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Stability Indicating RP-HPLC-PDA Method for Simultaneous Estimation of Olmesartan Cilnidipine and Chlorthalidone with Forced Degradation Behavior Study in Bulk and in Its Tablet Dosage Form

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ABSTRACT

An accurate, efficient Stability indicating reversed-phase high-perfomance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous estimation of Olmesartan, Cilnidipine and Chlorthalidone. All the drugs were separated on a KROMASIL 250 x 4.6 mm, column packed with 5 Âµm particles. The mobile phase, optimized through an experimental design, was a 45:55 (v/v) mixture of acetonitrile and Ortho phosphoric acid buffer(0.1%OPA), pumped at a flow rate of 1 ml/min. UV detection was performed at 230 nm. The retention time of Olmesartan, Cilnidipine and Chlorthalidone was found to be 2.280min, 8.356 min and 2.804min respectively. The method was validated in the sample concentration ranges of 20-120 µg/ml for Olmesartan and 5-30 µg/ml for Cilnidipine, and Chlorthalidone 6.25-37.5µg/ml. The method demonstrated to be robust, resisting to small deliberate changes in pH and flow rate of the mobile phase. The LOD values were 0.08µg/ml, 0.04µg/ml and 0.05 µg/ml, while the LOQ values were 0.24µg/ml, 0.12µg/ml and 0.16µg/ml for Olmesartan, Cilnidipine and Chlorthalidone respectively.

Keywords: RP-HPLC, Olmesartan, Cilnidipine and Chlorthalidone, tablet dosage form.

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INTRODUCTION

Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers. It is indicated for the treatment of high blood pressure 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. Cilnidipinea 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 Olmesartan, Cilnidipine and Chlorthalidone 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 Olmesartan, Cilnidipine and Chlorthalidone alone or in combination with other antihypertensive agents in pharmaceutical formulations. They include UV-VIS spectroscopy[1-2], high performance liquid chromatography[3-6], high performance thin layer chromatography[7] 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 Olmesartan, Cilnidipine 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 Olmesartan, Cilnidipine and Chlorthalidonein 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).

MATERIALS AND METHOD

Chemicals and Reagents

Pharmaceutically pure samples of Olmesartan C ilnidipine and Chlorthalidone were obtained as a gift samples from Dr. Reddy's, Hyderabad used as such without further purification. A combination of Olmesartan Cilnidipine and Chlorthalidone40/10/12.5mg in tablet formulations (Olmin Trio 40) was procured from Indian market, HPLC grade methanol, Acetonitrile, water and Ortho phosphoric 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 230 nm. Compounds were separated on a KROMASIL, column (250 × 4.6 mm i.d., 5µm particle size) under reversed phase partition conditions. The mobile phase was a Acetonitrile and OPA buffer. The flow rate was 1ml/min and the run time was 8 minutes. Samples were injected using Rheodyne injector with 20 µL loop and detection was carried out at 230 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 Olmesartan Cilnidipine 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 Olmesartan 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 Cilnidipine working standard or reference standard in to a 20 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 – III

Weight and transfer about 12.5 mg of Chlorthalidone working standard or reference standard in to a 20 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 stir 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 Olmesartan, 10µg/mL Cilnidipine and 20 µg/mL Chlorthalidone concentration 10µlwereinjectedintothe 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 solution Olmesartan and Cilnidipine 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 Olmesartan and Cilnidipine 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 Olmesartan and Cilnidipine 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 6h 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 Olmesartan Cilnidipine 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 OPA buffer. This mobile phase used gave a very satisfactory and good resolution of Olmesartan Cilnidipine 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 Olmesartan Cilnidipine 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 Olmesartan Cilnidipine 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. To inject the standards on above finalized chromatographic conditions and their results was mentioned on below Table 2.

Table 1: Finalized chromatographic conditions

Flow rate: 1 ml/min	**Wave length**: 230 nm	**Injection Volume**:20µL
Column temperature: 30˚C	**Sample temperature**: Ambient	**Run time**: 8 minutes
Mobile phase: Buffer and Acetonitrile in the ratio 55:45		
Column: KROMASIL 250 x 4.6 mm, 5µ.		

Table 2: Results from system suitability study of Olmesartan Cilnidipine and Chlorthalidone

System Suitability Parameters	Results			Acceptance Criteria
	Olmesartan	Cilnidipine	Chlorthalidone	
Retention time	2.280	8.356	2.804	
%RSD for area of Olmesartan Cilnidipine and Chlorthalidone for five replicate injections of standard solution	1.15	0.40	0.43	NMT 2.0
Tailing factor for Olmesartan Cilnidipine and Chlorthalidone peak	1.18	1.03	1.09	NMT 2.0
Theoretical plates for Olmesartan Cilnidipine and Chlorthalidone	4263	86213	5041	NLT 2000

Results of Degradation Study

The degradation study indicated that Olmesartan Cilnidipine and Chlorthalidone was susceptible to acid, base, oxidation, and photo, thermal and neutral degradation. Typical chromatograms of stressed samples are shown in below figures 6 to 11 and all the results are shown in below Table 6. 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.

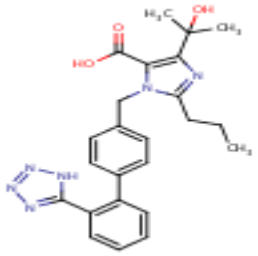
Method Validation

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

Linearity

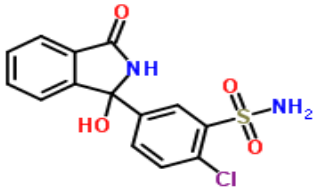
Aliquots 0.25,0.5,0.75,1.0,1.25 and 1.50mlof stock solution of Working standard solution Olmesartan Cilnidipine and Chlorthalidone were transferred in a series of 10 mL volumetric flasks for 25,50,75,100,125 and 150% levels. Finally the volume was made up to the mark with the diluent. Two replicates per concentration were injected and chromatograms were recorded. The peak area ratios of Olmesartan Cilnidipine and Chlorthalidone were calculated and respective calibration curves were plotted of response against concentration of each drug. Calibration curves for Olmesartan Cilnidipine and Chlorthalidone were plotted separately of response against respective concentration of Olmesartan Cilnidipine and Chlorthalidone. The slope and intercept

value for calibration curve were y =15669x +924.91 (R^2 = 0.9996) for Olmesartan, y =16220x + 1225.6 (R^2 = 0.9999) for Cilnidipine and y=14759x +762.65 (R^2 = 0.9995) 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 Olmesartan Cilnidipine and Chlorthalidone are given in Figures 3 and 4 respectively.



(A) Olmesartan

(B) Cilnidipine



(C) Chlorthalidone

Figure 1: The Chemical Structures of Olmesartan (A), Cilnidipine (B) and Chlorthalidone (C).

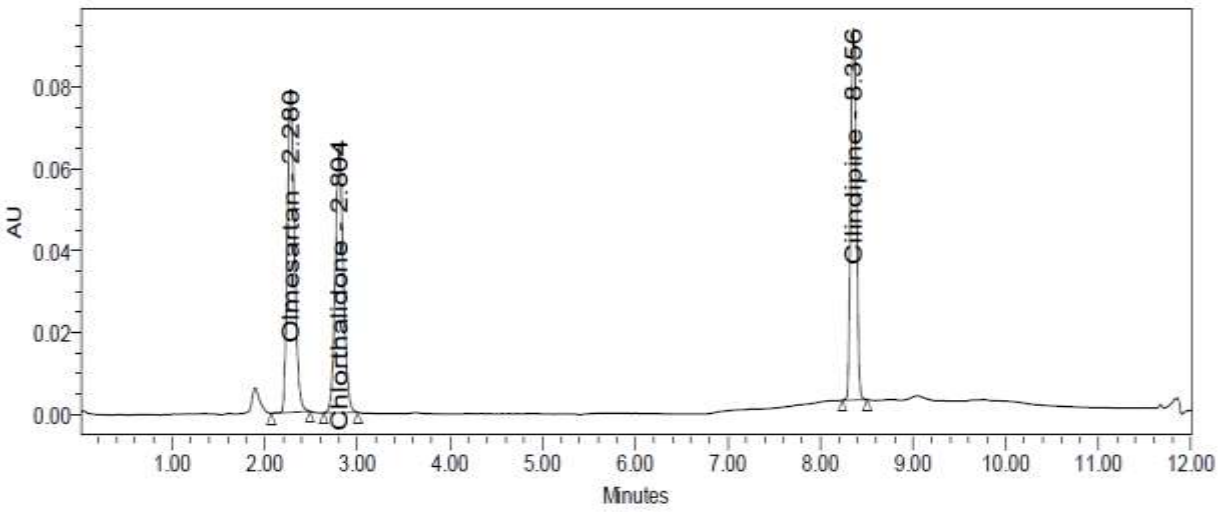


Figure 2: Optimized chromatograms for Olmesartan Cilnidipine and Chlorthalidone

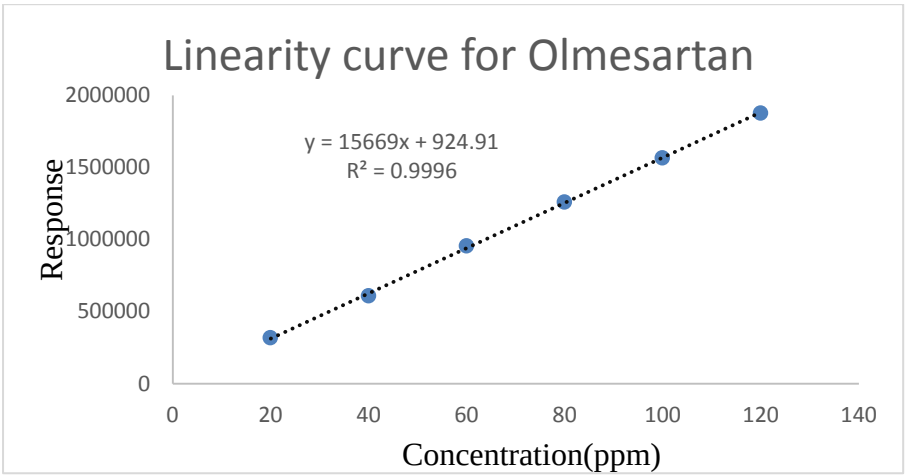


Figure 3: Linearity curve for Olmesartan

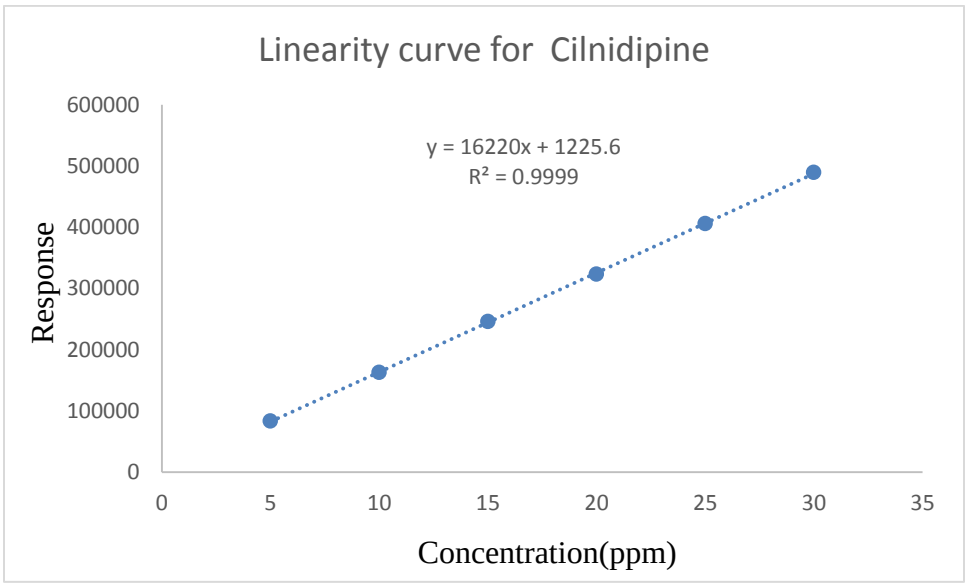


Figure 4: Linearity curve for Cilnidipine

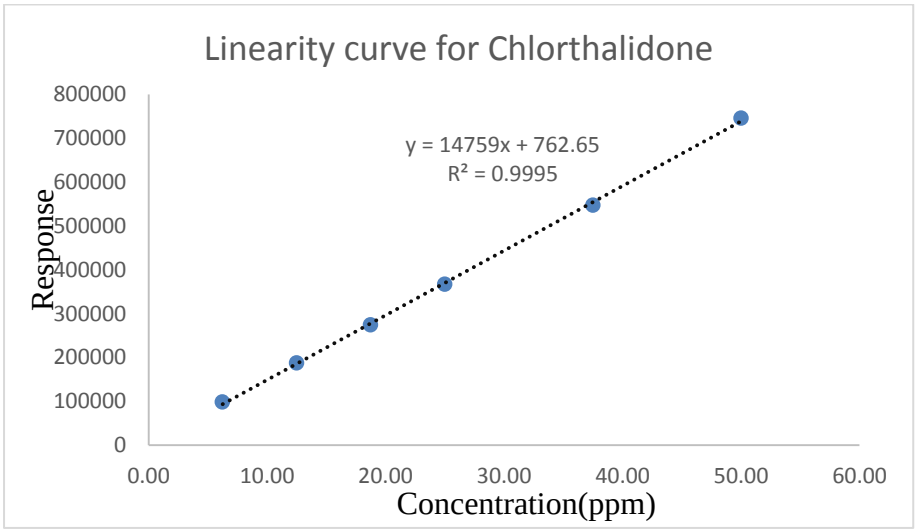


Figure 5: Linearity curve for Chlorthalidone

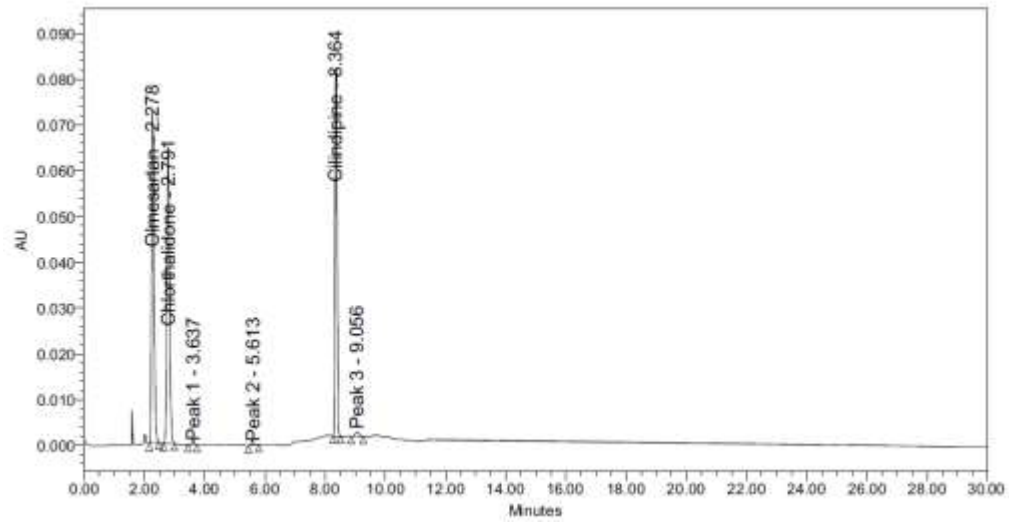


Figure 6: Chromatograms of acid degradation study.

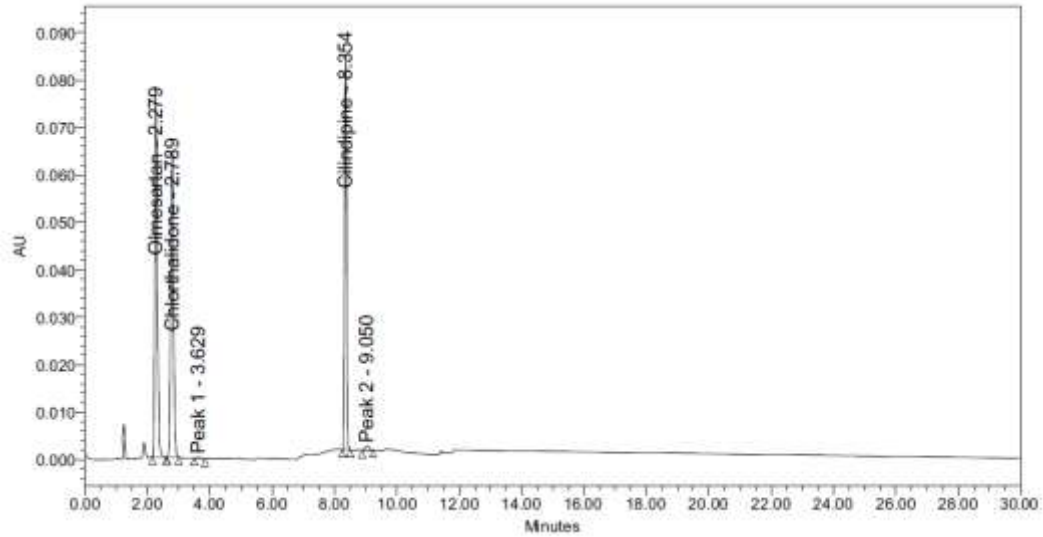


Figure7: Chromatograms of base degradation study

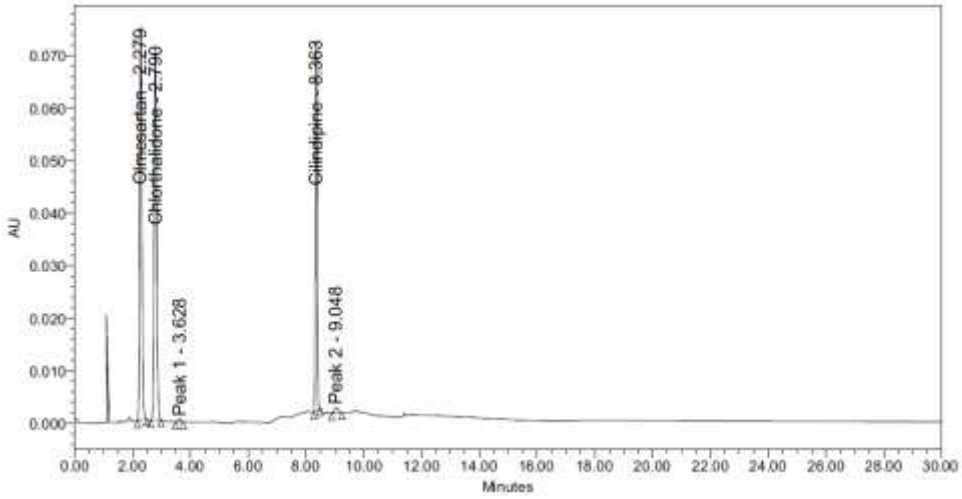


Figure 8: Chromatograms of oxidative degradation study

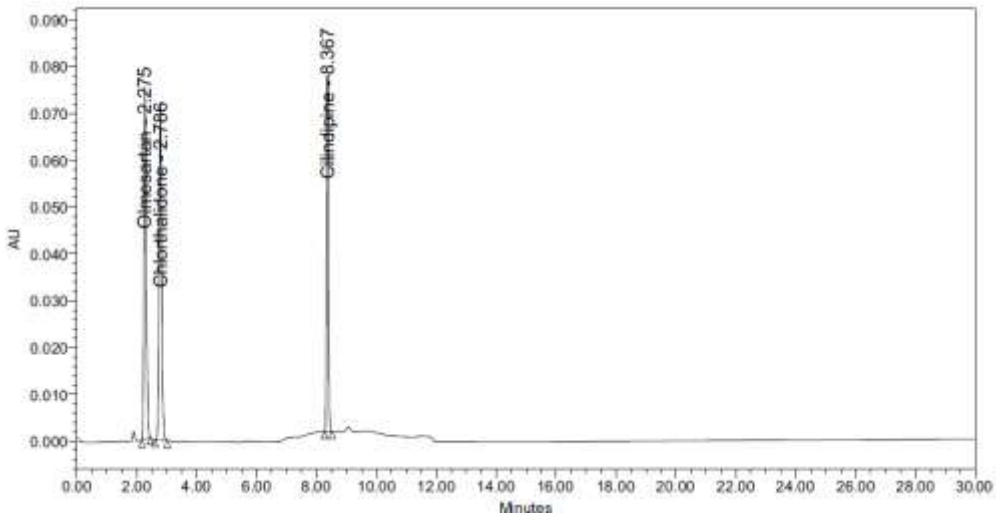


Figure 9: Chromatograms of thermal degradation study

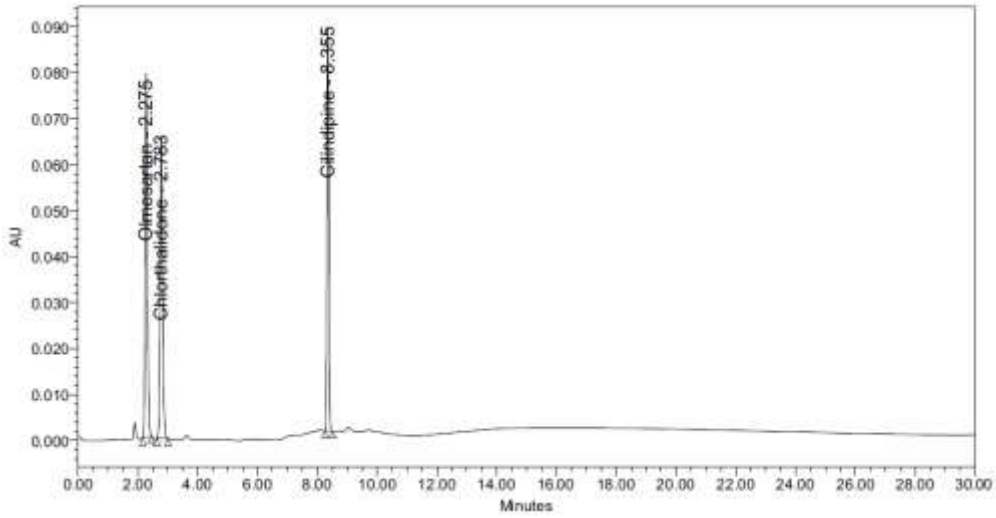


Figure 10: Chromatograms of photo degradation study.

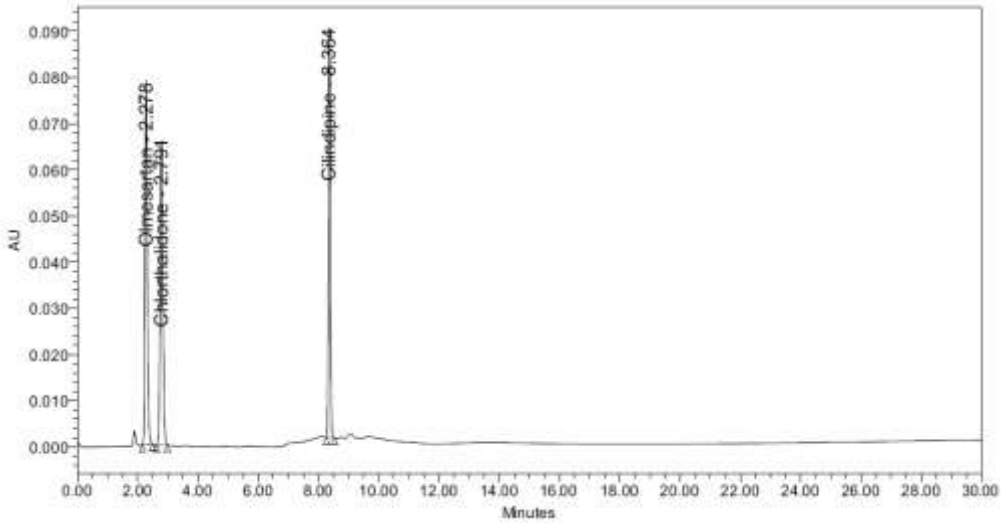


Figure 11: Chromatograms of neutral degradation study.

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 0.85, 0.71 and 0.68 for Olmesartan Cilnidipine and Chlorthalidone, respectively. The %RSD of intraday precision was0.86, 0.71 and 0.68 for Olmesartan Cilnidipine and Chlorthalidone and Interday precision was 0.43, 0.41 and 0.35 for Olmesartan Cilnidipine and Chlorthalidone respectively and overall %RSD for Olmesartan Cilnidipine and Chlorthalidone are 1.03, 0.56 and 0.97(Table3)

Table3: Precision studies

S. No	% Assay(n=6)					
	Olmesartan		Cilnidipine		Chlorthalidone	
	Intraday precision	Interda-precision	Intraday precision	Interday precision	Intraday precision	Interday precision
1	99.8	98.4	99.3	100.2	100.9	99.4
2	99.4	99.1	98.9	99.0	100.8	100.0
3	100.4	97.9	100.1	99.5	101.3	99.5
4	98.9	98.4	99.6	99.6	102.1	99.2
5	101.4	98.5	98.2	99.8	99.9	99.6
6	99.7	98.2	99.8	99.2	101.2	99.1
Mean	99.9	98.42	99.3	99.55	101.0	99.49
%RSD	0.86	0.43	0.71	0.41	0.68	0.35
Over all % RSD (n=12)	1.03		0.56		0.97	

Tab- is 40/10/25 mg of Olmesartan, Cilnidipine and Chlorthalidonerespectively

Table 4: Recovery studies of OlmesartanCilnidipine and Chlorthalidone

Product name		Level of % Recovery		
		50	100	150
Olmesartan	% Mean Recovery*	100.94	100.41	98.57
	% R.S.D*	0.24	0.42	0.47
Cilnidipine	% Mean Recovery*	100.62	100.27	100.69
	% R.S.D*	1.88	0.54	0.76
Chlorthalidone	% Mean Recovery*	100.41	99.44	100.74
	% R.S.D*	2.03	1.21	0.98

*Avg. of three determinations for 50, 150 &100%, R.S.D. is relative standard deviation

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

Parameters	Olmesartan	Cilnidipine	Chlorthalidone
Linearity range (µg/mL)	20-120	5-30	6.25-37.5
Correlation co-efficient	0.9996	0.9999	0.9995
LOD [a] (µg/mL)	0.08	0.04	0.05
LOQ [b] (µg/mL)	0.24	0.12	0.16
Accuracy (% Recovery)	98.57-100.94	100.27-100.69	99.44-100.74
Precision (% RSD)[c]			
Intraday (n^d= 6)	0.86	0.71	0.68
Interday (n^d= 6)	0.43	0.43	0.35

Table 6: Forced Degradation Studies

Drug substance	Sample treatment	% assay	% degradation	Purity Angle	Purity Threshold
Olmesartan	Acid	96.65	3.35	0.175	0.30
	Base	97.95	2.05	0.289	0.342
	Peroxide	95.00	5.00	0.181	0.311
	Thermal	97.65	2.35	0.185	0.311
	UV	98.44	1.56	0.200	0.314
Cilnidipine	Acid	95.62	4.38	0.093	0.261
	Base	97.77	2.23	0.102	0.270
	Peroxide	94.53	5.47	0.168	0.274
	Thermal	97.79	2.21	0.106.	0.275
	UV	98.34	1.66	0.102	0.271
Chlorthalidone	Acid	96.05	3.95	0.122	0.311
	Base	98.47	1.53	0.138	0.326
	Peroxide	94.83	5.17	0.115	0.310
	Thermal	97.02	2.98	0.132	0.329
	UV	98.27	1.73	0.130	0.325

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 50%, 100% and 150%. The percentages of recoveries were calculated, results of which are represented in Table 4.

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 Olmesartan Cilnidipine and Chlorthalidone in sample solution.

[a] *LOD = Limit of detection.*

[b] *LOQ =Limit of quantitation.*

[c] *RSD = Relative standard deviation.*

[d] *n = Number of determination*

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 Olmesartan Cilnidipine and Chlorthalidone in combined tablet dosage form.

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REFERENCES

1. Sidhdhapara, Mital, Patel, BirajuParmar, Ashok, Vekariya, Hitesh, Patel, Piyush.Derivative spectrophotometric method for simultaneous determination of cilnidipine and Olmesartan medoximil in tablet dosage form, Der PharmaChemica. 2014; 6(3): 175.
2. Nehal G, Krutika B, Hemangi P, Ketan D. Development and validation of spectrophotometric method for simultaneous estimation of olmesartan medoxomil and cilnidipine by simultaneous equation method, Inventi rapid.2014.
3. Amit S Minase, Manjusha Dole N and Sanjay Sawant D. Development and validation of analytical method for simultaneous estimation of cilnidipine and Olmesartan medoxomil in bulk and tablet dosage form by RP-HPLC. Int J Pharm Pharma Sci 2014; 6(7): 508.
4. NehalGhelani C, KetanDadhania, ShitalFaldu. Analytical method development and validation for simultaneous estimation of Olmesartan medoxomil and cilnidipine in their combined

pharmaceutical dosage form by RP-HPLC method, PharmaTutor.2014 ;2(7).

5. Nirmal M , ThakkerHaresh, Panchal B, Dinesh R. Rakholiya, Murugan R, Vishnu P. Choudhari, and Bhanudas S. Kuchekar. Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Olmesartan Medoxomil and Metoprolol Succinate in pharmaceutical dosage form. Pharm Methods. 2012; 3(2): 84–9.
6. Sidhdhapara, Mital, Patel Biraju, Parmar Ashok. Development and validation of RP-HPLC method for simultaneous estimation of clinidipine and olmesartan in their combined tablet dosage form, Int J Pharm Biological Sci. 2014; 4(1):157-60.
7. Amit S. Minase, Manjusha N. Dole. Development and validation of analytical method for simultaneous estimation of cilnidipine and Olmesartan medoxomil in bulk and tablet dosage form by HPTLC. J Advanced Scientific Res 2014; 5(3): 34-8.