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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CLINIDIPINE AND OLMESARTAN WITH FORCED DEGRADATION STUDIES IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Stability indicating reversed-phase high-perfomance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of cilinidipine and olmesartan. All the drugs were separated on ODS 250x 4.6 mm, 5µ. The mobile phase was a 60:40 (v/v) mixture of acetonitrile and 0.1%orthophosphoric acid buffer, pumped at a flow rate of 1 ml/min. UV detection was performed at 270 nm. The retention time of cilinidipine and olmesartan was found to be 2.382 min and 3.687 min respectively. The method was validated in the sample concentration ranges of 50-300µg/ml for cilinidipine and 25 – 150 µg/ml for Olmesartan. The method demonstrated to be robust, resisting to small deliberate changes in pH and flow rate of the mobile phase. The LOD values were 0.26 µg/ml and 10.3 µg/ml, while the LOQ values were 0.87 µg/ml and 31.1 µg/ml for cilinidipine and olmesartan respectively. The recoveries for all three levels were above 99%.

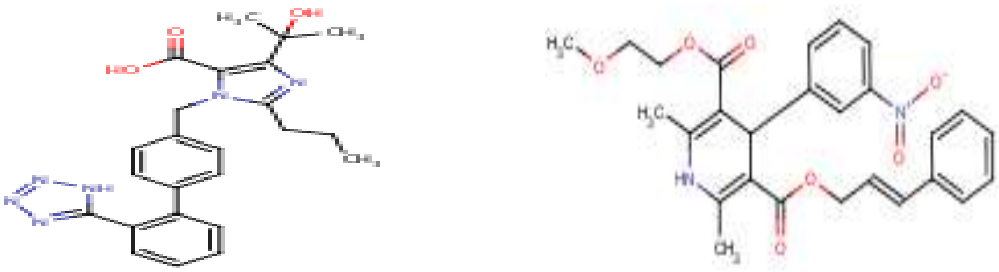
Keywords: RP-HPLC, cilinidipine and olmesartan, Tablet dosage form, forced degradation.

INTRODUCTION

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 and it is a unique Ca^{2+} channel blocker with an inhibitory

action on the sympathetic N-type Ca^{2+} channels, which is used for patients with hypertension. Olmesartan is an angiotensin II receptor blocker and chemically it is 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylic acid. It selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure. Literature survey reveals High Performance Liquid Chromatographic (HPLC) for determination of cilinidipine and olmesartan Hydrochloride combination are not official in Pharmacopeias of USP and BP. And their determination is official as single compound in Pharmacopeias.

Various analytical methods have been reported for the assay of cilinidipine and olmesartan alone or in combination with each other and also with other antihypertensive agents in pharmaceutical formulations. But there is no RP-HPLC method with stability indicating methods with these combination They include UV-VIS spectroscopy, high performance liquid chromatography,[1-6] high performance thin layer chromatography[7-8] and LC - MS/ MS.[9]



(A) Olmesartan **(B) Cilnidipine**

Figure 1: The Chemical Structures of Olmesartan (A) and Cilinidipine (B)

As on only few methods is available for their simultaneous determination, however, it is essential to develop a suitable analytical method for simultaneous estimation of cilinidipine and olmesartan 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 cilinidipine and olmesartan in pharmaceutical dosage forms with stability indicating studies. 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).

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutically pure samples of cilinidipine and olmesartan Hydrochloride were obtained as a gift samples from Dr.Reddy's, Hyderabad used as such without further purification. A combination of cilinidipine and olmesartan 10/20 mg in tablet formulations (CELAVI O) was procured from Indian market, HPLC grade methanol, Acetonitrile, water and orthophosphoric acid 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 270 nm. Compounds were separated on a ODS (250 × 4.6 mm i.d., 5µm particle size) under reversed phase partition conditions. The mobile phase was a Acetonitrile and orthophosphoric acid buffer. The flow rate was 1ml/min and the run time was 8 minutes. Samples were injected using Rheodyne injector with 10µL loop and detection was carried out at 270nm. 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 cilinidipine and olmesartan 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

Weigh and transfer about 10mg of cilinidipine working standard or reference standard in to a 10 ml volumetric flask, add diluent and sonicate for 5 min to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of standard stock solution – II

Weigh and transfer about 20 mg of olmesartan working standard or reference standard into a 10 ml volumetric flask, add about 10 ml of Diluent and sonicate for 5 minutes to dissolve the material completely and make up the volume with diluent and mix well.

Preparation of standard solution

Pipette out 1 ml of above stock solution –I and II into 10 mL volumetric flask and diluted upto the volume with diluent.

Procedure for Analysis of Tablet Formulation

10 tablets were weighed and powdered and take 380mg (equivalent to 20mg of olmesartan and 10 mg of cilindipine) was transferred into a 10mL volumetric flask, 5mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. Filter the solution through the 0.45N nylon filter. From the filtered solution 1 ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent. 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 200µg/mL Olmesartan, 100µg/mL Cilnidipine 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 Olmesartan and Cilnidipine 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.

Alkali Degradation Condition

To 1 ml of stock solution Olmesartan and Cilnidipine 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 Olmesartan and Cilnidipine 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 hr 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.

RESULTS AND DISCUSSION

Method development

Several tests were performed in order to get satisfactory separation-resolution cilinidipine and olmesartan in different mobile phases with various ratios of buffers and organic phases by using different columns. The ideal mobile phase was found to be an Acetonitrile and orthophosphoric acid buffer. This mobile phase used gave a very satisfactory and good resolution of cilinidipine and olmesartan. 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 cilinidipine and olmesartan on the analytical column was evaluated at a flow rate of 1 ml/min. The injection volume was 10 µL. The retention time of standard and sample for cilinidipine and olmesartan 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 were mentioned on below Table-1.

Table 1: Finalized chromatographic conditions

Flow rate:1 ml/min	**Wave length**:270 nm	**Injection Volume**:10µL
Column temperature: 30±5˚C	Sample temperature: Ambient	Run time:8 minutes
Column	ODS 250x 4.6 mm, 5µ.	Which column
Mobile phase ratio	Mobile phase-A (%v/v) (Orthophosphoric acid buffer)	Mobile phase-B (%v/v) (Acetonitrile)
	40	60

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 cilinidipine and olmesartan

System Suitability Parameters	Results		Acceptance Criteria
	cilinidipine	olmesartan	
Retention time	2.382	3.687	
%RSD for area of cilinidipine and olmesartan Hydrochloride for five replicate injections of standard solution	0.54	0.36	NMT 2.0
Tailing factor for cilinidipine and olmesartan peak	1.47	1.31	NMT 2.0
Theoretical plates for cilinidipine and olmesartan	2982	4045	NLT 2000

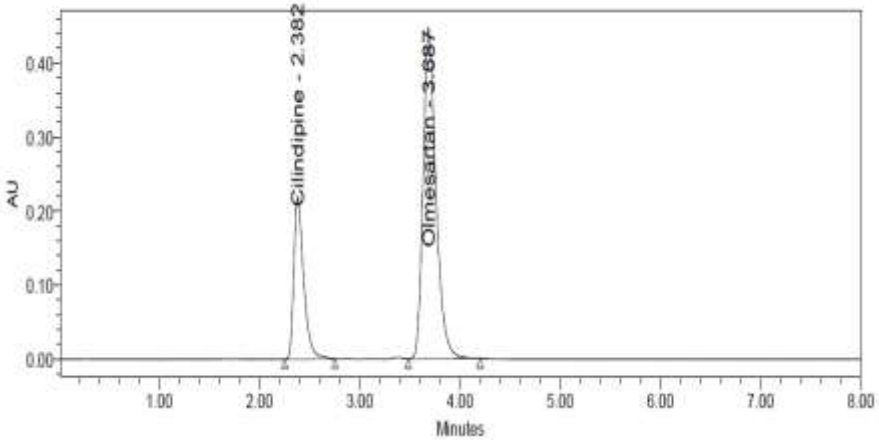


Figure 2: Optimized chromatograms for cilinidipine and olmesartan

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 0.25,0.50,0.75,1.25 and 1.50ml of Working standard solution cilinidipine and olmesartan were transferred in a series of 10 mL volumetric flasks respectively for 25, 50, 75,

100, 125 and150% 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 cilinidipine and olmesartan were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for cilinidipine and olmesartan were plotted separately of response against respective concentration of cilinidipine and olmesartan. The slope and intercept value for calibration curve were y = 14305x + 794.93 (R^2 = 0.9995) for cilinidipine and y = 17831x + 398.33 (R^2 = 0.9993) for olmesartan 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 cilinidipine and olmesartan are given in Figures 3 and 4 respectively.

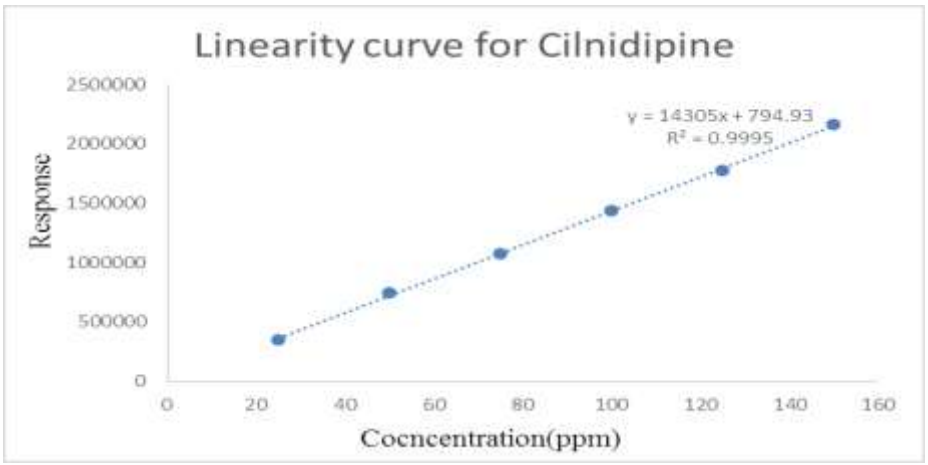


Figure 3: Linearity curve for cilinidipine

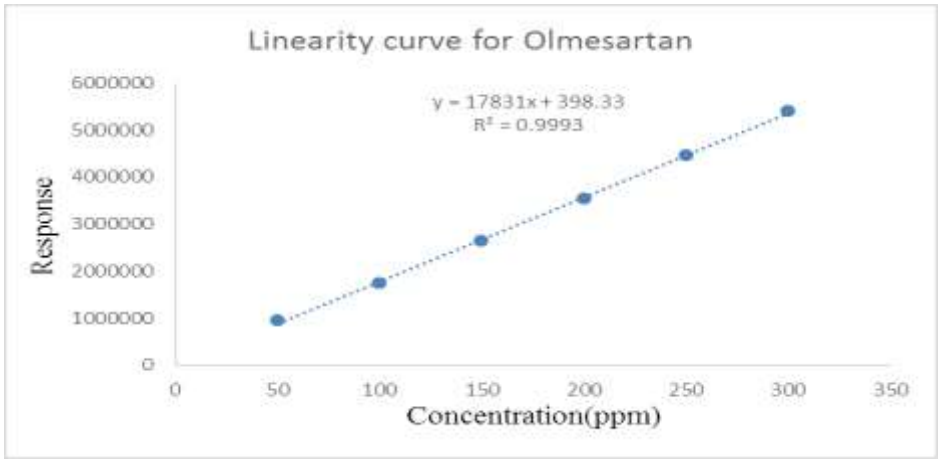


Figure 4: Linearity curve for olmesartan

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. For strength 10/20 mg, the %RSD of intraday precision of cilinidipine and olmesartan are 0.87 and 1.70 respectively. The %RSD of interday precision of cilinidipine and olmesartan are 1.24 and 0.22 respectively and overall %RSD for cilinidipine and olmesartan are 1.29, 0.84 (Table3)

Table3: Precision studies

No. of Tablets	% Assay			
	cilinidipine		olmesartan	
	Intraday precision	Interday precision	Intraday precision	Interday precision
1	100.3	100.3	98.0	99.5
2	101.3	100.3	100.0	99.4
3	100.6	99.2	98.7	99.2
4	99.9	99.5	98.4	99.2
5	102.1	100.1	100.0	99.8
6	99.8	103.9	101.2	99.4
Mean	100.7	100.6	99.4	99.4
%RSD	0.87	1.70	1.24	0.22
Over all % RSD (n=12)	1.29		0.84	

ACCURACY

To check the accuracy of the method, recovery studies were carried out by the 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 clinidipine and olmesartan

Level of % Recovery	% Mean Recovery*		% R.S.D.*	
	cilinidipine	olmesartan	cilinidipine	olmesartan
50	99.46	100.54	0.51	2.09
100	99.67	99.31	0.74	1.73
150	101.80	100.97	0.22	1.14

**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 standard and sample solutions 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 cilinidipine and olmesartan in sample solution.

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

Parameters	**cilinidipine**	**olmesartan**
Linearity range (µg/mL)	25-150	50-300
Correlation co-efficient	0.9995	0.9993
LOD [a] (µg/mL)	0.06	0.03
LOQ [b] (µg/mL)	0.19	0.10
Accuracy (% Recovery)	99.46 – 101.80	99.31 – 100.97
Precision (% RSD)		
Intraday (n^{d}= 6)	0.87	1.24
Interday (n^{d}= 6)	1.70	0.22

[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 Olmesartan and Cilnidipine was susceptible to acid, base, oxidation, and photo, thermal and neutral degradation. Typical chromatograms of stressed samples are shown in figs.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 hr, 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
Olmesartan	Acid	99.43	5.57	0.255	0.394
	Base	95.36	4.64	0.267	0.394
	Peroxide	95.59	4.41	0.378	0.550
	Thermal	96.16	3.84	0.284	0.419
	UV	97.34	2.66	0.208	0.393
Cilnidipine	Acid	95.59	4.41	0.504	0.596
	Base	96.05	3.95	0.721	0.867
	Peroxide	97.62	2.38	0.683	0.760
	Thermal	98.45	1.55	0.057	0.671
	UV	99.27	0.73	0.576	0.553

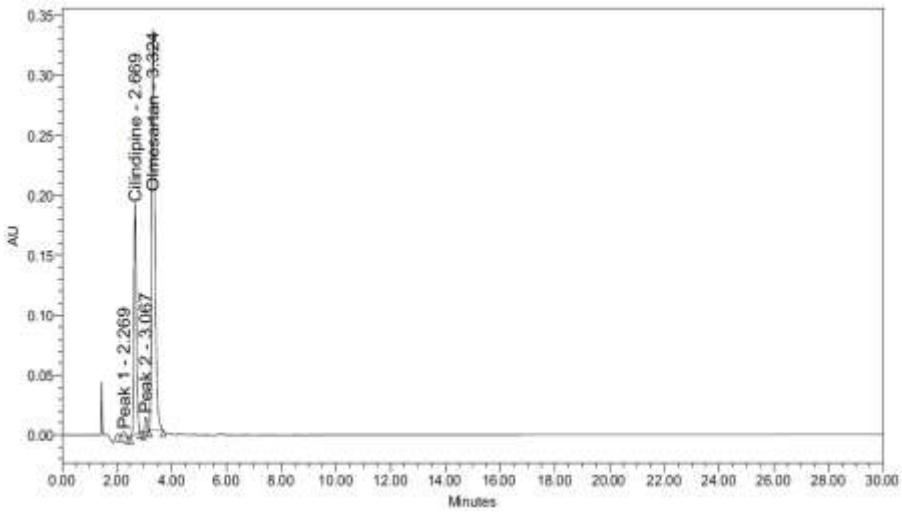


Fig. 6: Chromatograms of acid degradation study.

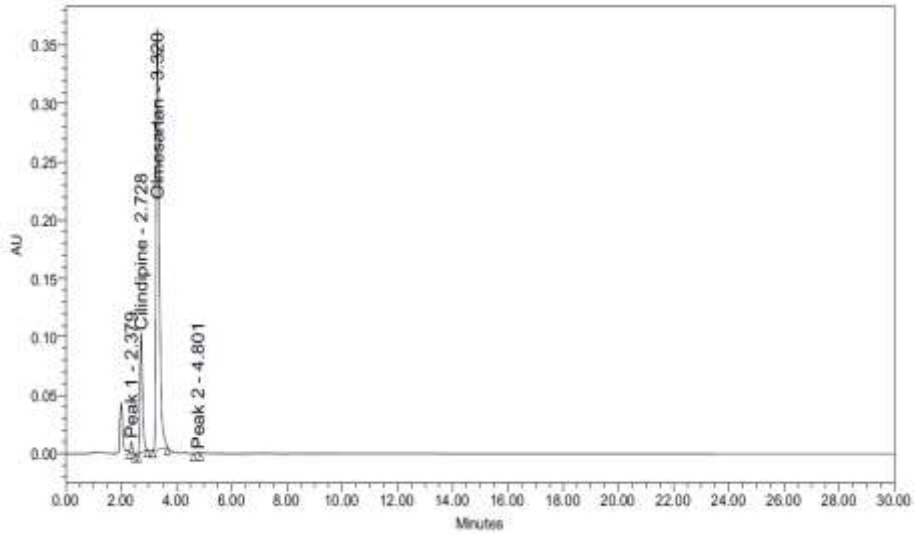


Figure7: Chromatograms of base degradation study.

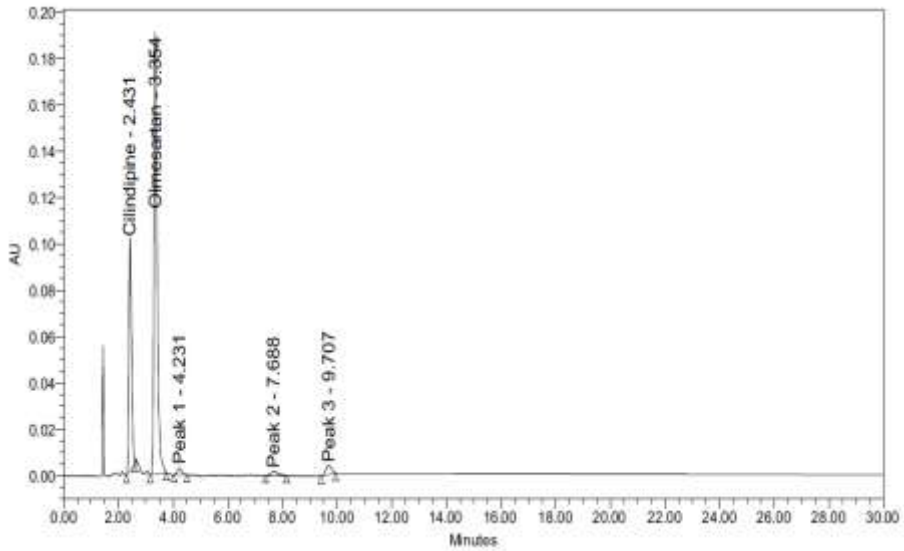


Figure 8: Chromatograms of oxidative degradation study.

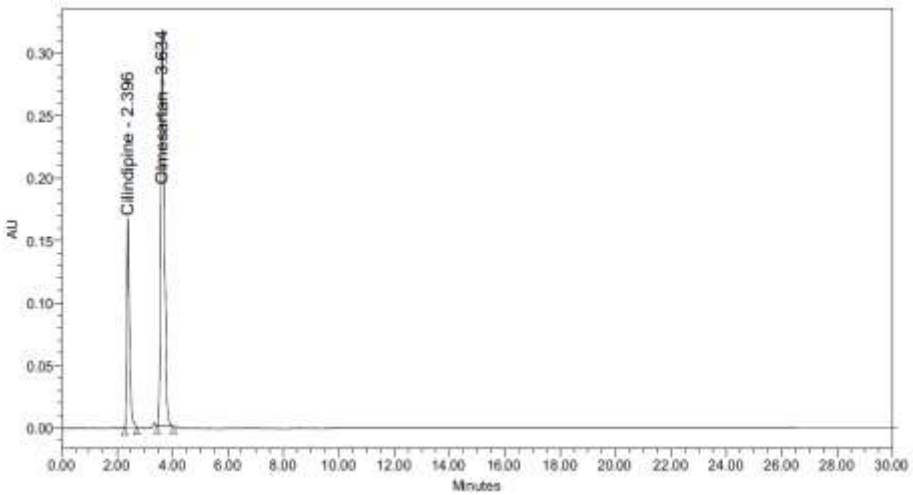


Figure 9: Chromatograms of thermal degradation study.

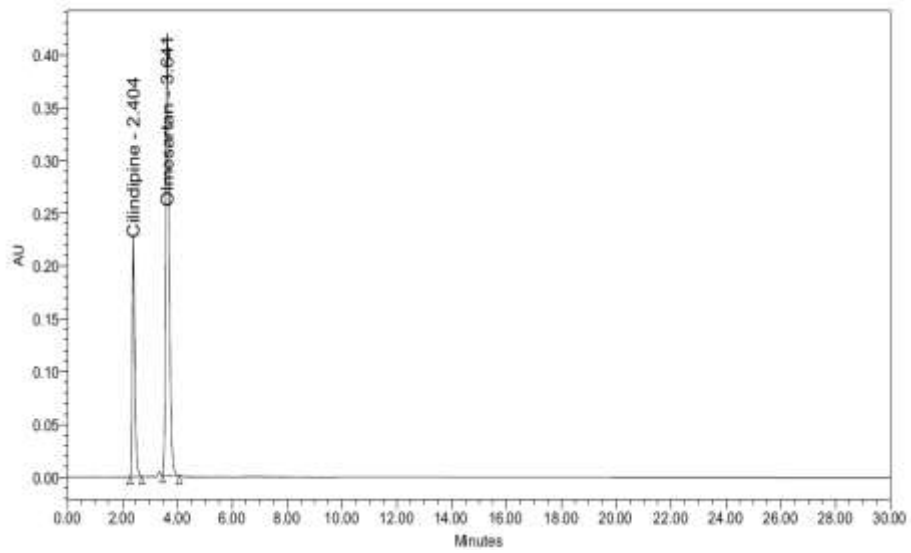


Figure 10: Chromatograms of photo degradation study.

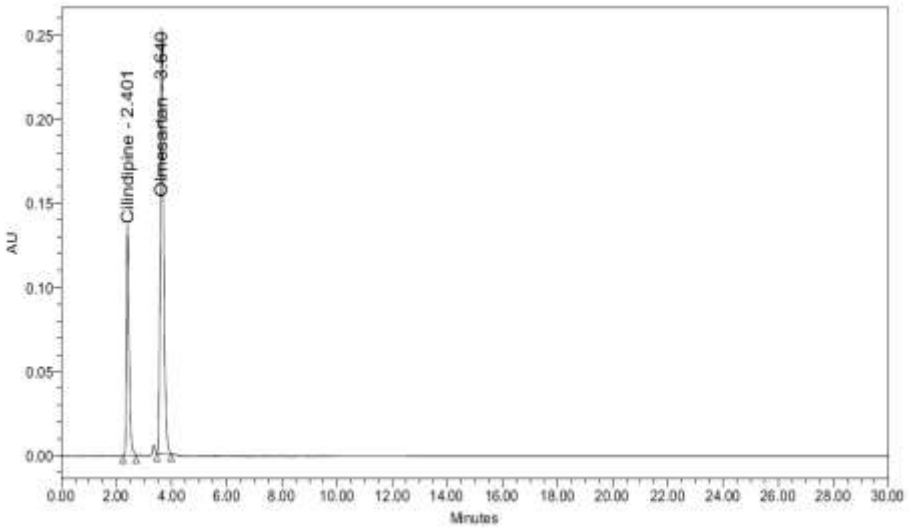


Figure 11: Chromatograms of neutral degradation study.

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 cilinidipine and olmesartan in combined tablet dosage form.

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