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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN CILINIDIPINE AND CHLORTHALIDONE IN BULK AND THEIR COMBINED TABLET DOSAGE FORM WITH FORCED DEGRADATION STUDIES

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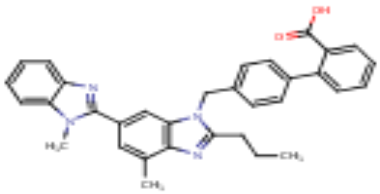
ABSTRACT

An accurate reproducible and efficient stability indicating reversed-phase high-perfomance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of Telmisartan, cilinidipine and chlorthalidone. All the drugs were separated on a ODS 150 x 4.6 mm, column packed with 5 Âµm particles. The mobile phase, optimized through an experimental design, was a 50:50 (v/v) mixture of sodium dihydrogen Ortho phosphate buffer and Acetonitrile (pH 3.0), pumped at a flow rate of 1 ml/min. UV detection was performed at 240 nm. The retention time of Telmisartan, cilinidipine and chlorthalidone was found to be 3.468min, 7.178min and 2.161min respectively. The method was validated in the sample concentration ranges of 20-120 µg/ml for Telmisartan, 5-30 µg/ml for cilinidipine and 6.25-37.5 µg/ml for chlorthalidone. The method demonstrated to be robust, resisting to small deliberate changes in pH and flow rate of the mobile phase. The LOD values were 0.02µg/ml,0.15 µg/ml and 0.04µg/ml, while the LOQ values were 0.06µg/ml, 0.45µg/ml and 0.12µg/ml for telmisartan, cilinidipine and chlorthalidone respectively.

KEYWORDS: RP-HPLC, Telmisartan Cilinidipine and Chlorthalidone, Tablet dosage form, forced degradation.

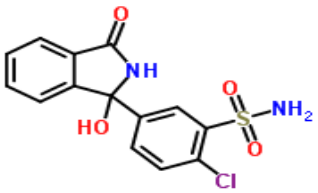
1. INTRODUCTION

Telmisartan is an angiotensin II receptor antagonist (ARB) used in the management of hypertension. Generally, angiotensin II receptor blockers (ARBs) such as telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing inhibition of the action of angiotensin II on vascular smooth muscle, ultimately leading to a reduction in arterial blood pressure and it 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. Cilinidipine is a dihydropyridine calcium channel blocker and chemically it is 3-O-(2-Methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl]2, 6-dimethyl-4-(3-nitrophenyl) -1,4-dihydropyridine-3,5-dicarboxylate. Chlorthalidone is an oral antihypertensive/diuretic. It is a monosulfamyl diuretic that differs chemically from thiazide diuretics in that a double-ring system is incorporated in its structure. It is 2-chloro-5(1-hydroxy-3-oxo-1- isoindolinyl) benzenesulfonamide and chemically it is 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzenesulfonamide. It prevents reabsorption of sodium and chloride by inhibiting the Na+/Cl- symporter in the distal convoluted tubule. Literature survey reveals High Performance Liquid Chromatographic (HPLC) for determination of Telmisartan, cilinidipine and chlorthalidone combination are not official in Pharmacopeias of USP and BP. And their determination is official as single compound in Pharmacopeias.



(A) Telmisartan

(B) Cilinidipine



(C) Chlorthalidone

Figure 1. The Chemical Structures of Telmisartan (A),Cilinidipine(B) and chlortalidone (C).

Various analytical methods have been reported for the assay of Telmisartan, cilinidipine and chlorthalidone alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV-VIS[1] spectroscopy, high performance liquid chromatography,[2-9] high performance thin layer chromatography[10] and LC - MS/ MS.

No methods are available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of Telmisartan cilinidipine and chlorthalidone in bulk and in pharmaceutical preparations, because HPLC methods have been widely used for routine quality control assessment of drugs, because of their accuracy, repeatability, selectivity, sensitivity and specificity. We have developed a simple, accurate method of Telmisartan cilinidipine and chlorthalidone in pharmaceutical dosage forms. Because analytical methods must be validated before use by the pharmaceutical industry, the proposed HPLC- UV detection method was validated in accordance with International conference on Harmonization (ICH).

2. MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of Telmisartan cilinidipine and chlorthalidone were obtained as a gift samples from Dr.Reddy's, Hyderabad used as such without further purification. A combination of telmisartan cilinidipine and chlorthalidone 40/10/12.5 mg in tablet formulations (Lntrio 10) was procured from Indian market, HPLC grade methanol, Acetonitrile, water and triethylammonium phosphate buffer (AR grade) purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

Instrumentation and Chromatographic Conditions

Analysis was performed with a Waters 2695 separation module equipped with Empower-2 software and loop of injection capacity of 80µL, and waters-PDA detector set at 226 nm. Compounds were separated on ODS column (150 × 4.6 mm i.d., 5µm particle size) under reversed phase partition conditions. The mobile phase was a Acetonitrile and phosphate buffer (pH 3.0 ±0.05, adjusted with orthophosphoric acid). The flow rate was 1 ml/min and the run time was 11 minutes. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 240 nm. Before analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45µ nylon filter. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was

performed in column temperature maintained at 30±5◦c. The UV spectrum of Telmisartan cilinidipine and chlorthalidone selecting the working wavelength of detection was taken using a shimadzu UV-1800, With UV Probe software UV-Visible spectrophotometer (shimadzu, Kyoto, Japan). All Weighing were done on Shimadzu balance (Model AY-120).

Preparation of Standard Stock Solutions

Preparation of standard stock solution – I

Weight and transfer about 40 mg of telmisartan working standard or reference standard in to a 50 ml volumetric flask, add about 20 ml of diluent and sonicate for 3 min to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of standard stock solution – II

Weight and transfer about 10mg of cilinidipine working standard or reference standard in to a 50 ml volumetric flask, add about 10 ml of Diluent and sonicate for 3 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of standard stock solution – II

Weight and transfer about 12.5 mg of chlorthalidone working standard or reference standard in to a 50 ml volumetric flask, add about 10 ml of Diluent and sonicate for 3 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of Standard solution

Pipette out 1ml of standard stock solution –I,II&III into 10 mL volumetric flask and diluted up to the volume with diluent.

Procedure for Analysis of Tablet Formulation

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 380mg was transferred into a 50ml volumetric flask, 20ml of diluent added and sonicated for 25 min, with intermittent vigorous shaking and stirr with the aid of magnetic stirrer, further the volume was made up to volume with diluent, mix and allow the sample solution to settle down. Dilute 1 ml of supernatant solution to 10 ml with diluent and mix. Filter the solution through the 0.45N nylon filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solutions were injected, chromatogram was obtained and the peak areas were recorded. The

injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curves.

Degradation Study

The drug content was employed for acidic, alkaline, and oxidant media and also for thermal and photolytic stress conditions. After the degradation treatments were completed, the stress content solutions were allowed to equilibrate to room temperature and diluted with diluent to attain 80µg/mL Telmesartan, 20µg/mL clinidipine and 25µg/mL chlorethalidon concentration 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. Specific degradation conditions were described as follows.

Acidic Degradation Condition

To 1 ml of stock ssolution Telmisartan and Cilinidipine and chlorthalidone, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.

Alkali Degradation Condition

To 1 ml of stock solution Telmisartan and Cilinidipine and chlorthalidone, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c.

Oxidative Degradation Condition

To 1 ml of stock solution of Telmisartan and Cilinidipine and chlorthalidone, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c.

Thermal Degradation Condition

The standard drug solution was placed in oven at 105 °C for 6 h to study dry heat degradation.

Photolytic Degradation Condition

The photochemical stability of the drug was also studied by exposing the 300µg/ml&10µg/ml&25µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º.

3. RESULTS AND DISCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution Telmisartan Cilinidipine and Chlorthalidone in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be a Acetonitrile and phosphate buffer (pH 3.0 ±0.05, adjusted with orthophosphoric acid). This mobile phase used gave a very satisfactory and good resolution of Telmisartan Cilinidipine and Chlorthalidone. Increasing or decreasing pH of mobile phase by ± 0.2 did not show significant change in retention time of each analyte. The retention time of Telmisartan Cilinidipine and Chlorthalidone on the analytical column was evaluated at a flow rate of 1 ml/min. The injection volume was 20 µL. The retention time of standard and sample for Telmisartan Cilinidipine and Chlorthalidone were satisfactory with good resolution. This work was focused on optimization of the conditions for the simple and rapid as well as low cost effective analysis including a selection of the proper column or mobile phase to obtain satisfactory results. Solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients. Finalized chromatographic conditions was mentioned on below Table-1.

Table 1: Finalized chromatographic conditions

Flow rate: **1 ml/min**	**Wave length**: **240 nm**	**Injection Volume**:**10µL**
Column temperature: 30˚C	**Sample temperature**: Ambient	**Run time**: 11 minutes
Mobile phase: Buffer and Acetonitrile in the ratio 50:50		
Column: ODS150 x 4.6 mm, 5µ.		

To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table-2

Table2: Results from system suitability study of Telmisartan Cilinidipine and Chlorthalidone

System Suitability Parameters	Results			Acceptance Criteria
	Telmisartan	Cilinidipine	Chlorthalidone	
Retention time	3.468	7.178	2.161	
%RSD for area of Telmisartan Cilinidipine and Chlorthalidone for five replicate injections of standard solution	0.73	0.83	0.59	NMT 2.0
Tailing factor for Telmisartan Cilinidipine and Chlorthalidone peak	1.15	1.14	1.34	NMT 2.0
Theoretical plates for Telmisartan Cilinidipine and Chlorthalidone	24815	3040	2851	NLT 2000

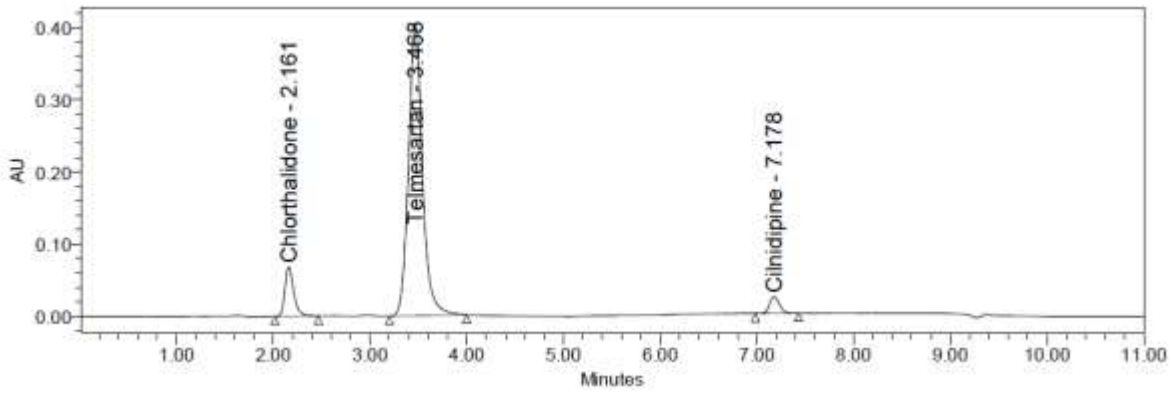


Figure 2: Optimized chromatograms for Telmisartan Cilinidipine and Chlorthalidone

4. METHOD VALIDATION

The method was validated for specificity, linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines.

Linearity

Aliquots 0.25,0.5,0.75,1.0,1.25 and 1.50 ml of stock solution of Working standard solution Telmisartan Cilinidipine and Chlorthalidone were transferred in a series of 10 mL volumetric flasks for 25,50,75,100,125 and 150% levels. Finally the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of Telmisartan Cilinidipine and Chlorthalidone were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for Telmisartan Cilinidipine and Chlorthalidone were plotted separately of response against respective concentration of Telmisartan Cilinidipine and Chlorthalidone. The slope and intercept value for calibration curve were y = 52537x + 1187.5 (R^2 = 0.9994) for telmisartan, y =7691.1x +1049.4 (R^2 = 0.9993) for Cilinidipine and y =

18568x + 654.73 (R^2 = 0.9998) for Chlorthalidone , where Y represents the peak area of analyte and X represents analyte concentration. The results are satisfactory, because there is a significant correlation between response factor and concentration of drugs within the concentration range. The calibration curves of Telmisartan Cilinidipine and Chlorthalidone are given in Figures 3,4 and 5 respectively.

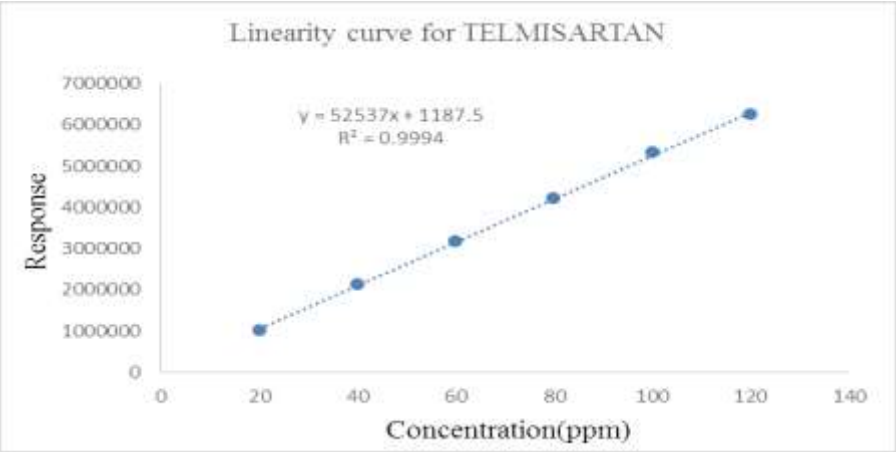


Figure 3: Linearity curve for Telmisartan

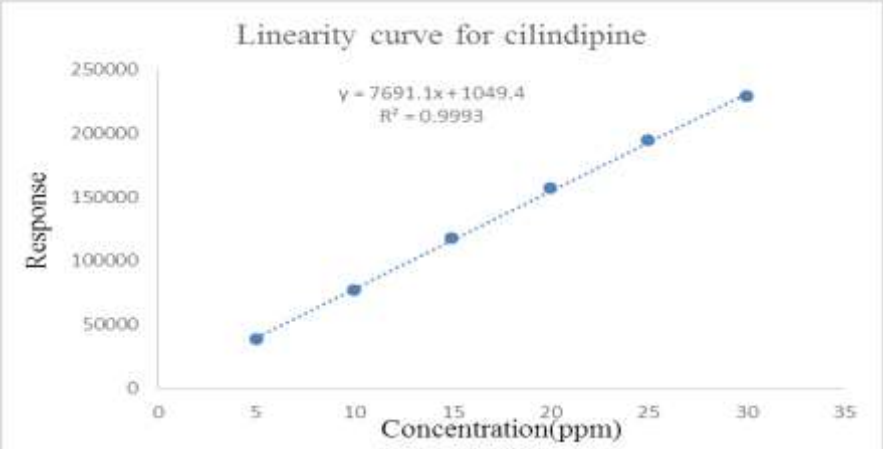


Figure 4: Linearity curve for cilinidipine

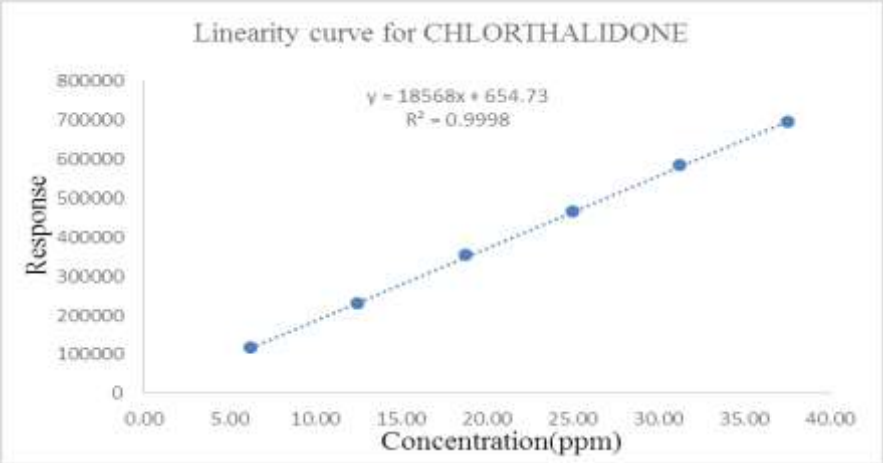


Figure 5: Linearity curve for chlorthalidone

Precision

Precision of the method was confirmed by the repeated analysis of formulation for six times. The% RSD values were found to be satisfactory. The low % RSD values indicated that drugs showed good agreement with the label claim ensures the precision of the method.

Intraday and Interday precision was determined by preparing six (n=6) replicate samples and analyzed on same day for intraday and on different days for interday precision. (Table3). The peak areas were recorded and Relative standard deviation (RSD) was calculated for both series of analyses. The %RSD of intraday precision was 0.94, 0.53 and 1.23 for Telmisartan Cilinidipine and Chlorthalidone respectively. The %RSD of interday precision was for Telmesartan Clinidipine and Chlorthalidon 1.59, 0.82 and 0.39 respectively and overall %RSD for Telmesartan Clinidipine and Chlorthalidon are 1.2,0.8 and 1.3 (Table3).

Table3: Precision studies

S. No	% Assay(n=6)					
	Telmesartan		Clinidipine		Chlorthalidon	
	Intraday precision	Interda-precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	99.01	99.32	99.06	98.07	101.4	101.56
2	101.18	98.73	100.22	99.57	99.6	102.05
3	100.70	103.24	99.98	98.14	102.07	102.26
4	100.44	100.06	100.15	99.79	99.51	102.76
5	100.19	99.61	99.23	98.28	99.28	102.18
6	98.85	99.78	99.23	98.00	99.23	102.37
Mean	100.06	100.12	99.64	98.64	100.20	102.20
%RSD	0.94	1.59	0.53	0.82	1.23	0.39
Over all % RSD (n=12)	1.2		0.8		1.3	

Tab-is 40/10/12.5 mg of Telmesartan Clinidipine and Chlorthalidon respectively.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 25%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

Table 4: Recovery studies of Telmisartan Cilinidipine and Chlorthalidon

Product name		Level of % Recovery		
		50	100	150
Telmisartan	% Mean Recovery*	101.01	100.53	100.46
	% R.S.D*	1.32	1.03	0.60
Cilinidipine	% Mean Recovery*	100.36	100.12	100.43
	% R.S.D*	0.85	0.71	1.25
Chlorthalidone	% Mean Recovery*	100.93	99.61	100.11
	% R.S.D*	0.32	1.09	0.40

**Avg. of six determinations for 25 & 150, three determinations for 100%, R.S.D. is relative standard deviation.*

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ /S and 10 σ /S respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Robustness

As defined by ICH, The robustness of an analytical procedure describes to its capability to remain unaffected by small and deliberate variations in method parameters. Robustness was performed to injected the standard and samples by small variation in the chromatographic conditions and found to be unaffected by small variations like ± 2% variation in volume of mobile phase composition with respect to acetonitrile, ± 0.2 mL/min in flow rate of mobile phase ,± 0.5 variation in pH, different type of filters and ± 5 column temperature variation. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

Specificity

Specificity was tested against standard compounds and against potential interferences. Specificity was determined by comparing the responses of standard and sample solution. No interference was detected at the retention times of both Telmisartan Cilinidipine and Chlorthalidone in sample solution.

Table 5: Summary of validation parameters of proposed RP-HPLC method

Parameters	Telmisartan	Cilinidipine	Chlorthalidone
Linearity range (µg/mL)	20-120	5-30	6.25-37.5
Correlation co-efficient	0.9994	0.9993	0.9998
LOD [a] (µg/mL)	0.02	0.15	0.04
LOQ [b] (µg/mL)	0.06	0.45	0.12
Accuracy (% Recovery)	100.46-101.01	100.12-100.43	99.61-100.93

Precision (% RSD)[c]			
Intraday (n[d]= 6)	0.94	0.53	1.23
Interday (n[d]= 6)	1.59	0.82	0.39

[a] *LOD = Limit of detection.*

[b] *LOQ =Limit of quantitation.*

[c] *RSD = Relative standard deviation.*

[d] *n = Number of determination*

The degradation study indicated that Telmisartan Cilinidipine and Chlorthalidone was susceptible to acid, base, oxidation, photo, thermal and neutral degradation. Typical chromatograms of stressed samples are shown in figs. fig. 6-11. In all degradations the drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the non-degraded drug, without giving any additional degradation peaks. Both the drugs showed no degradation at 0 h, in all the degradation conditions. In that percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under non-degradation condition. It also showed retention time of degraded products which were observed in different degradation conditions for both drugs.

Table 5. Forced Degradation Studies

Drug substance	**Sample treatment**	**% assay**	**% degradation**	**Purity Angle**	**Purity Threshold**
Telmesartan	Acid	96.17	3.83	0.064	0.296
	Base	98.29	1.71	0.074	0.294
	Peroxide	96.55	3.45	0.070	0.291
	Thermal	99.11	0.89	0.060	0.293
	UV	98.80	1.20	0.063	0.291
Cilinidipine	Acid	93.68	6.32	1.077	1.297
	Base	97.43	2.57	1.040	1.132
	Peroxide	93.57	6.43	0.980	1.111
	Thermal	96.36	3.64	1.085	1.261
	UV	97.21	2.79	0.912	1.078
Chlorthalidone	Acid	93.07	6.93	0.265	0.400
	Base	96.85	3.15	0.237	0.375
	Peroxide	95.24	4.76	0.252	0.390
	Thermal	97.38	2.62	0.269	0.401
	UV	99.45	0.55	0.244	0.377

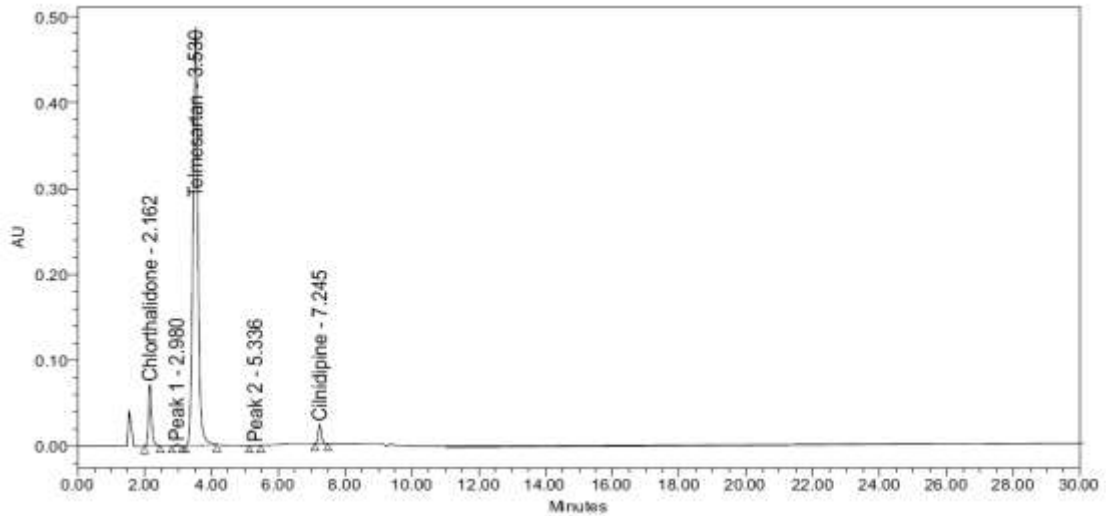


Fig. 6: Chromatograms of acid degradation study

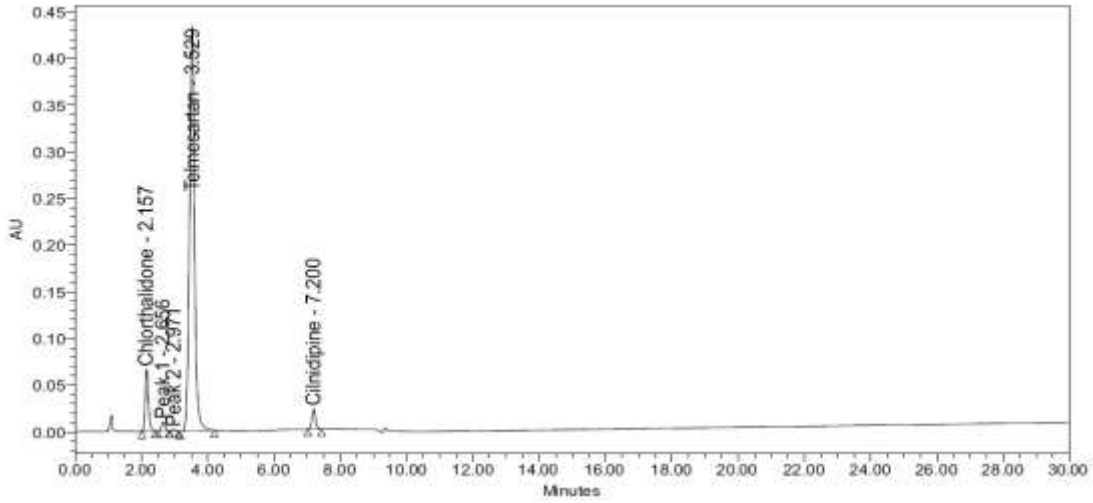


Fig.7: Chromatograms of base degradation study.

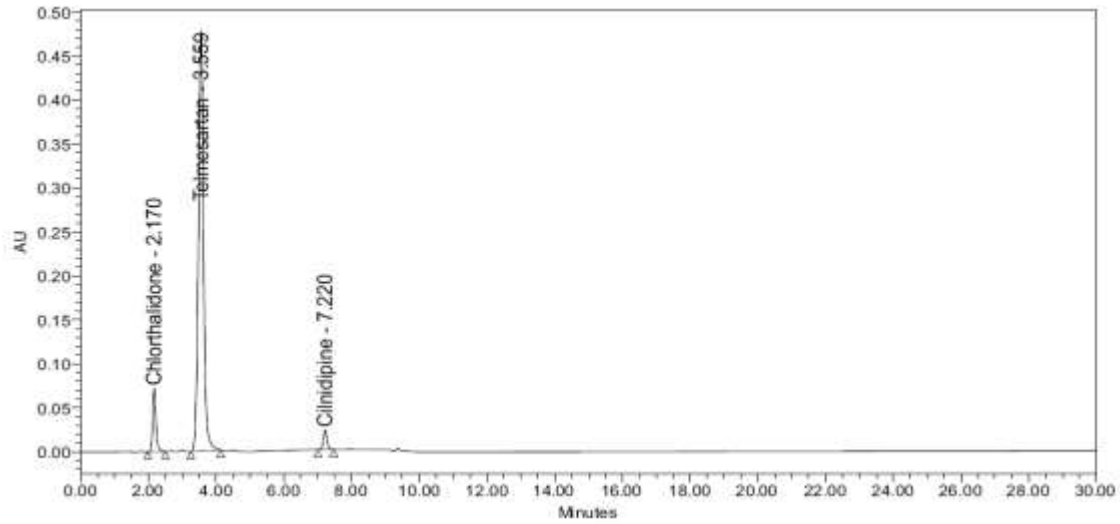


Fig.8: Chromatograms of oxidative degradation study.

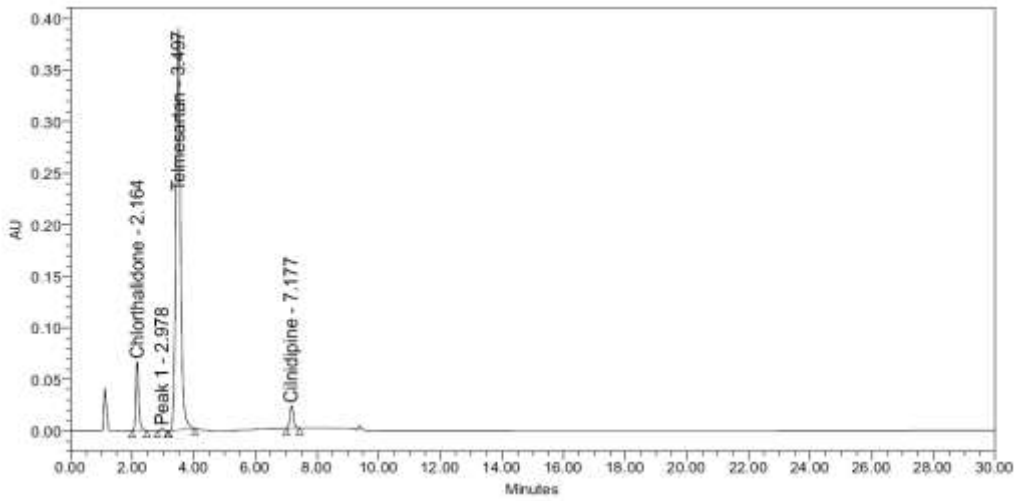


Fig.9: Chromatograms of thermal degradation study.

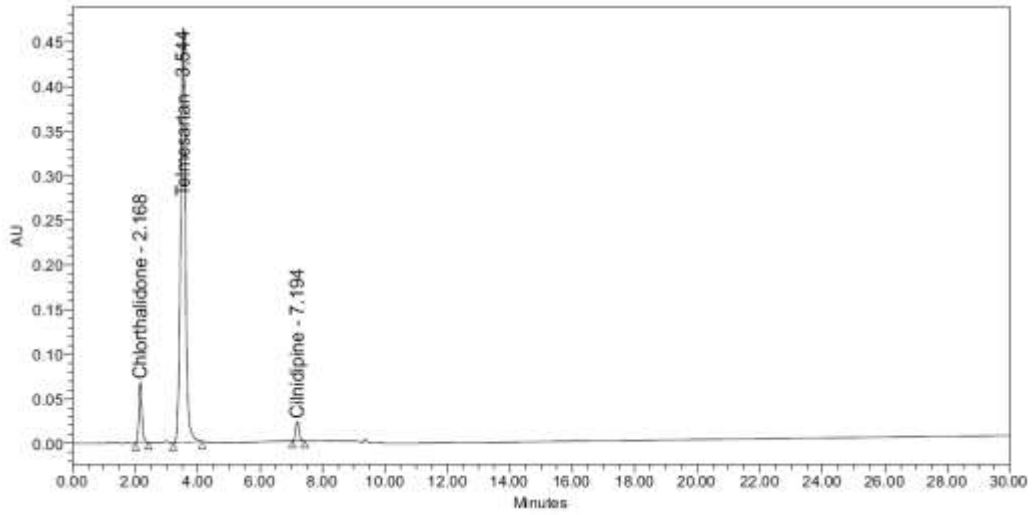


Fig.10: Chromatograms of photo degradation study.

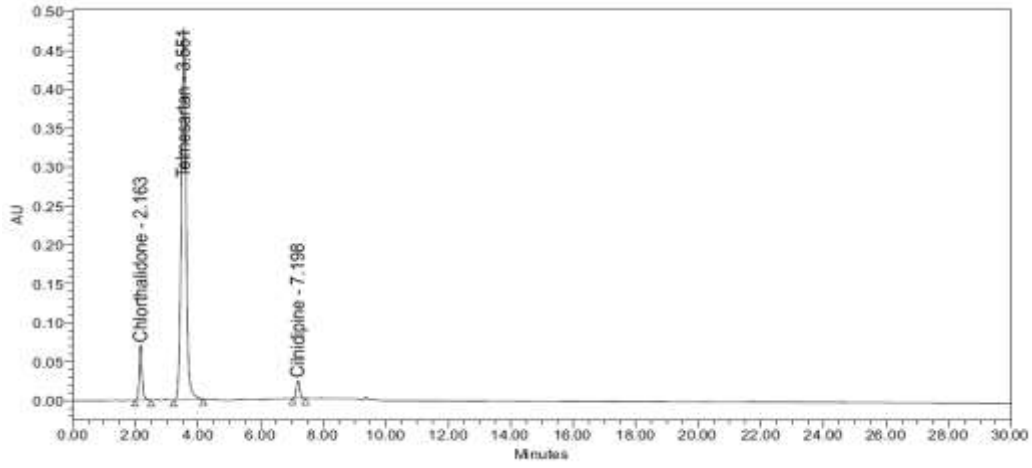


Fig.11: Chromatograms of neutral degradation study.

5. CONCLUSION

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of Telmisartan Cilinidipine and Chlorthalidone in combined tablet dosage form.

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