WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 15, 897-910. Research Article ISSN 2277– 7105

STABILITY INDICATING HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF OLMESARTAN AND CILNIDIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Article Received on
12 June 2018,
Revised on 03 July 2018,
Accepted on 24 July 2018
DOI: 10.20959/wjpr201815-13028

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Olmesartan and Cilnidipine in tablet dosage form. Chromatogram was run through Std BDS 150 x 4.6 mm, 5 µm. Mobile phase containing buffer and Acetonitrile taken in the ratio 60:40 was pumped through the column at a flow rate of 1 ml/min. The buffer used in this method was 0.1% OPA Buffer. The temperature was maintained at 30°C. Optimized wavelength selected was 210 nm. The retention time of Olmesartan and Cilnidipine were found to be 2.098 min and 2.928 min. %RSD of the Olmesartan and Cilnidipine were and found to be 0.2 and 0.2 respectively. %Recovery was obtained as 99.95% and 100.81% for Olmesartan and Cilnidipine respectively. LOD, LOQ values obtained from regression equations of Olmesartan and Cilnidipine were 0.01, 0.36 and 0.03, 1.09 respectively. Regression equation of Olmesartan is y = 22366x + 877.5, and y = 10431x + 4275 of Cilnidipine. Retention times were decreased and the run time was decreased. The method was validated for its linearity, precision and accuracy. The proposed method was successfully applied for the determination of Olmesartan and Cilnidipine in pure form, pharmaceutical formulations.

KEYWORDS: Olmesartan, Cilnidipine, RP-HPLC, Validation, Method.

INTRODUCTION

Olmesartan chemically 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylicacid4-(hydroxy-1-methylethyl)-2-

propyl-1-{[2'-(1Htetrazol-5-yl)-1,1'-biphenyl-4-yl]methyl}-1H-imidazole-5-carboxylic acid is an antihypertensive agent [1], which belongs to the class of medications called angiotensin II receptor blockers. It is indicated for the treatment of high blood pressure and is marketed under the name Olmetec[2]. The FDA label includes a black-box warning of injury and death to the fetus, so women of child-bearing age need to be warned and take the necessary precautions.

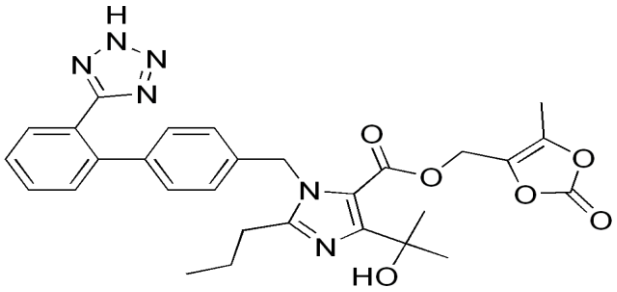

Fig. 1: Chemical structure of Olmesartan.

Cilnidipine chemically 3-O-(2-methoxyethyl) 5-O-[(E)-3-phenylprop-2-enyl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate is a calcium channel blocker. Clinidipine is the novel calcium antagonist accompanied with L-type and N-type calcium channel blocking function. It was jointly developed by Fuji Viscera Pharmaceutical Company, Japan and Ajinomoto, Japan and approved to come into market for the first time and used for high blood pressure treatment in 1995. Compared with other calcium antagonists, clinidipine can act on the Ntype calcium-channel that existing sympathetic nerve end besides acting on L-type calciumchannel that similar to most of the calcium antagonists.[3]

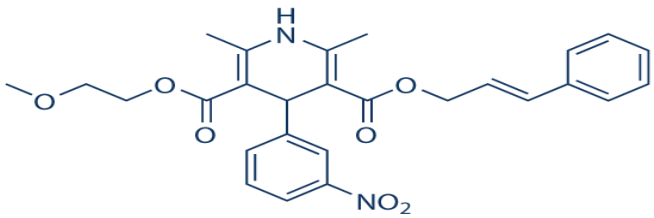

Fig. 2: Chemical structure of Cilnidipine.

The main aim of the present study is to develop an accurate, precise, sensitive, selective, reproducible and rapid analytical technique for simultaneous estimation of Olmesartan and Cilnidipine in bulk ant tablet dosage form.

There are very less analytical methods reported for the simultaneous estimation of Olmesartan and Cilnidipine.[4-11] However, there is a need for developing an economical, fast and accurate method for estimation of Olmesartan and Cilnidipine.

MATERIALS AND METHODS

Materials

Olmesartan and Cilnidipine pure drugs (API), Combination Olmesartan and Cilnidipine tablets (Cilidin-o), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dehydrogenate ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments

Electronics Balance-Denver, pH meter -BVK enterprises, India. Ultrasonicator-BVK enterprises WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Olmesartan and Cilnidipine solutions.

Chromatography conditions

The chromatographic separation was performed on Standard BDS C18 (4.6 x 150mm, 5µm)at an ambient column temperature. The samples were eluted using OPA Buffer : Acetonitrile in ratio of 50:50 v/v as the mobile phase at a flow rate of 1ml/min the mobile phase and samples were degassed by ultrasonication for 30 min and filtered through 0.45µm Nylon 47mm membrane filter. The measurements were carried out with an injection volume of 10µL, flow rate was set to 1 mL/min and PDA detection was carried out at 240 nm. All determinations were done at ambient column temperature (30°C). The chromatograms of the prepared standard stock solutions of Ertugliflozin and Sitagliptin were recorded under optimized chromatographic conditions.

Diluent

Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50.

Preparation of Standard stock solutions

Accurately weighed 50mg of Olmesartan, 25mg of Cilnidipine and transferred to individual 25ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (2000µg/ml of Olmesartan and 1000µg/ml of Cilnidipine).

Preparation of Standard working solutions (100% solution)

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (200µg/ml Olmesartan of and 100µg/ml of Cilnidipine).

Preparation of Sample stock solutions

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (2000µg/ml of Olmesartan and 1000µg/ml of Cilnidipine).

Preparation of Sample working solutions (100% solution)

1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (200µg/ml of Olmesartan and 100µg/ml of Cilnidipine).

Preparation of buffer

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

METHOD VALIDATION[12-13]

System suitability

System suitability tests are a fundamental part of liquid chromatographic method. It ensures that system is working correctly. System suitability parameters such as number of theoretical plates, retention time, and tailing factor were evaluated. This was performed by injecting mixture of standard in six replicates.

Linearity

The linearity of the proposed method was determined by quantitative dilution of the standard solution of olmesartan and cilnipine to obtain solution in concentration range of 50-300 µg/ml and 25-150 µg/ml for olmesartan and cilnipine respectively. A graph of peak area

versus concentration in µg/ml was plotted for all three drugs in triplicate. The slope, intercept, and correlation coefficient of regression line were determined.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ represent the concentration of analyte that would yield to signal-to-noise ratio of 3 for LOD and 10 for LOQ. LOD and LOQ were calculated using following formula,

$LOD=3.3\ \sigma/S$

$LOQ= 10\ \sigma\ /S$

where, σ = standard deviation of response (peak area) and S = average of slope of the calibration curve.

Method precision

The method precision of the proposed method was determined by injecting six replicates of sample and standard on the same day to ensure that the analytical method is repeatable.

System precision

The system precision is checked by injecting six replicates of standard solution to ensure that the analytical system is working properly.

Accuracy

The accuracy of this method was performed at three different levels (50%, 100%, 150%), by the addition of a known amount of standard to the sample at each level. Each level was repeated three times (n=3).

Robustness

Robustness is the measure of optimized method capacity to remain unaffected by small, but deliberate variations in method parameters such as mobile phase flow rate (±0.2 ml/min), wavelength nm (±1 nm), and column oven temperature (±1°C).

RESULTS AND DISCUSSION

Method development: Method development was done by changing various, mobile phase ratios, buffers et.

System suitability parameters

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. The results are summarized in **Table.1.**

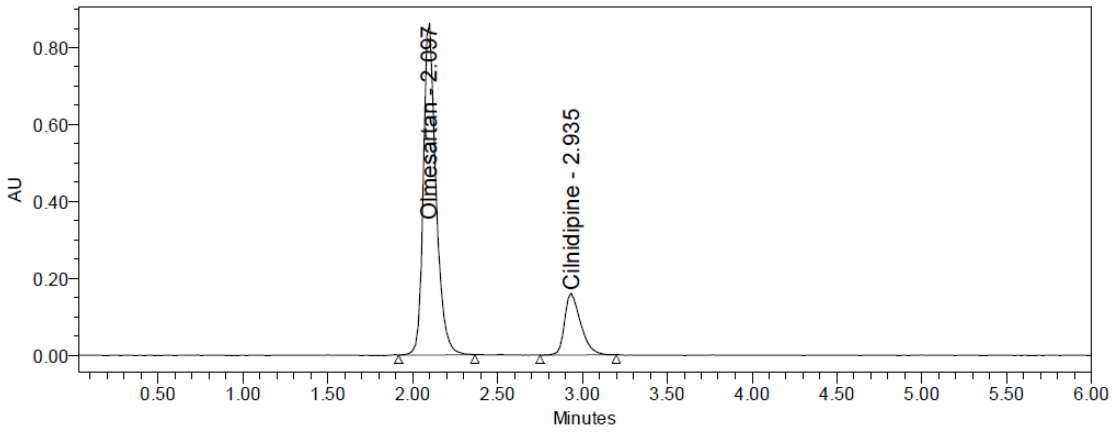

Fig. 3: Optimized Chromatogram.

Table 1: System suitability parameters for Olmesartan and Cilnidipine.

S. no.	Olmesartan			Cilnidipine			Resolution
Inj	RT (min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	
1	2.093	4011	1.29	2.926	4616	1.37	5.2
2	2.093	4033	1.28	2.927	4570	1.36	5.2
3	2.095	4008	1.25	2.928	4694	1.38	5.3
4	2.096	3957	1.24	2.928	4767	1.35	5.3
5	2.096	4027	1.25	2.929	4735	1.36	5.4
6	2.098	4100	1.25	2.930	4610	1.35	5.4

Specificity

Retention times of Olmesartan and Cilnidipine were 2.098min and 2.928 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity

Six linear concentrations of Olmesartan (50-300µg/ml) and Cilnidipine (25-150µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Olmesartan was y =22366.x + 877.5 and of Cilnidipine was y = 10431x + 4275 Correlation coefficient obtained was 0.999 for the two drugs. The results are summarized in **Table.2.**

Table 2: Linearity table for Olmesartan and Cilnidipine.

Olmesartan		Cilnidipine	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
50	1095571	25	265429
100	2251803	50	528161
150	3366094	75	786110
200	4478741	100	1056518
250	5618778	125	1305957
300	6679863	150	1564150

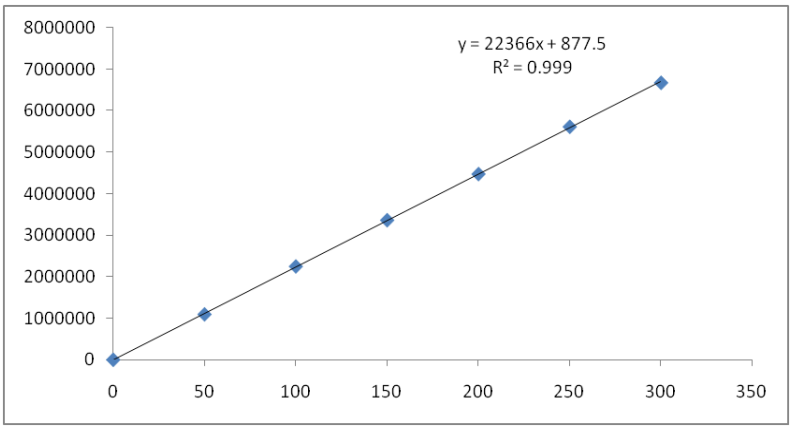

Fig. 4: Calibration curve of Olmesartan.

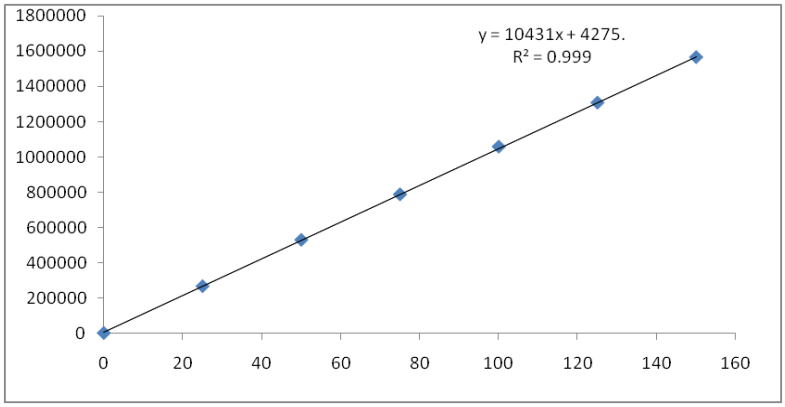

Fig. 5: Calibration curve of Cilnidipine.

Precision

System Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.6% and 0.9% respectively for Olmesartan and Cilnidipine .As the limit of Precision was less than "2" the system precision was passed in this method. The results are summarized in **Table.3.**

Table 3: System precision table of Olmesartan and Cilnidipine.

S. No.	Area of Olmesartan	Area of Cilnidipine
1.	4374991	1033483
2.	4433973	1037480
3.	4370504	1040265
4.	4387891	1030042
5.	4423266	1050247
6.	4415634	1024324
Mean	4401043	1035974
S.D	26741.2	8961.2
%RSD	0.6	0.9

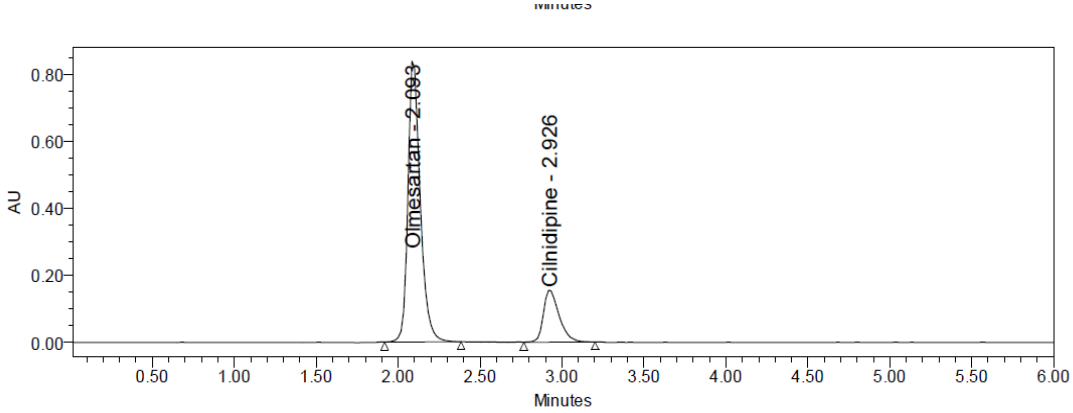

Fig. 6: System precision chromatogram.

Repeatability

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.2% and 0.2% respectively for Olmesartan and Cilnidipine. As the limit of Precision was less than "2" the system precision was passed in this method. The results are summarized in **Table.4.**

Table 4: Repeatability table of Olmesartan and Cilnidipine.

S. No	Area of Olmesartan	Area of Cilnidipine
1.	4367190	1029852
2.	4386768	1027669
3.	4374598	1032251
4.	4388843	1029148
5.	4394880	1028385
6.	4378512	1032018
Mean	4381799	1029887
S.D	10212.8	1889.6
%RSD	0.2	0.2

Intermediate precision (Day_ Day Precision)

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.5% and 0.3% respectively for Olmesartan and Cilnidipine. As the limit of Precision was less than "2" the system precision was passed in this method. The results are summarized in **Table.5.**

Table 5: Intermediate precision table of Olmesartan and Cilnidipine.

S. No	Area of Olmesartan	Area of Cilnidipine
1.	4360328	1018896
2.	4343389	1021204
3.	4375945	1024753
4.	4377782	1024427
5.	4366262	1025627
6.	4409915	1028462
Mean	4372270	1023895
S.D	22243.4	3379.6
%RSD	0.5	0.3

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.95 % and 100.81% for Olmesartan and Cilnidipine respectively. The results are summarized in **Table 6 and 7.**

Table 6: Accuracy table of Olmesartan.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	100	99.39647	99.40	99.95 %
	100	99.21986	99.22	
	100	99.58842	99.59	
100%	200	201.7938	100.9	
	200	199.2568	99.6	
	200	199.3183	99.7	
150%	300	299.7876	99.9	
	300	302.7471	100.9	
	300	301.0141	100.3	

Table 7: Accuracy table of Cilnidipine.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.917	99.83	100.81%
	50	50.144	100.29	
	50	49.502	99.00	
100%	100	99.884	99.88	
	100	100.489	100.49	
	100	99.677	99.68	
150%	150	150.64	100.42	
	150	151.21	100.81	
	150	150.53	100.36	

Table 8: Sensitivity table of Olmesartan and Cilnidipine.

Molecule	LOD	LOQ
Olmesartan	0.01 µg/mL	0.03 µg/mL
Cilnidipine	0.36 µg/mL	1.09 µg/mL

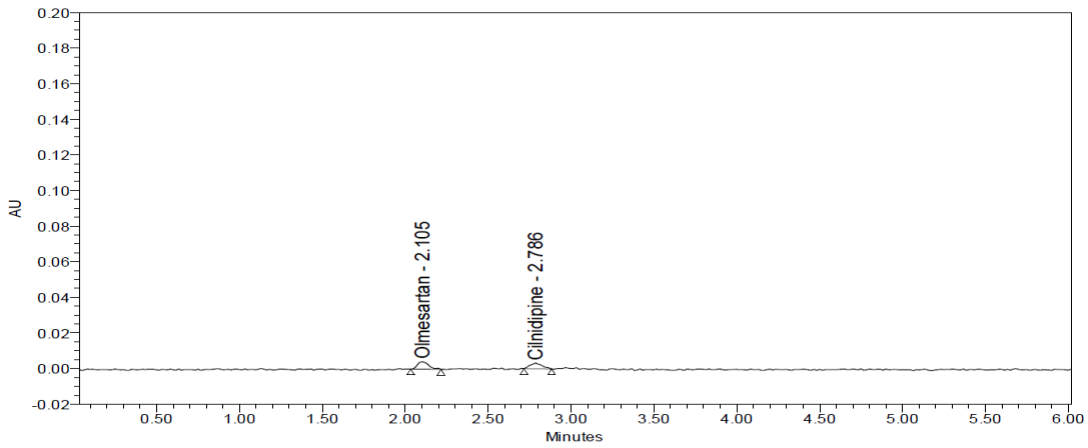

Fig. 7: LOD Chromatogram of Standard.

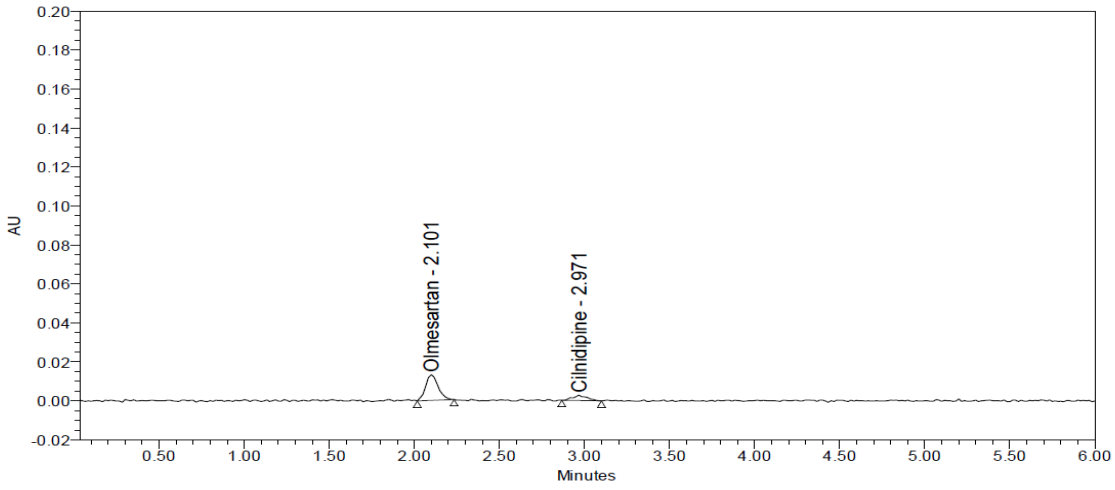

Fig. 8: LOQ Chromatogram of Standard.

Robustness

Robustness conditions like Flow minus (0.9 mL/min), Flow plus (1.1 mL/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit. The results are summarized in **Table 9.**

Table 9: Robustness data for Olmesartan and Cilnidipine.

S. No.	Condition	%RSD of Olmesartan	%RSD of Cilnidipine
1	Flow rate (-) 0.9ml/min	0.6	0.6
2	Flow rate (+) 1.1ml/min	0.7	0.9
3	Mobile phase (-) 55B:45A	0.1	0.1
4	Mobile phase (+) 65B:35A	0.2	0.3
5	Temperature (-) 25°C	0.6	0.2
6	Temperature (+) 35°C	0.3	0.1

Assay of formulation

Rhodes pharmaceuticals, bearing the label claim Olmesartan 500mg, Cilnidipine 50mg. Assay was performed with the above formulation. Average % Assay for Olmesartan and Cilnidipine obtained was 99.46% and 99.31% respectively. The results are summarized in **Table 10 and 11.**

Table 10: Assay Data of Olmesartan.

S. No	Standard Area	Sample area	% Assay
1	4374991	4367190	99.13
2	4433973	4386768	99.58
3	4370504	4374598	99.30
4	4387891	4388843	99.62
5	4423266	4394880	99.76
6	4415634	4378512	99.39
Avg	4401043	4381799	99.46
Stdev	26741.2	10212.8	0.23
% RSD	0.6	0.2	0.23

Table 11: Assay Data of Cilnidipine.

S. No	Standard Area	Sample area	% Assay
1	1033483	1029852	99.31
2	1037480	1027669	99.10
3	1040265	1032251	99.54
4	1030042	1029148	99.24
5	1050247	1028385	99.17
6	1024324	1032018	99.52

Avg	1035974	1029887	99.31
Stdev	8961.2	1889.6	0.1822
%RSD	0.9	0.2	0.2

DEGRADATION

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation. The results are summarized in **Table.12 and 13.**

Table 12: Degradation Data of Olmesartan.

S. No.	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.69	0.097	0.282
2	Alkali	4.41	0.097	0.260
3	Oxidation	6.13	0.097	0.283
4	Thermal	4.18	0.098	0.286
5	UV	2.77	0.091	0.282
6	Water	2.77	0.090	0.282

Table 13: Degradation Data of Cilnidipine.

S. No	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	5.49	0.346	0.571
2	Alkali	4.82	0.351	0.561
3	Oxidation	3.03	0.379	0.594
4	Thermal	2.94	0.363	0.600
5	UV	2.52	0.393	0.626
6	Water	0.87	0.523	0.771

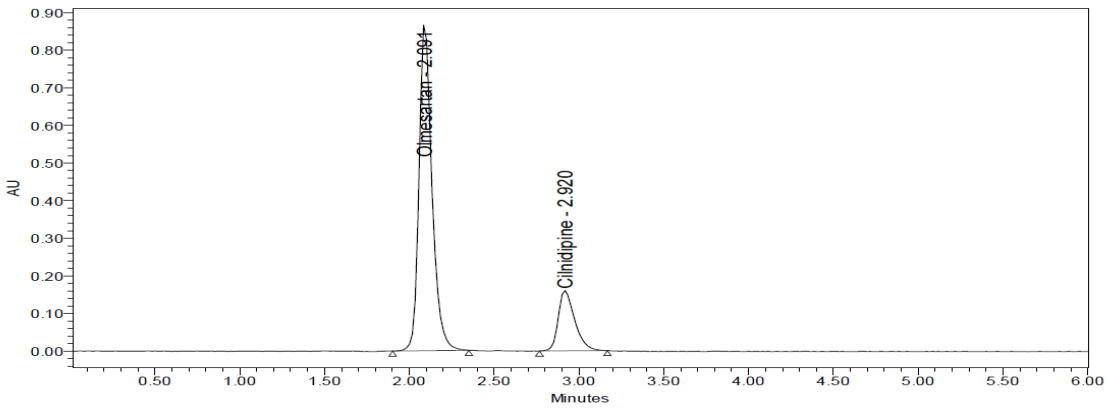

Fig. 9: Acid degradation chromatogram of Olmesartan and Cilnidipine.

Table 14: Summary Table.

Parameters		Olmesartan	Cilnidipine	LIMIT
Linearity Range (µg/ml)		50-300µg/ml	25-150 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		22366	10431	
Intercept(c)		877.5	4275	
Regression equation (Y=mx+c)		y = 22366x + 877.5	y = 10431x + 4275	
Assay (% mean assay)		99.46%	99.31%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.6	0.9	NMT 2.0%
Method precision %RSD		0.2	0.2	NMT 2.0%
Accuracy %recovery		99.95%	100.81%	98-102%
LOD		0.01	0.36	NMT 3
LOQ		0.03	1.09	NMT 10
Robustness	**FM**	0.6	0.6	% RSD NMT 2.0
	FP	0.7	0.9	
	MM	0.1	0.1	
	MP	0.2	0.3	
	TM	0.6	0.2	
	TP	0.3	0.1	

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Olmesartan and Cilnidipine in Tablet dosage form. Retention time of Olmesartan and Cilnidipine were found to be 2.098 min and 2.928 min. %RSD of the Olmesartan and Cilnidipine were and found to be 0.2 and 0.2 respectively. %Recovery was obtained as 99.95% and 100.81% for Olmesartan and Cilnidipine respectively. LOD, LOQ values obtained from regression equations of Olmesartan and Cilnidipine were 0.01, 0.36 and 0.03, 1.09respectively. Regression equation of Olmesartan is y = 22366x + 877.5, and y = 10431x + 4275 of Cilnidipine. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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