Advances in Bioresearch
Adv. Biores., Vol 8 (5) September 2017: 195-203
©2017 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.5.195203

Advances in Bioresearch

ORIGINAL ARTICLE

Simultaneous Estimation of Telmisartan and Hydrochloro-thiazide by RP-HPLC

P. Harshalatha*[1], K.B.Chandrasekhar[2] and M.V.Chandrasekhar[3]
[1]Lecturer in Chemistry, Government College (UG&PG), Anantapuramu, A.P., India
[2]Professor of Chemistry & Director, Oil Technological Research institute, JNTUA, Anantapuramu, A.P., India
[3]Reader in Chemistry, Government College (UG&PG), Anantapuramu, A.P., India
*Corresponding author e-mail: harsha.pankaj@yahoo.in

ABSTRACT
The aim of present study is to develop a validated high performance liquid chromatographic method for the simultaneous analysis of two anti-hypertensive drugs, Telmisartan and Hydrochlorothiazide in their tablet dosage form. Chromatographic process was carried on a Hypersil gold C18, 25× 4.6 mm, 5 µm SS column employing degassed and filtered mobile phase of buffer: methanol in their 10:90 ratio. Flow rate was maintained at 0.7ml/min and the eluent was monitored at 327nm. Developed method was validated for the various validation parameters such as precision, specificity, accuracy, linearity, robustness, ruggedness and stability. A good linearity was observed for the drugs Telmisartan and Hydrochlorothiazide over the concentration range of 48-72 µg/ml and 32-48 µg/ml respectively. Results of accuracy and specificity studies assured that there was no interference of excipients from a dosage form with the analyte. The % recoveries of Telmisartan and hydrochlorothiazide were 98.3% and 100%respectively. Robustness and ruggedness results implied that the method was not affected even with the variations in the chromatographic column and analyst respectively. Thus the developed HPLC method was found to be precise, simple, accurate and economical for the simultaneous analysis of the antihypertensive drugs in their tablet dosage forms.
Keywords:RP-HPLC, Anti hypertensive drugs, Hypertension, Telmisartan, Hydrochlorothiazide

Received 06/04/2017 Revised 05/06/2017 Accepted 29/08/2017

How to cite this article:
P. Harshalatha, K.B.Chandrasekhar and M.V.Chandrasekhar. Simultaneous Estimation of Telmisartan and Hydrochloro-thiazide by RP-HPLC. Adv. Biores., Vol 8 [5] September 2017: 195-203

INTRODUCTION:

Hypertension is a chronic medical condition where the blood pressure in arteries is raised gradually [5]. It is considered as an important cause of cardiovascular morbidity and mortality and affects one in four adults worldwide [3].Hypertension is usually age-related structural change and its occurrence is more common in older patients [6]. A relative increase in systolic and decrease in diastolic blood pressures is observed in older patients with reduced arterial elasticity. This particular condition is referred as isolated systolic hypertension and accounts a major portion of hypertensive cases [8]. To achieve a control on blood pressure, long acting antihypertensive drugs or their combinations are essential [4]. Among all other treatments available, Angiotensin II receptor blockers are considered the first line treatment for controlling and minimizing cardiovascular morbidity and mortality in hypertensive patients whereas thiazide diuretics are helpful in initiating the therapy [1].

Telmisartan is an angiotensin II receptor antagonist and is chemically referred as 41-[1,41-dimethyl-21-propyl-[2,61-bi-1H-benzimidazole]-11-yl)methyl][1,11-biphenyl]-2-carboxylic acid [2]. It acts by vasodilatingthe blood vessels, therefore the blood vessels get relaxed while at the same time blood pressure gets lowered allowing heart to pump more easily and increased supply of oxygen to heart and other different organs [11]. Telmisartan is usually prescribed in combination with hydrochlorothiazide, a thiazide derivative. Chemically hydrochlorothiazide is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-

7-sulfonamide 1, 1-dioxide. It reduces reabsorption of electrolytes from the distal convoluted tubule of kidney; therefore relatively less amount of water isreabsorbed form the collecting ducts as a result of increased osmolarity in lumen allowing more urine flow from body, lowering blood pressure [10].

As per the literature survey, various works have been reported for the analysis of telmisartan and hydrochlorothiazide individually but there are only few works that represent the simultaneous estimation of telmisartan and hydrochlorothiazide by LC-MS, HPLC, capillary electrophoresis etc. Therefore, it is desirable to develop a simple, accurate, rapid and of course an economical procedure for the simultaneous analysis of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms. Thus the current work presents an accurate, precise and reliable method for the simultaneous analysis of selected anti-hypertensive drugs in its pharmaceutical dosage form by RP-HPLC method with ultra-violet detection.

MATERIALS AND METHODS:

Materials

Telmisartan and Hydrochlorothiazide were procured from Yarrow Chem Products, Mumbai. Potassium dihydrogenorthophosphate, Methanol and Ortho Phosphoric acid were purchased from Merck. All the reagents and solvents used were of HPLC grade.

Marketed formulation:

Arbitel-H (Micro Labs Ltd) was selected for the study which contains Telmisartan- 40 mg and Hydrochlorothiazide- 12.5 mg.

Mode of separation and stationary phase selected:

Chromatographic separation was achieved by using a Shimadzu separation module and a UV-Visible detector. Reverse phase - HPLC method was preferred as mode of separation. Stationary phase used for the study was Hypersil gold C_{18}, 25× 4.6 mm, 5 µm SS column.

Selection of Detector Wavelength:

Detection wavelength selected for the study was 327nm and the same λmax was continued throughout the study.

Preparation of Mobile Phase

Potassium dihydrogen orthophosphate buffer: methanolwere mixed in a ratio of 50:50 and degassed.

Standard preparation

For the preparation of standard solution, 40mg of Telmisartan and 12.5 mg of Hydrochlorothiazide were dissolved in 20ml of mobile phase in a graduated flask. Above solution was sonicated and final volume was made using the same mobile phase. From the above stock, 5ml of solution was transferred to a 25ml graduated flask and made up to the final volume using mobile phase to give a concentration of 60µg/ml and 40µg/ml of Telmisartan and Hydrochlorothiazide respectively.

Sample preparation

100mg of powdered sample (Arbitel-H), equivalent to 40mg of Telmisartan and 12.5 mg of Hydrochlorothiazide was weighed accurately and transferred to a 100ml volumetric flask. It was dissolved in 15ml of mobile phase and subjected to sonication to dissolve the drug completely. Final volume was made with the same mobile phase and the solution was filtered. From this stock, 5ml was transferred to a graduated flask and dissolved in 25ml of mobile phase. Final volume was made to give a concentration of 60µg/ml and 40µg/ml of Telmisartan and Hydrochlorothiazide respectively.

Percentage purity:

10 µl of both standard and sample solutions containing Telmisartan and Hydrochlorothiazide was injected into the HPLC system and the Peak area for both the drugs was measured and % Assay was calculated by using the assay formula. Chromatograms and data of Quantitative determination for Telmisartan and Hydrochlorothiazide are reported below Table 1,

Percentage purity of Telmisartan and Hydrochlorothiazide samples was 98.17 % w/v and 98.00 % w/v respectively.

Table 1: Quantitative Determination of Telmisartan and Hydrochlorothiazide

S.No.	Sample Name	Peak Area of standard	Average Peak Area of standard	Peak Area of sample	Average Peak Area of Sample	Amount found	% Purity
1	Telmisartan 40 mg	2236362	2263087	2107274	2110524	**39.27 mg**	**98.17 % w/v**
		2295727		2105726			
		2257174		2118572			
2.	Hydrochlorothiazide 12.5 mg	12762443	12625237	12572657	12537555	**12.25 mg**	**98.00 % w/v**
		12473645		12357352			
		12639625		12682657			

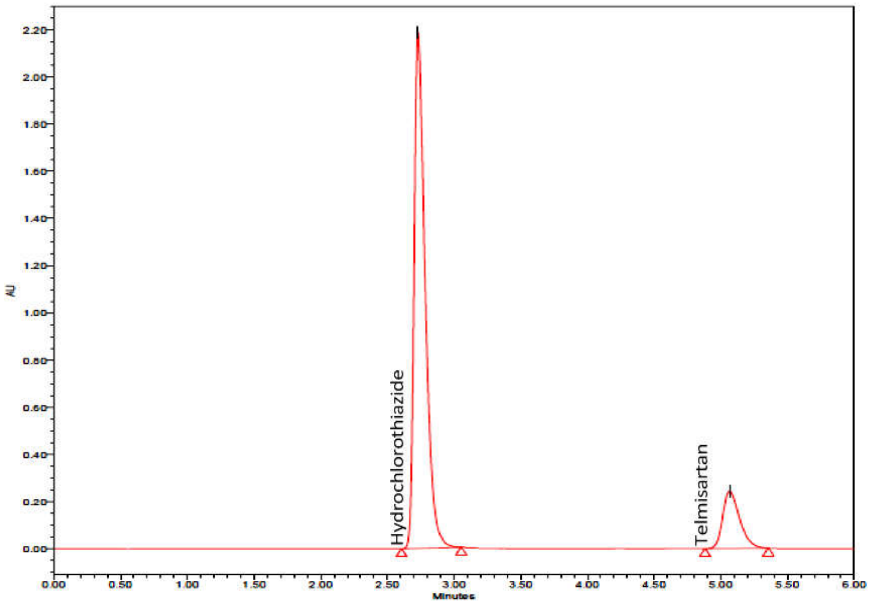

Figure 1: Chromatogram Showing Standard Solution of Telmisartan and Hydrochlorothiazide for Quantitative Determination

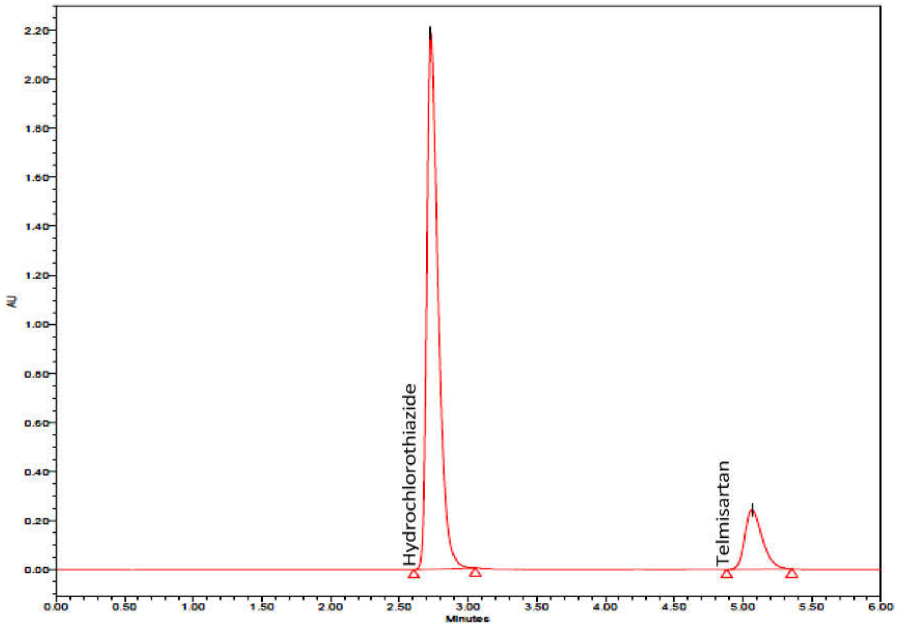

Figure 2: Chromatogram Showing Sample Solution of Telmisartan and Hydrochlorothiazide for Quantitative determination

RESULTS AND DISCUSSION

Method validation:

Proposed HPLC method was validated for the following parameters;

Specificity:
Specificity of a method is assessed to ensure that the excipients present in a pharmaceutical dosage form do not interfere with the analyte. It is studied by injecting 10 µl of Placebo and standard solutions of Telmisartan into the HPLC system and chromatograms were recorded. Chromatograms were compared and checked visually for any interference. From the results, an excellent specificity was observed with Telmisartan and Hydrochlorothiazide eluting at retention times 2.42 and 4.54respectively. Peak of analyte was found pure which indicates that the excipients did not interfere with the analyte results shown in Table 2.

Table 2: Specificity data for Hydrochlorothiazide and Telmisartan

S.No	Standard solution of Telmisartan and Hydrochlorothiazide for specificity		Sample solution of Telmisartan and Hydrochlorothiazide for specificity		Solution of placebo for specificity	
	Name of the peak	Rt in Minutes	Name of the peak	Rt in Minutes	Name of the peak	Rt in Minutes
1.	Hydrochlorothiazide	2.426	Hydrochlorothiazide	2.463	-	-
2.	Telmisartan	4.544	Telmisartan	4.532	-	-

System suitability:
System suitability test confirms whether the system is tolerable to carry on the analysis to be performed. It monitors the quality of a chromatographic column by considering the following system suitability parameters: resolution, tailing factor, theoretical plates. The test was performed by injecting 6 replicates of 10 µl of standard solution containing 60 and 40 µg/ml of Telmisartan and Hydrochlorothiazide respectively and the data obtained was tabulated below in Table 3.

Table 3: System Suitability Parameters Result of Telmisartan and Hydrochlorothiazide

Parameters	Telmisartan	Hydrochlorothiazide
Tailing factor	1.63	1.41
Resolution	6.42	
Retention time	2.436	4.53
Theoretical plates per unit	4152.2	6572.4

Precision:
Reproducibility of test method was analyzed by injecting 10µl of 6 replicates of individual standards of Telmisartan and Hydrochlorothiazide into the HPLC system and assayed for determining method precision; similarly 6 replicates of standard mixture was analyzed for system precision. % RSD for Retention time, Peak area, Peak height and assays were calculated and tabulated below in Table 4.

Table 4: System Precision data

Injections	Telmisartan			Hydrochlorothiazide		
	Rt in Minutes	Peak Area	Height	Rt in Minutes	Peak Area	Height
1	2.452	12576462	2172583	4.524	2295835	259356
2	2.486	12562650	2165835	4.584	2215837	257134
3	2.464	12658245	2152967	4.514	2228375	257825
4	2.473	12625967	2169275	4.528	2217535	258324
5	2.468	12576592	2175625	4.542	2218573	254772
6	2.452	12572655	2173658	4.525	2217375	253851
Mean	2.465	12595428	2168323	4.536	2232255	256877
S.D.	0.013	37910.72	8280.48	0.025	31471.61	2135.02
% RSD	0.528	0.300	0.381	0.553	1.409	0.831

Linearity:
Linearity of the proposed method was estimated by injecting 10µl of five standard solutions of concentrations ranging from 48-72µg/ml and 32-48 µg/ml of Telmisartan and Hydrochlorothiazide

respectively. A graph was plotted for concentration vs. peak area and the correlation coefficient was calculated. Results were tabulated in Table 5 and shown in Figure 3 and 4.

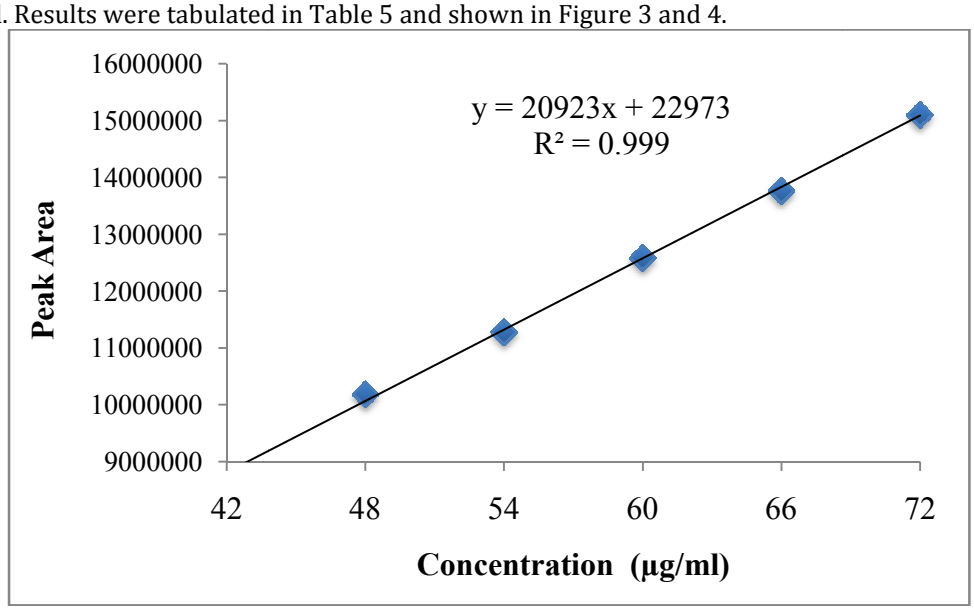

Figure 3: Linearity Curve for Telmisartan

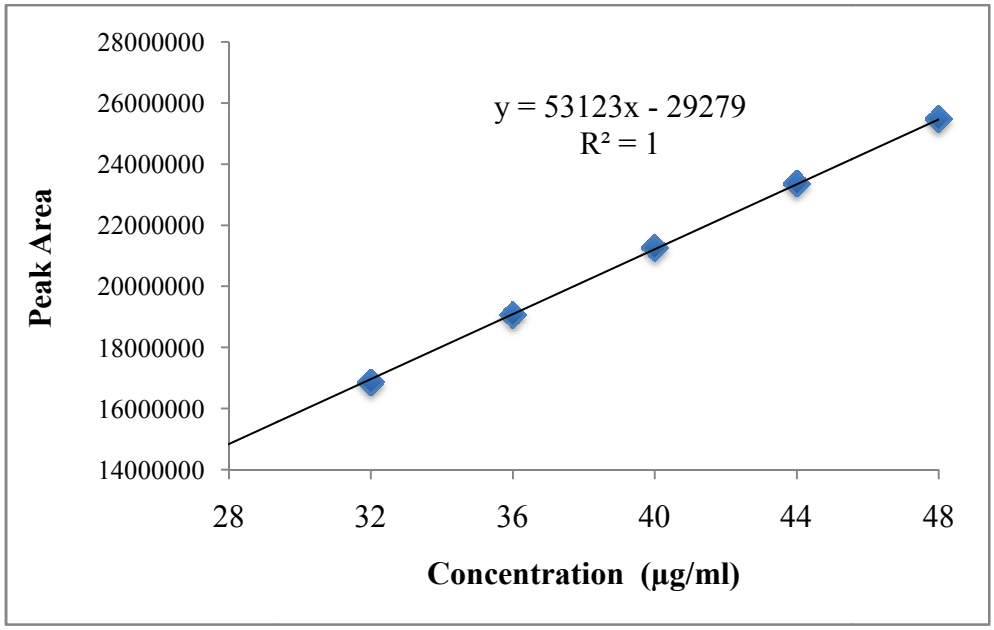

Figure 4: Linearity Curve for Hydrochlorothiazide

Table 5: Linearity and Range Results for Hydrochlorothiazide and Telmisartan

Parameters	Telmisartan	Hydrochlorothiazide
Linear Dynamic Range	48 - 72 µg/ml	32 - 48 µg/ml
CorrelationCo-efficient	0.99985	0.99996
Slope (m)	209235	531239

Accuracy:

Accuracy of a developed method can be studied by calculating the drug recoveries by standard addition method. This gives a measure of positive or negative interferences of excipients from a formulation. For this purpose, 10 µl of placebo and standard solutions of Accuracy - 50 %, Accuracy - 100 % and Accuracy - 150 % solutions of Telmisaratan and Hydrochlorothiazide were injected into HPLC and their individual recovery and mean recovery values were calculated and shown in Table 6 and 7.

Table 6: Data of % Recovery Studies forTelmisartan.

S.No	Spiked level	Sample area	µg/ml added	µg/ml found	% recovery	% mean recovery
1.	50 %	6284752	30	29.51	98.36	98.27
		6273652	30	29.65	98.83	
		6185726	30	29.29	97.63	
2.	100 %	12324573	60	59.73	99.55	99.34
		12386185	60	59.83	99.71	
		12374359	60	59.26	98.76	
3.	150 %	18051337	90	89.37	99.30	99.12
		17996607	90	88.86	98.73	
		18032149	90	89.42	99.35	

Table 7: Data of % Recovery Studies forHydrochlorothiazide.

S.No	Spiked level	Sample area	µg/ml added	µg/ml found	% recovery	% mean recovery
1.	50 %	1019500	20	20.32	101.60	99.11
		1029250	20	19.62	98.10	
		1015949	20	19.53	97.65	
2.	100 %	2105403	40	40.25	100.62	100.93
		2125124	40	40.73	101.82	
		2110525	40	40.14	100.35	
3.	150 %	3144338	60	60.52	100.86	101.25
		3111832	60	61.53	102.55	
		3142378	60	60.21	100.35	

Robustness:

Robustness for a HPLC method in this present study was evaluated by analyzing the samples under a variety of conditions such as flow rate variation and detection wavelength variation. 10 µl of 6 replicates of standard solution of Telmisartan and Hydrochlorothiazide was injected under varied flow rates (0.8 ml, 1.0 and 1.2 ml) and varied detection wavelengths (210, 215 and 220nm) and their % RSD for the Retention time and Peak area were calculated and presented in the Table 8, 9, 10 and 11.

Table8: Robustness Data of Flowrate Variation for Telmisartan

S.No	Flow rate 0.8 ml/min		Flow rate 1.0 ml/min		Flow rate 1.2 ml/min	
	Rt in Mins	Peak Area	Rt in Mins	Peak Area	Rt in Mins	Peak Area
1	2.935	12693628	2.463	12693476	2.043	10762554
2	2.952	12634945	2.472	12614963	2.072	10836525
3	2.993	12689365	2.414	12582583	2.052	10696552
4	2.948	12635909	2.482	12573963	2.083	10793558
5	2.973	12672063	2.416	12568835	2.084	10683886
6	2.952	12572446	2.476	12573952	2.063	10755945
Mean	2.958	12649726	2.453	12601295.33	2.066	10754836.67
S.D	0.020	45561.306	0.030	48130.280	0.016	57730.772
% R.S.D	0.700	0.360	1.251	0.381	0.804	0.536

Table 9: Robustness Data of Flow rate Variation for Hydrochlorothiazide

S.No	Flow rate 0.8 ml/min		Flow rate 1.0 ml/min		Flow rate 1.2 ml/min	
	Rt in Mins	Peak Area	Rt in Mins	Peak Area	Rt in Mins	Peak Area
1	5.026	2582751	4.520	2285624	4.392	2075915
2	5.195	2559432	4.517	2264825	4.371	1988625
3	5.052	2594625	4.501	2296014	4.315	2074156
4	5.147	2501757	4.592	2297635	4.352	2085265
5	5.027	2553920	4.520	2207561	4.388	2065867
6	5.185	2548463	4.571	2240762	4.361	2018562
Mean	5.105	2556824.66	4.536	2265403.5	4.363	2051398.33
S.D	0.079	32306.781	0.035	35603.465	0.028	38714.307
% R.S.D	1.552	1.263	0.791	1.571	0.644	1.887

Table 10: Robustness Data of Detection Wavelength Variation for Telmisartan

S.No	Detection wavelength 210 nm		Detection wavelength 215 nm		Detection wavelength 220 nm	
	Rt in Mins	Peak Area	Rt in Mins	Peak Area	Rt in Mins	Peak Area
1	2.481	12692756	2.491	12739375	2.481	12862753
2	2.423	12856364	2.475	12878457	2.416	12856636
3	2.483	12662558	2.415	12735932	2.482	12752395
4	2.418	12648258	2.485	12834662	2.463	12947635
5	2.476	12658264	2.462	12865625	2.481	12854855
6	2.464	12644352	2.481	12866236	2.486	12759255
Mean	2.457	12693758.67	2.468	12820047.83	2.468	12838921.5
S.D	0.029	81469.236	0.027	65451.369	0.026	73195.398
% R.S.D	1.198	0.641	1.128	0.510	1.085	0.570

Table 11: Robustness Data of Detection Wavelength Variation for Hydrochlorothiazide

S.No	Detection wavelength 210 nm		Detection wavelength 215 nm		Detection wavelength 220 nm	
	Rt in Mins	Peak Area	Rt in Mins	Peak Area	Rt in Mins	Peak Area
1	4.518	1867345	4.581	2209575	4.585	2075815
2	4.584	1867525	4.575	2285518	4.525	2075155
3	4.594	1858653	4.512	2256623	4.562	2085644
4	4.574	1860653	4.516	2294566	4.572	2099645
5	4.525	1876346	4.583	2284562	4.521	2073546
6	4.583	1865307	4.573	2296643	4.582	2075415
Mean	4.563	1865971.5	4.556	2271247.833	4.557	2080870
S.D	0.032	6232.472	0.033	33427.474	0.028	10167.634
% R.S.D	0.719	0.334	0.730	1.471	0.618	0.488

Ruggedness:

Ruggedness test is usually carried between two different analysts, columns and instruments. Analyst to analyst variability was carried out in the present study to determine the ruggedness of assay method. Six replicates of 10 µl of standard solution were injected into a HPLC system and the % RSD for the Retention time and Peak area of six replicate injections were calculated for Analyst – I and Analyst – II. The results are reported in Table 12.

Table 12: Ruggedness Data for Hydrochlorothiazide

S.No	Telmisartan				Hydrochlorothiazide			
	ANALYST – I		ANALYST – II		ANALYST – I		ANALYST – II	
	RT	AREA	RT	AREA	RT	AREA	RT	AREA
1	2.485	12636502	2.491	10682552	4.582	2285895	4.512	2088155
2	2.471	12699734	2.475	10663653	4.575	2271595	4.576	2057156
3	2.461	12682565	2.471	10665925	4.515	2274561	4.563	2087561
4	2.415	12644329	2.494	10693764	4.574	2271744	4.515	2045521
5	2.456	12695832	2.415	10683884	4.579	2218763	4.535	2096345
6	2.451	12694632	2.471	10693656	4.513	2285245	4.584	2065615
MEAN	2.456	12675599	2.469	10680572.33	4.556	2267967.167	4.547	2073392.167
S.D	0.023	27960.833	0.028	13121.713	0.032	24947.484	0.031	20230.813
%RSD	0.962	0.220	1.154	0.122	0.722	1.099	0.685	0.975

LOD and LOQ:

LOD and LOQ are dependent on the S/N values of the standard injection. S/N ratio value shall be 3 and 10 for LOD and LOQ respectively. Limit of quantification was found to be 0.00008ug/ml for Telmisartan and 0.000496 ug/ml for Hydrochlorothiazide respectively. The Chromatograms are shown in figure 5 and 6.

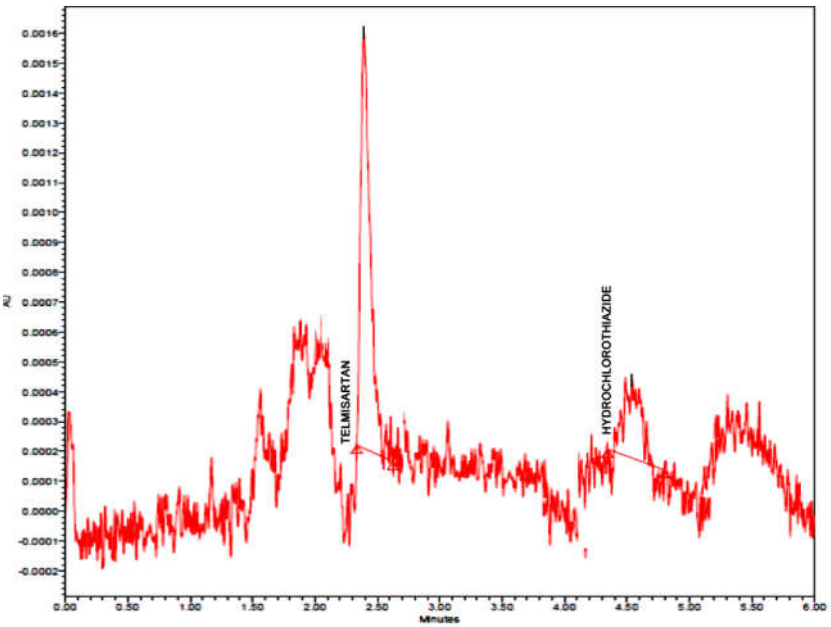

Figure 5: Chromatogram Showing Limit of Detection for Telmisartan and Hydrochlorothiazide

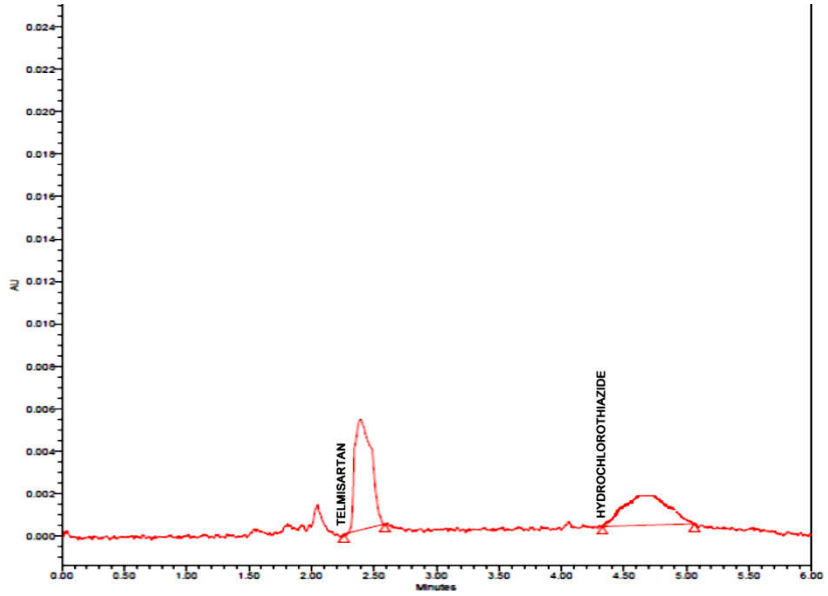

Figure 6: Chromatogram Showing Limit of Quantification for Telmisartan and Hydrochlorothiazide

Stability studies:

Stability studies are aimed at testing all the possible conditions that the drug might experience prior or during analysis. Sample solutions were subjected to acidic, basic, neutral, oxidative and photo degradation studies and peak area for the Telmisartan and Hydrochlorothiazide peaks were measured and % degradation calculated from % Assay. Results of the study have been reported in the Table 13.

Table 13: Results of Degradation Studies for Telmisartan and Hydrochlorothiazide

S. No.	Name	Sample weight	% Assay-Telmisartan	% Assay-Hydrochlorothiazide	% DEG-Telmisartan	% DEG-Hydrochlorothiazide
1	Acid	276	94	84	-6	-26
2	Base	276	91	96	-12	-12
3	Peroxide	276	89	93	-5	-9
4	Water	276	88	95	-7	-16
5	Light	276	92	94	-9	-15

CONCLUSION

A rapid, accurate, precise HPLC method was developed and validated for the simultaneous estimation of two anti-hypertensive drugs, Telmisartan and Hydrochlorothiazide in their tablet dosage form. An apparent resolution was obtained when the method was applied to pharmaceutical dosage from.

REFERENCES

1. Beckett NS, Peters R, Fletcher AE, Staessen JA, Liu L, Dumitrascu D, Stoyanovsky V, Antikainen RL, Nikitin Y, Anderson C, Belhani A, Forette F, Rajkumar C, Thijs L, Banya W, Bulpitt CJ, and HYVET Study Group.(2008). "Treatment of hypertension in patients 80 years of age or older." *The New England Journal of Medicine*, 1887–1898.
2. Deppe S, Boger RH, Weiss J, Benndorf RA. (2010)."Telmisartan: a review of its pharmacodynamic and pharmacokinetic properties ." *Expert Opinion on Drug Metabolism and Toxicology*, 863-871.
3. Glynn RJ, Chae CU, Guralnik JM, Taylor JO, Hennekens CH. (2000). "Pulse pressure and mortality in older people." *Archives of Internal Medicine*, : 2765–2772.
4. Gradman AH, Basile JN, Carter BL, Bakris GL. (2011). " Combination therapy in hypertension." *Journal of Clinical Hypertension*, : 146–154.
5. Kearney PM, Whelton M, Reynolds K, Whelton PK, He J.(2004). "Worldwide prevalence of hypertension: a systematic review." *Journal of Hypertension*: 11-19.
6. Lim MA, Townsend RR. (2009). "Arterial compliance in the elderly: its effect on blood pressure measurement and cardiovascular outcomes." *Clinics in Geriatric Medicine.*, 191–205.
7. LM., Ruilope. (2011). Telmisartan for the management of patients at high cardiovascular risk." *Current Medical Research and Opinion*, 1673–1682.
8. Owens P, Lyons S, O'Brien E. " Ambulatory blood pressure in the hypertensive population: patterns and prevalence of hypertensive subforms." *Journal of Hypertension*, 1998: 1735–1743.
9. Pimenta E, Oparil S. "Management of hypertension in the elderly." *Nature Reviews cardiology*, 2012: 286–296.
10. Rao AL, Swamy TG, Nagaraju K. (2011)."RP-HPLC Method for the Simultaneous Estimation of Telmisartan and Hydrochlorothiazide in Pharmaceutical Dosage Form." *International Journal of Drug Development & Research*,: 362-368.
11. Salama, Ismail. (2011). "Simultaneous HPLC–UV analysis of telmisartan and hydrochlorothiazide in human plasma." *Bulletin of Faculty of Pharmacy*,: 19–24.