:45) with the gradient as mentioned in **Table 1.** The mobile phase was degassed and filtered by using 0.45µm membrane filter. The flow rate is 1.2 mL/min with an injection volume of 10µL. The analysis was performed at a column temperature of 40°C with the detection at wavelength of 270nm (Telmisartan & Hydrochlorothiazide). For complete extraction of actives from formulations, trials were taken and 0.1N sodium hydroxide was finalized as diluent-1 and

Preparation of Diluent-2:

2 g/L ammonium dihydrogen phosphate solution (Adjusted the pH to 3.0± 0.2 with orthophosphoric acid): Acetonitrile: methanol (50:25:25).

Table 1:- Mobile Phase Gradient Programme for Chromatographic Method.

Time (min)	Mobile Phase A	Mobile Phase B
0.00	100	0
1.50	100	0
1.51	0	100
8.00	0	100
8.01	100	0
14.00	100	0

Solution preparations:

Preparation of Standard Solution:

Accurately weigh about 40mg of Telmisartan, 12.5 mg of Hydrochlorothiazide standards were weighed and taken in 100 mL volumetric flask to this add 50mL of methanol, sonicate to dissolve completely, then dilute to volume with methanol and mix well. Further take 5mL of this solution into 50mL volumetric flask, then dilute to volume with diluent-2 and mix well (Concentration of Telmisartan and Hydrochlorothiazide 40µg/mL and 12.5 µg/mL respectively.

Preparation of sample solution:

Take randomly 10 tablets into 250mL volumetric flask, add 12.5mL of 0.1N sodium hydroxide solution (diluent-1) and shake for 30 minutes mechanical shaker, until the tablets completely disperse, add about 200mL of methanol, sonicate for 20 minutes with intermediate shaking at every 5 minutes of time intervals. Then make up to the volume with methanol and mix well. Centrifuge the sample solution at 5000rpm for 10 minutes and use clear supernatant solution. take 5mL of clear sample solution into 200mL of volumetric flask dilute to volume with diluent-2 and mixed well.

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). [10]

Specificity:

Specificity-Blank and Placebo interference:

To establish the interference of blank, placebo, degradation impurities, study was conducted. Assay was performed on placebo in duplicate equivalent to concentration of test preparation as per proposed method. Established the degradation studies on different conditions and reported mass balance.

Linearity:

Established the Linearity by plotting a graph of concentration versus peak response and determining the correlation coefficient, slope and Y-intercept. A series of solutions of Telmisartan and Hydrochlorothiazide, the standard solutions were prepared in the concentration range from 20.28µg/mL to 81.14 µg/mL for Telmisartan and 6.25 µg/mLto 25.7 µg/mL for Hydrochlorothiazide.

Method Precision and Intermediate Precision:

The precision study was confirmed by preparing six preparations, %RSD of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different day with different analyst and column.

Accuracy:

The (%) recovery level was confirmed 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:

Conducted the solution stability of Standard and Sample Solutions at room temperature, and refrigerator conditions, as per proposed method. % Difference between the areas obtained for Telmisartan and Hydrochlorothiazide at initial and different time interval should not be more than 2.0. So sample and standard solution was stable up to 48hrs on room temperature and refrigeration conditions.

Robustness:

The robustness studies were evaluated by deliberate changes in Chromatographic conditions. The conditions studied were flow rate (altered by ±0.10 mL/min), wavelength (Altered by ±2 nm), variation in mobile phase buffer pH (3.0±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 and also 2 injections of check standard solutions. The column efficiency as determined from Telmisartan and Hydrochlorothiazide peaks is not less than 1500 USP plate count, the USP Tailing for the same peaks are 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% and similarity factor between Standard solution and check standard solution should be (0.98 to 1.02).

Results And Discussion:-

Analytical Method Validation:
The content test method is validated for Specificity, Linearity, Precision, Accuracy (Recovery), solution stability, Robustness and System Suitability and was found to be meeting the predetermined acceptance criteria.

Specificity:
Specificity-blank and placebo interference:
Interference study:
From the chromatograms of blank, placebo, and degradation impurity solutions showed there is no inference at the retention time of Telmisartan and Hydrochlorothiazide peaks.The chromatogram of blank, placebo, standard and sample using the proposed method is shown in Figure 3, Figure 4, Figure 5 and Figure 6.

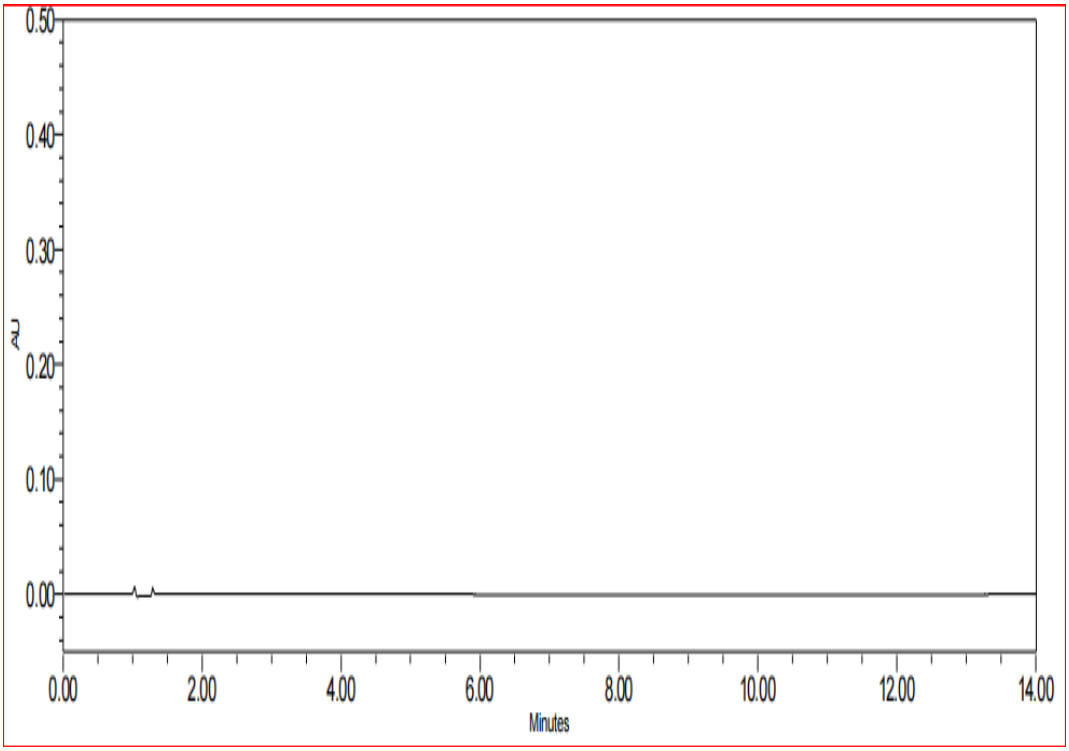


Figure 3:- (Blank solution Chromatogram).

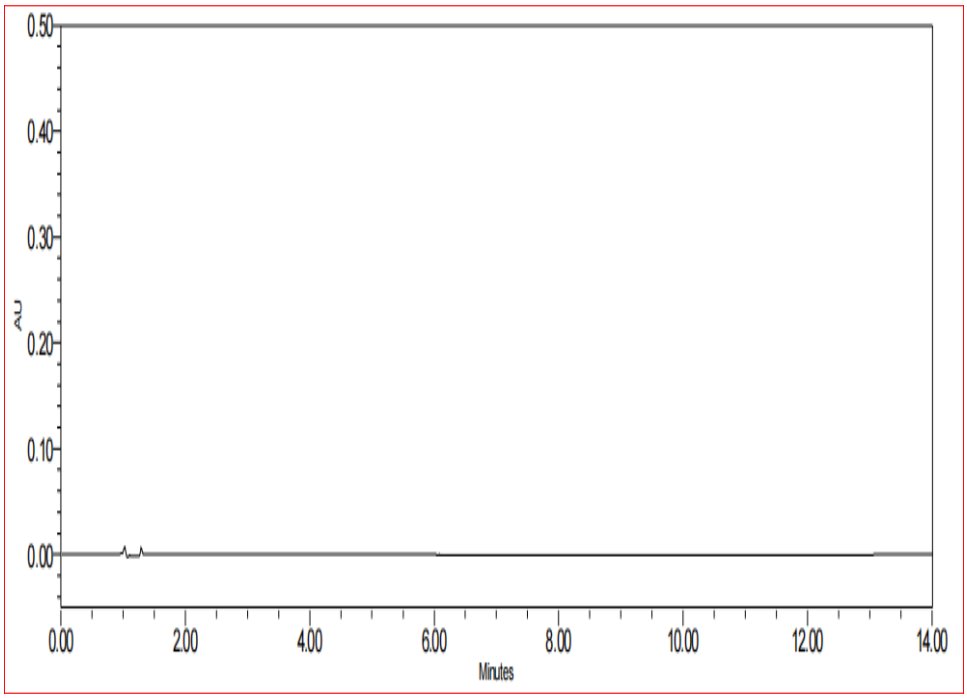


Figure 4:- (Placebo solution Chromatogram).

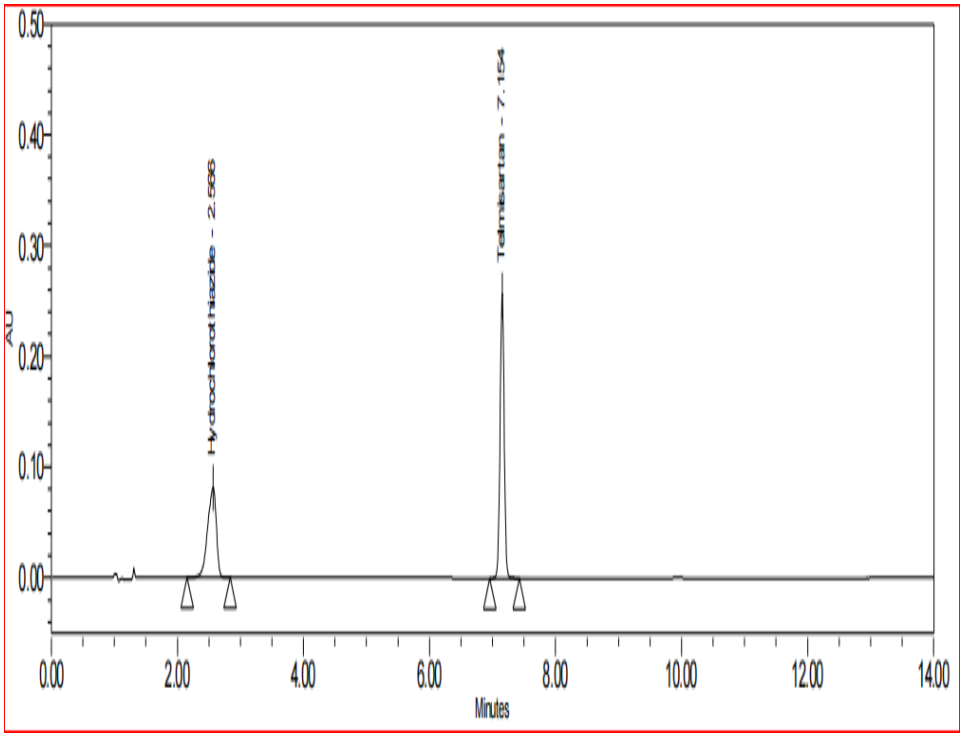


Figure 5:- (Standard solution Chromatogram).

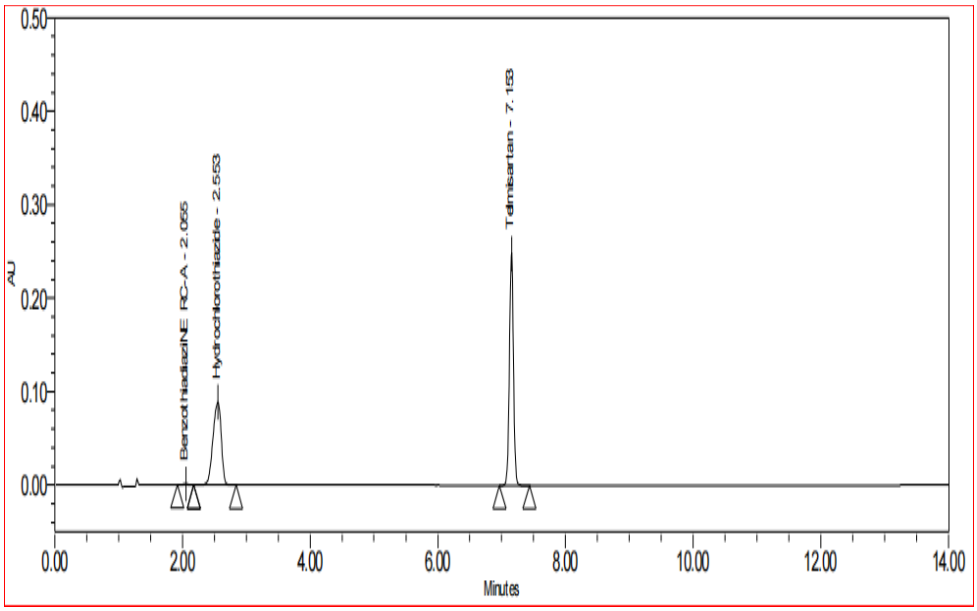


Figure 6:- (Sample solution Chromatogram).

Force degradation study:

Table 2:- Degradation results summary.

Telmisartan						
Degradation Conditions		Degradation Content (%)	Total Degradation	(%) Mass Balance	Purity Angle	Purity Threshold
Controlled Sample		97.2	0.04	97.2	0.096	0.274
Acid Degradation	1mol/L HCl-2ml-24h	96.5	0.09	99.3	0.093	0.277
Base Degradation	1mol/LNaOH-2ml-24h	97.6	0.04	100.4	0.099	0.274
Oxidation Degradation	10%H_2O_2-2ml-4h	95.7	0.08	98.5	0.092	0.275
Temperature (Solid)	60℃-14h	101.8	0.04	104.7	0.096	0.301
Temperature (Liquid)	60℃-3h	97.8	0.03	100.6	0.095	0.276
Light (Solid)	4500LUX -24h	97.0	0.04	99.8	0.100	0.275
Light (Liquid)	4500LUX -24h	97.6	0.04	100.4	0.101	0.274
Humidity	92.5%RH -24h	95.7	0.04	98.5	0.091	0.275
Hydrochlorothiazide						
Degradation Conditions		Degradation Content (%)	Total Degradation	(%) Mass Balance	Purity Angle	Purity Threshold
Controlled Sample		101.7	0.29	102.0	0.147	0.434
Acid Degradation	1mol/L HCl-2ml-24h	89.4	8.51	96.0	0.137	0.369
Base Degradation	1mol/LNaOH-2ml-24h	96.6	2.30	97.0	0.141	0.360
Oxidation Degradation	10%H_2O_2-2ml-4h	94.1	7.46	99.6	0.348	0.396
Temperature (Solid)	60℃-14h	42.0	66.03	105.9	0.392	1.124
Temperature (Liquid)	60℃-3h	101.7	2.41	102.1	0.142	0.357

Light (Solid)	4500LUX -24h	95.8	4.79	98.6	0.131	0.368
Light (Liquid)	4500LUX -24h	101.5	0.79	100.3	0.141	0.347
Humidity	92.5%RH -24h	94.5	0.33	93.0	0.136	0.365

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.Correlation obtained between peak area and concentration of Telmisartan and Hydrochlorothiazide. Linearity graph of Telmisartan and Hydrochlorothiazide and are shown in table 3 and table 4.

Table 3:- Linearity Results for Telmisartan.

Level (%)	Concentration (µg/mL)	Peak Area
50	20.2881	625644
80	32.4609	1000268
100	40.5761	1242773
120	48.6913	1504070
200	81.1522	2471810
Linear equation	y=30330.5837x+15146.5234	
Correlation Coefficient	0.9999	

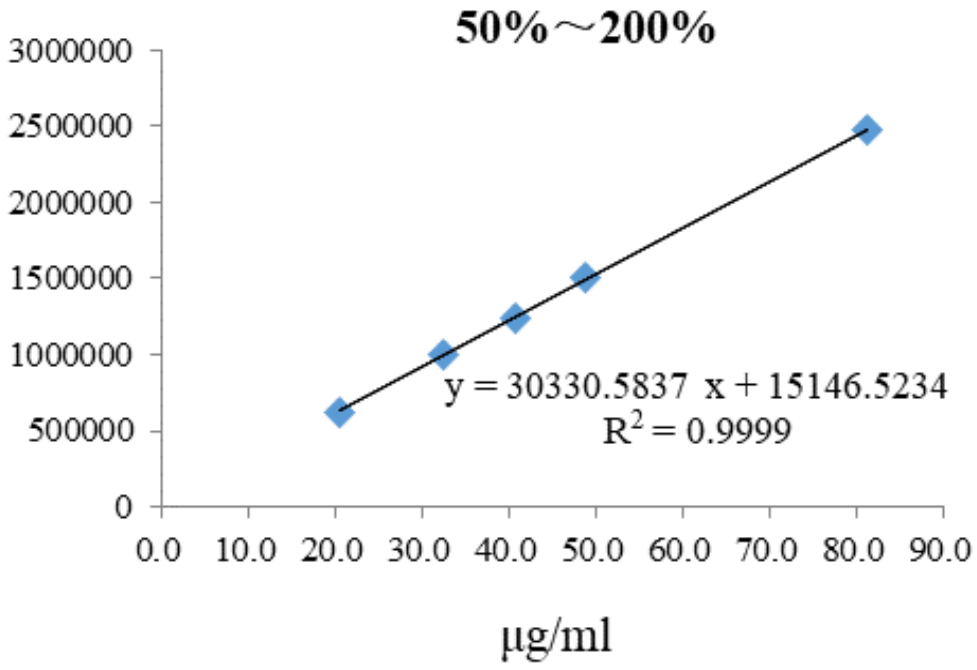
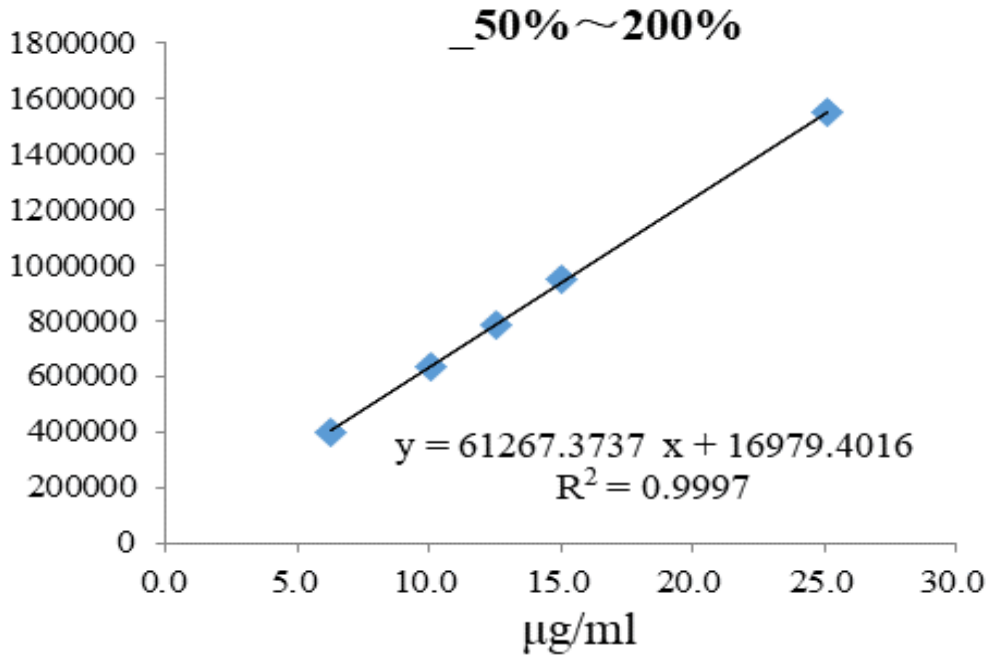


Table 4:-Linearity Results for Hydrochlorothiazide.

Level (%)	Concentration (µg/mL)	Peak Area
50	6.2635	394311
80	10.0216	631796
100	12.5271	784383
120	15.0325	948907
200	25.0541	1546747
Linear equation	y=61267.3737x+16979.4016	
Correlation Coefficient	0.9999	



Method Precision and Intermediate Precision:

The average % contents of Telmisartan and Hydrochlorothiazide in tablets were found to be 98.1 and 102.9. The %RSD found to be 0.2 and 0.2. The average results between method precision and intermediate precision have also shown less than 1.0% RSD. The results were given in Table 5.

The results were given in Table 5.

S.No.:	% Content (Telmisartan)		% Content (Hydrochlorothiazide)	
	Method Precision	Intermediate precision	Method Precision	Intermediate precision
1	98.9	97.6	103.3	102.3
2	98.3	98.1	102.6	103.7
3	98.4	97.9	102.6	103.5
4	98.5	97.5	102.9	102.1
5	98.5	97.7	102.8	102.4
6	98.4	97.7	102.7	103.3
Mean	98.5	97.8	102.8	102.9
SD	0.2098	0.2168	0.2639	0.6940
%RSD	0.2	0.2	0.3	0.7
Overall mean	98.1		102.9	
%RSD	0.4		0.5	

Accuracy:

Accuracy was conducted 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 6.

Table 6:- Recovery on Synthetic Mixture of Both Drug Substances as excipients.

Level (%)	Telmisartan		%RSD	Hydrochlorothiazide	AVG	%RSD
50%-1	101.1	101.1	0.1	99.6	99.7	0.1
50%-2	101.0			99.8		
50%-3	101.1			99.8		
100%-1	100.5	100.4	0.1	99.7	99.6	0.3

100%-2	100.3			99.9		
100%-3	100.3			99.3		
150%-1	100.0	99.8	0.2	99.7	99.5	0.2
150%-2	99.7			99.3		
150%-3	99.7			99.4		

Solution Stability:
The reference solution and the test sample solution considered, were respectively placed at room temperature and refrigerator for a period of48 hours. The results were given in Table 7 and Table 8.

Table 7:- Solution stability in reference solution.

Stability of reference solution (Telmisartan)				
Time	(~25°C)Room Temperature		（~5°C）Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	1248970	NA	1248970	NA
24h	1257174	0.7	1255911	0.6
48h	1258468	0.8	1261527	1.0
Stability of reference solution (Hydrochlorothiazide)				
Time	(~25°C) Room Temperature		（~5°C）Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	788681	NA	788681	NA
24h	793683	0.6	792423	0.5
48h	794490	0.7	796811	1.0

Table 8:- Solution stability in samplesolution.

Test Solution Stability (Telmisartan)				
Time	(~25) Room Temperature		(~5°C）Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	1217788	NA	1217788	NA
24h	1222462	0.4	1224353	0.5
48h	1225346	0.6	1224522	0.6
Test Solution Stability (Hydrochlorothiazide)				
Time	(~25°C) Room Temperature		（~5°C）Refrigerator	
	Peak Area	% Difference	Peak Area	% Difference
0h	812577	NA	812577	NA
24h	815922	0.4	817052	0.6
48h	817653	0.6	816731	0.5

Robustness:
The reference solution were injected in different conditions and there is no abnormal results, in all the conditions system suitability is good. The results were given in Table 9 and Table 10.

Table 9:- Robustness results of reference solution (Telmisartan).

Telmisartan					
Condition		Retention time	RSD% Peak Area	Theoretical Plates	Tailing Factor
Normal Condition		9.950	0.0	50126	1.1
Flow	1.1ml/min	7.974	0.0	14763	1.1
	1.3 ml/min	6.969	0.0	15336	1.2
Wavelength	268 nm	7.417	0.0	15293	1.3
	272nm	7.431	0.0	15296	1.3
Column Temperature	35 ℃	7.370	0.1	14901	1.3

	45℃	7.453	0.0	15597	1.2
Sample Temperature	15 ℃	7.421	0.0	15316	1.1
	25 ℃	7.423	0.1	15325	1.1
Organic phase % in mobile phase A	86:14	7.016	0.1	67939	1.1
	84:16	9.976	0.1	60660	1.1
Buffer preparation (pH)	2.8	6.652	0.1	73851	1.1
	3.2	7.377	0.1	55594	1.2

Table 10:- Robustness results of reference solution (Hydrochlorothiazide).

Hydrochlorothiazide					
Condition		Retention time	RSD% Peak Area	Theoretical Plates	Tailing Factor
Normal Condition		2.543	0.2	5720	1.3
Flow	1.1ml/min	2.723	0.0	2876	1.4
	1.3 ml/min	2.332	0.1	2562	1.5
Wavelength	268 nm	2.505	0.0	2592	1.2
	272nm	2.505	0.0	2578	1.2
Column Temperature	35℃	2.737	0.0	2589	1.3
	45℃	2.305	0.0	2418	1.1
Sample Temperature	15℃	2.506	0.1	2461	1.2
	25℃	2.505	0.1	2425	1.2
Organic phase % in mobile phase A	86:14	2.729	0.1	7795	1.3
	84:16	2.414	0.1	6896	1.3
Buffer preparation (pH)	2.8	2.519	0.2	7860	1.2
	3.2	2.571	0.1	7475	1.2

Conclusion:-

The Validated HPLC results shows that the of Telmisartan and Hydrochlorothiazide in bulk and tablets dosage forms. The method, specific, precise, robust, stable, and can be applied for the routine and stability analysis for commercially available formulation.

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