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THREE DRUG COMBINATION OF OLMESARTAN, CILNIDIPINE AND CHLORTHALIDONE QUANTITATIVE DETERMINATION BY USING UPLC

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ISSN 0799-3757

http://caribjscitech.com/

Abstract: Olmesartan, Cilnidipine and Chlorthalidone medicinal products are used to treat calcium channel blocking, high blood pressure and diuretic treatment. UPLC method was developed and validated. Chromatographic conditions are 1mL of OPA in a 1000ml of water and Acetonitrile 45:55 v/v as mobile phase, flow rate 0.3 ml/min, column SB C8 100x 3.0, 1.8mm, wave length 260nm, column temperature 30°C, injection volume 5µL, run time 4 min, diluent water and acetonitrile (50:50 v/v). Validated UPLC method can be used to quantify these three drugs in three combination product.

Keywords:

Abstract:
Olmesartan, Cilnidipine and Chlorthalidone medicinal products are used to treat calcium channel blocking, high blood pressure and diuretic treatment. UPLC method was developed and validated. Chromatographic conditions are 1mL of OPA in a 1000ml of water and Acetonitrile 45:55 v/v as mobile phase, flow rate 0.3 ml/min, column SB C8 100x 3.0, 1.8mm, wave length 260nm, column temperature 30°C, injection volume 5µL, run time 4 min, diluent water and acetonitrile (50:50 v/v). Validated UPLC method can be used to quantify these three drugs in three combination product.

Introduction:
Olmesartan is an angiotensin receptor blocker and used to reduce the risk from high blood pressure (hypertension)[1-3]. Olmesartan chemical name is (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate. This can be used alone or combination with other antihypertensive drug products[4-5].

Cilnidipine is used as Calcium channel blocker. It works as Calcium antagonist with L-type and N-type Calcium channel blocking functions[6-8]. Chemical of Cilnidipine is 3-(*E*)-3-Phenyl-2-propenyl 5-2-methoxyethyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Cilnidipine reduce the blood pressure and is used to treat hypertension and its comorbidities[9-10]. The main side effects of this drug are dizziness, heartbeat speed, face, hands and legs swelling.

Chlortalidone is used to treat diuretic medication and high blood pressure, enlargement of the main pumping chamber of the heart, swelling and fluid retention[11-13]. Chemical name is (*RS*)-2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol-1-yl) benzene-1-sulfonamide. Chlortalidone is more effect use than hydrochlorothiazide for lowering blood pressure[14-15]. It is also used with the combination of angiotensin converting enzyme inhibitor or angiotensin II receptor blocker. Chemical structure of the three components Olmesartan, Cilnidipine and Chlortalidone were represented in figure-1.

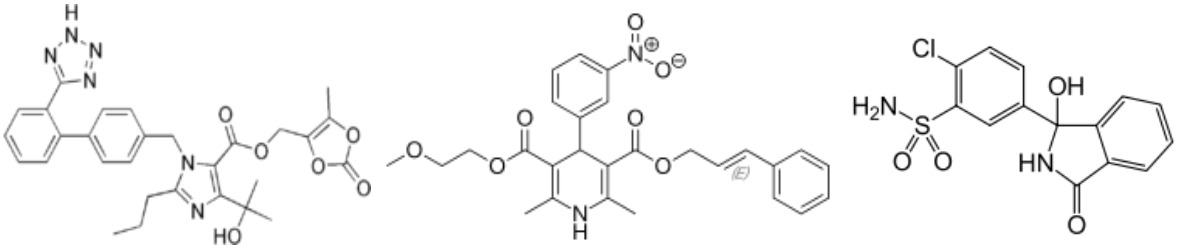

Figure-1: Chemical structure of Olmesartan Cilinidipine and Chlortalidone

Literature reports confirmed that there are few HPLC methods reported for two drug combination[16-17] or combination with other drug products[18-19]. The main objective of this study was to develop a simple and stability indicating UPLC method to quantify the three drugs in single method.

Materials and Method:

Buffer: (0.1%OPA)
1mL of ortho phosphoric acid solution in a 1000ml of volumetric flask add about 100ml of milli-Q water and final volume make up to 1000 ml with milli-Q water

Mobile phase:
Buffer and Acetonitrile taken in the ratio 45:55 v/v
Chromatographic conditions:

Flow rate	**:**	0.3 ml/min
Column	**:**	SB C8 100x 3.0, 1.8mm.
Detector wave length	**:**	260nm
Column temperature	**:**	30°C
Injection volume	**:**	5µL
Run time	**:**	4 min
Diluent	:	Water: Acetonitrile (50:50 v/v)

Preparation of Solutions:
Standard Preparation:
Accurately Weighed and transferred Olmesartan 10mg, Cilnidipine 5mg & and Chlorthalidone 6.25mg working Standards into a separate 10 ml clean dry volumetric flasks, 5ml of diluent added and sonicated for 30 minutes and make up to the final volume with diluents. 1 ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluents. (Olmesartan 100ppm & Cilnidipine 50ppm& Chlorthalidone 62.5ppm)
Sample Preparation:
5 tablets were weighed and calculated the average weight of each tablet then the equivalent to 1 tablet weight powder was transferred into a 50 mL volumetric flask, 20mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. 2.5ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluents.

Results and Discussion:
Method Development:
UPLC method development was initiated based on the literature published reports and physical and chemical properties of the analytes. Initial method development was progressed with ammonium acetate buffer and methanol as organic modifier.
Method development trial-1:
Conditions: 1. 1.3g of ammonium acetate buffer salt in 1000 ml of water used as buffer; 2. Buffer and acetonitrile 50: 50 v/v used as mobile phase A; 3. methanol used as mobile phase B; 4. Zorbax C18 150x3.0mm, 1.8µ column; 5. Flow rate 0.4ml/min, 30°C column temperature, 260 nm; 6. Mobile phase: A:B 50:50 v/v 7. Diluent: water and acetonitrile 45:55 v/v.
Observation: Olmesartan and Cilnidipine were eluted but chlorthalidone not eluted. Cilnidipine peak shape was poor. Method development trail chromatogram was represented in figure-2.

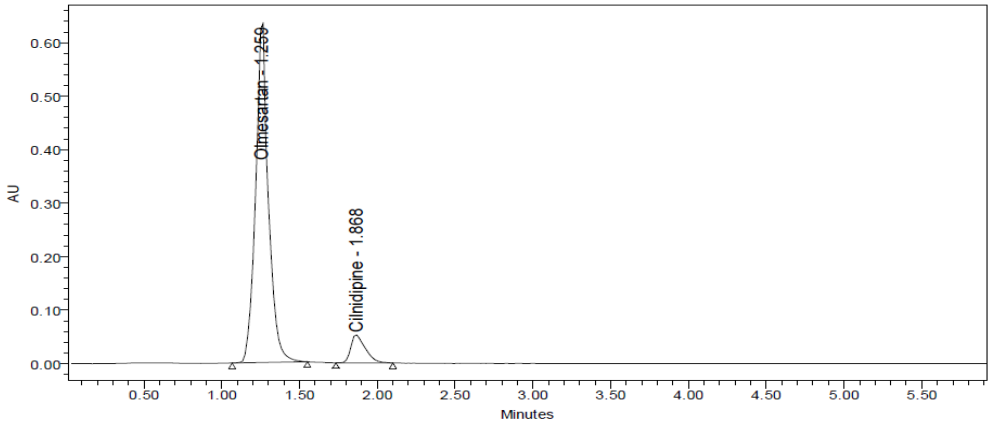

Figure-2: Method development trail-1 chromatogram

Method development trial-2:

Conditions: 1. 3.4g of K_2HPO_4 in 1000 ml of water used as buffer; 2. Buffer and acetonitrile 80: 20 v/v used as mobile phase A; 3. acetonitrile used as mobile phase B; 4. Zorbax C8 150x3.0mm, 1.8µ column; 5. Flow rate 0.4ml/min, 30°C column temperature, 230 nm; 6. Gradient program at 0 min 20% mobile phase B, at 10 min 43%, at 15 min 83%, at 25 min 83%, at 28 min 20% and at 35 min 20%; 7. Diluent: water and acetonitrile 45:55 v/v.

Observation: Olmesartan peak was eluted with multiple tops and peak shape was poor. Chlorthalidone was not eluted. Method development trail chromatogram was represented in figure-3.

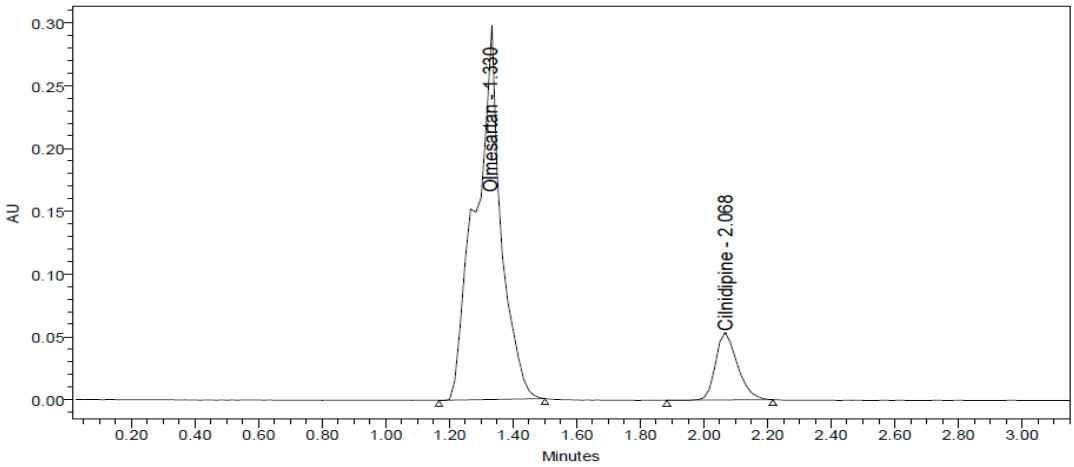

Figure-3: Method development trail-2 chromatogram

Method development trial-3:

Conditions: 1. Ortho phosphoric acid 1ml in 1000 ml of water used as buffer; 2. Buffer used as mobile phase A; 3. acetonitrile used as mobile phase B; 4. Zorbax C18 150x4.6mm, 5µ column; 5. Flow rate 1.0ml/min, 30°C column temperature, 230 nm; 6. Gradient program at 0 min 20% mobile phase B, at 10 min 43%, at 15 min 83%, at 25 min 83%, at 28 min 20% and at 35 min 20%; 7. Diluent: water and acetonitrile 45:55 v/v.

Observation: All three analytes Olmesartan, Cilnidipine and Chlorthalidone were eluted but Olmesartan and Cilnidipine base line separation was poor. Chlorthalidone peak shape was poor. Method development trail chromatogram was represented in figure-4.

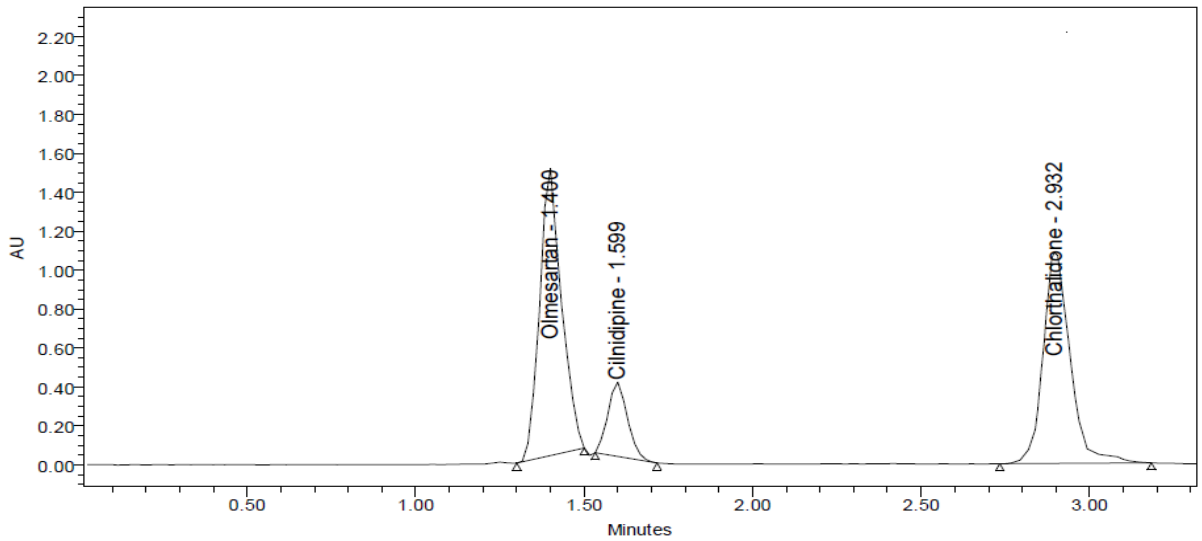

Figure-4: Method development trail-3 chromatogram

Method development trial-4:

Conditions: Buffer: 1mL of OPA in a 1000ml of volumetric flask, water added and final volume make up with milli-Q water. Mobile phase: Buffer and Acetonitrile 45:55 v/v. Flow rate: 0.3 ml/min, Column: SB C8 100x 3.0, 1.8mm, Detector wave length: 260nm, Column temperature: 30°C, Injection volume: 5mL, Run time: 4 min, Diluent: Water: Acetonitrile (50:50v/v).

Observation: Separation of three components was achieved and peak shape of each ingredient also good. Hence these chromatographic conditions were finalized and further method validation was performed. Method development trail chromatogram was represented in figure-5.

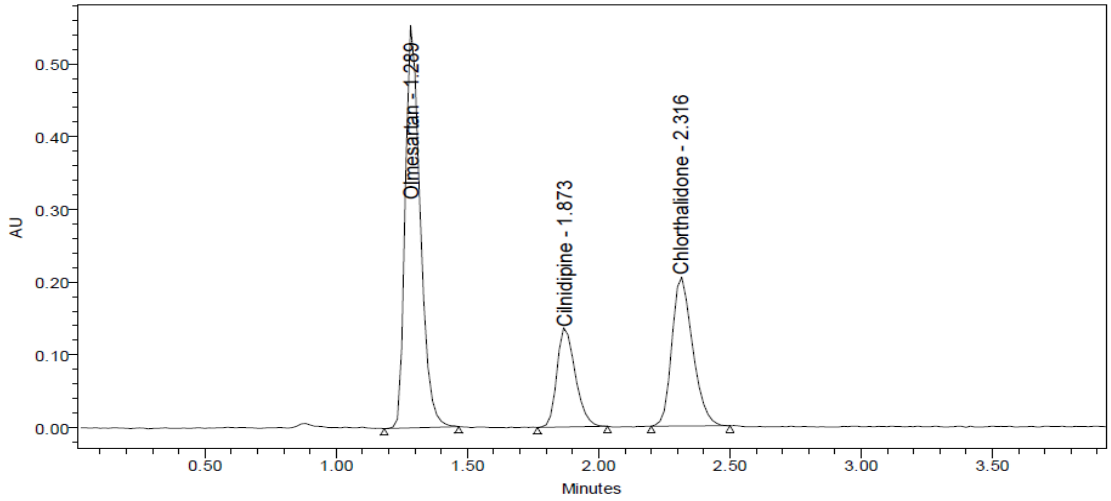

Figure-5: Method development trail-4 chromatogram

Method Validation:

Method validation was carried out with system suitability evaluation, precision, linearity, accuracy, robustness and ruggedness.

System suitability:

UPLC method system suitability was evaluated to confirm the method consistency and system suitability parameters. Blank, placebo and standard solution chromatograms were represented in figure-6 to 8. Table-1 represented the system suitability results. Retention time of all three analytes is Olmesartan 1.28 min, Cilnidipine 1.89 min and Chlorthalidone 2.34 min. Six replicate injections peak area average value and %RSD were calculated and results found within the limit.

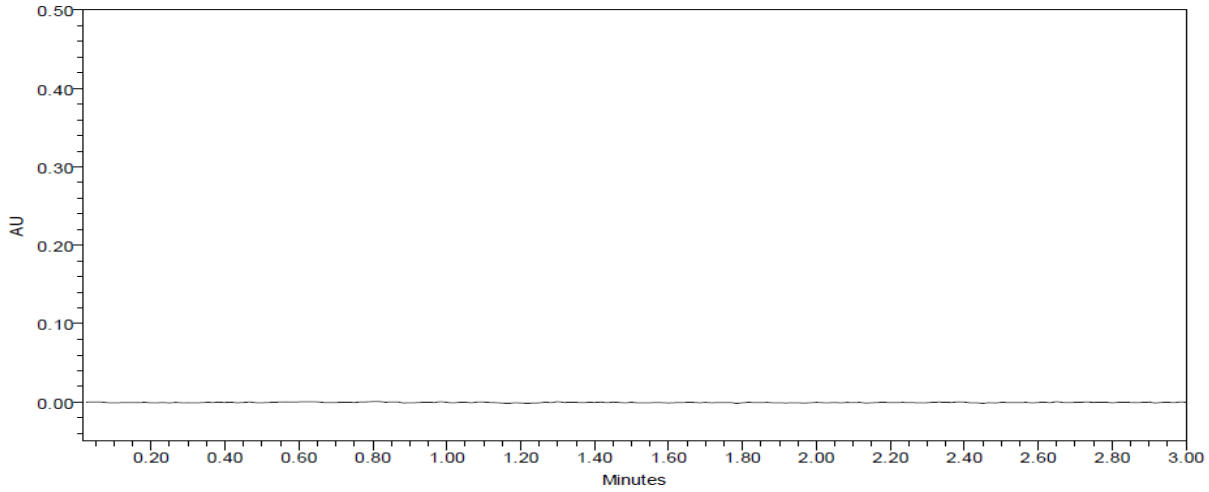

Figure-6: Blank chromatogram

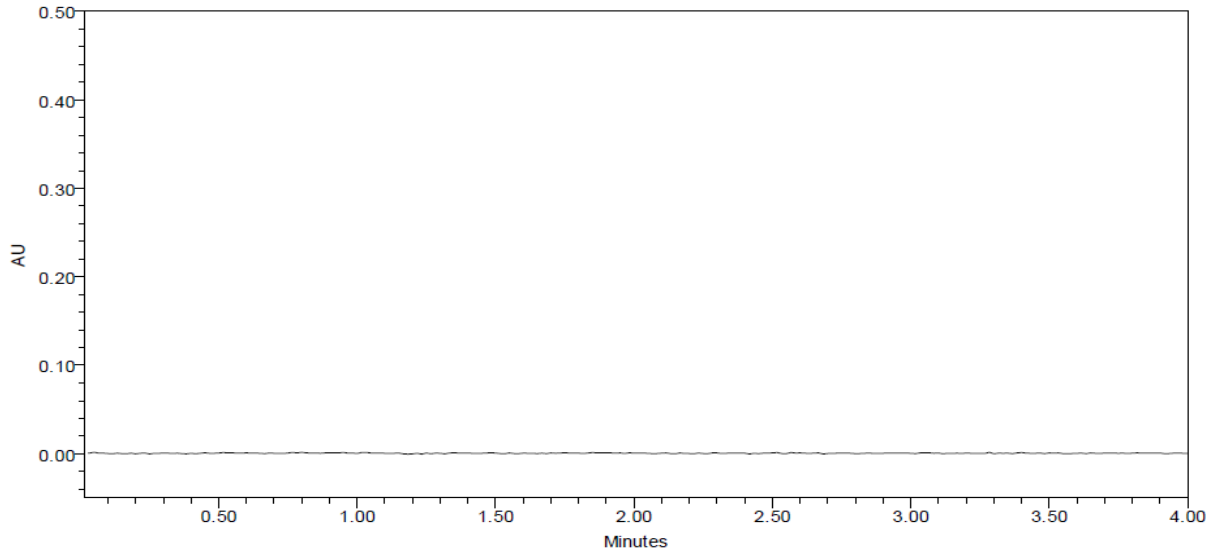

Figure-7: Placebo chromatogram

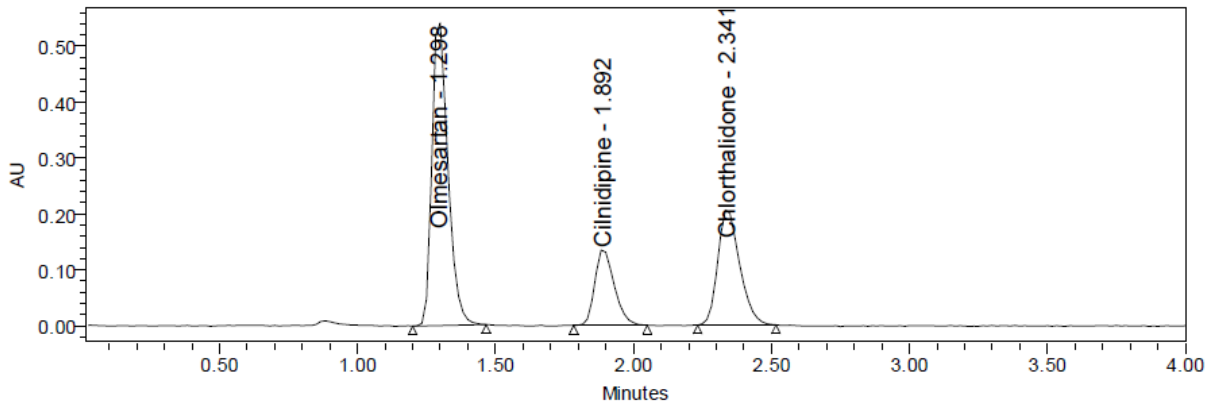

Figure-8: Standard chromatogram

System suitability results						
Injection	RT (min)			Area		
	Olmesartan	Cilnidipine	Chlorthalidone	Olmesartan	Cilnidipine	Chlorthalidone
1.	1.286	1.874	2.321	2144629	644952	1127107
2.	1.286	1.875	2.322	2165238	655350	1101592
3.	1.291	1.88	2.334	2178116	661056	1130096
4.	1.291	1.889	2.335	2202580	653413	1126627
5.	1.298	1.892	2.40	2194699	658357	1129405
6.	1.302	1.892	2.341	2169716	661169	1133358
Average				2175830	655716	1124698
%RSD			NA	1.0	0.9	1.0
Tailing factor	1.26	1.24	1.25	NA		

Table-1: System suitability results

Precision:

Method precision and system precision (intermediate precision) was evaluated with six replicate preparations. Precision samples were prepared as per the test procedure mentioned Six preparations assay values were represented in table-2. Intermediate precision was evaluated with different analyst, different UPLC system and different lot column.

S.No.	Precision assay (%)			Intermediate precision assay (%)		
	Olmesartan	Cilnidipine	Chlorthalidone	Olmesartan	Cilnidipine	Chlorthalidone
1	101.87	101.47	100.02	100.21	99.96	100.31
2	100.59	101.04	100.02	101.20	100.26	101.01
3	100.2	98.81	101.18	100.26	100.31	100.25
4	99.13	99.37	100.55	100.35	101.21	100.61
5	100.13	100.41	100.67	100.61	100.12	99.98
6	99.29	99.75	100.42	100.25	100.36	100.21
Avg.	100.14	100.48	100.48	100.48	10037	100.39
%RSD	0.99	1.02	0.44	0.38	0.43	0.36

Table-2: Precision and intermediate results

Specificity:

Specificity was evaluated with different degradation conditions such as acid, base, peroxide, thermal, water, UV/visible and humidity conditions. Test solutions were stressed with different stress conditions. Peak purity results were calculated. Specificity results and peak purity results were satisfactory. Specificity chromatograms were represented in figure-9 to 15. Specificity results were tabulated in table-3.

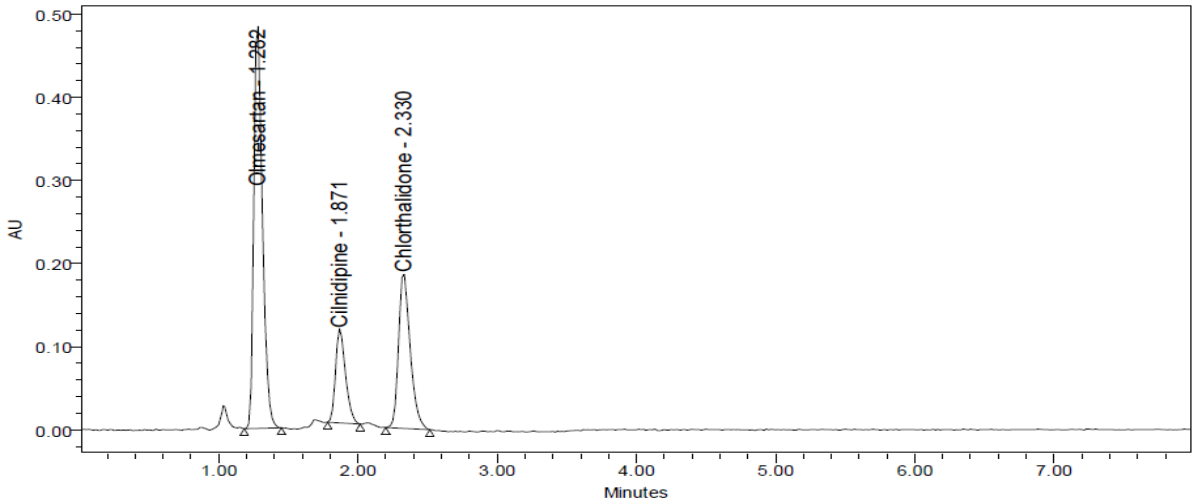

Figure-9: Acid Degradation chromatogram

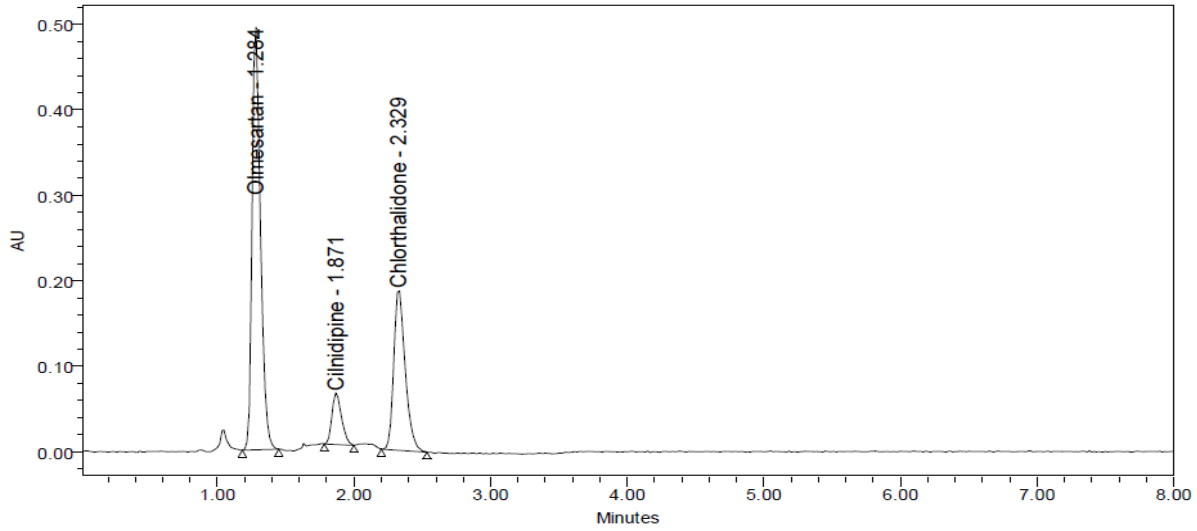

Figure-10: Base Degradation chromatogram

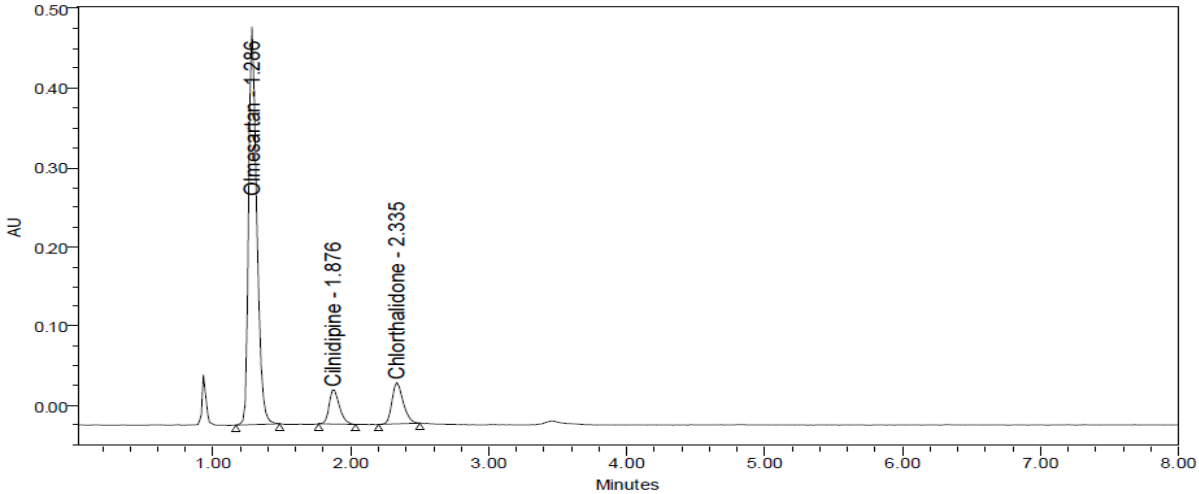

Figure-11: Peroxide Degradation chromatogram

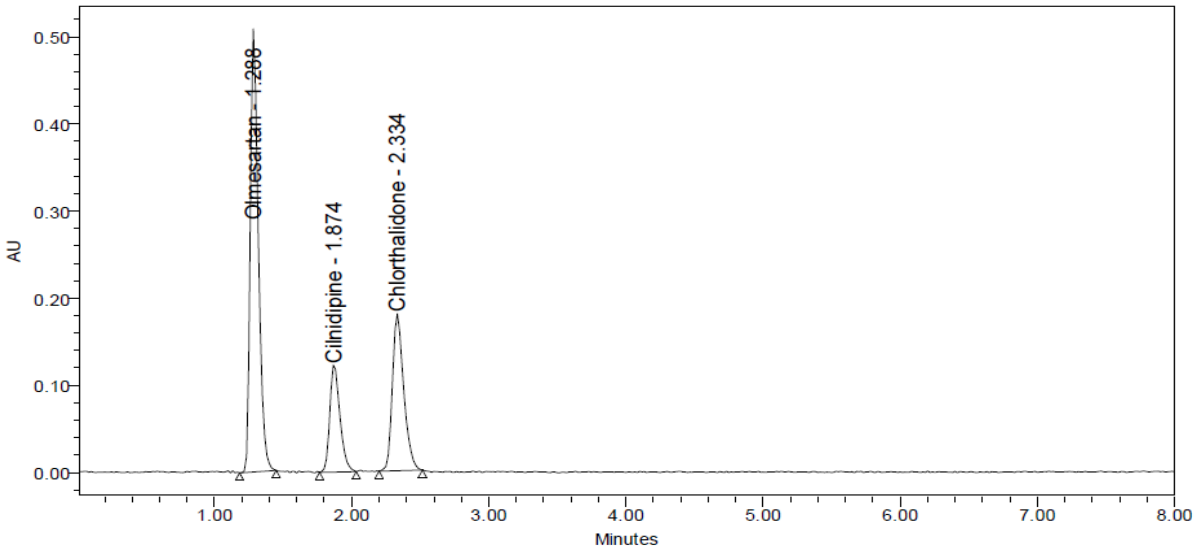

Figure-12: Thermal Degradation chromatogram

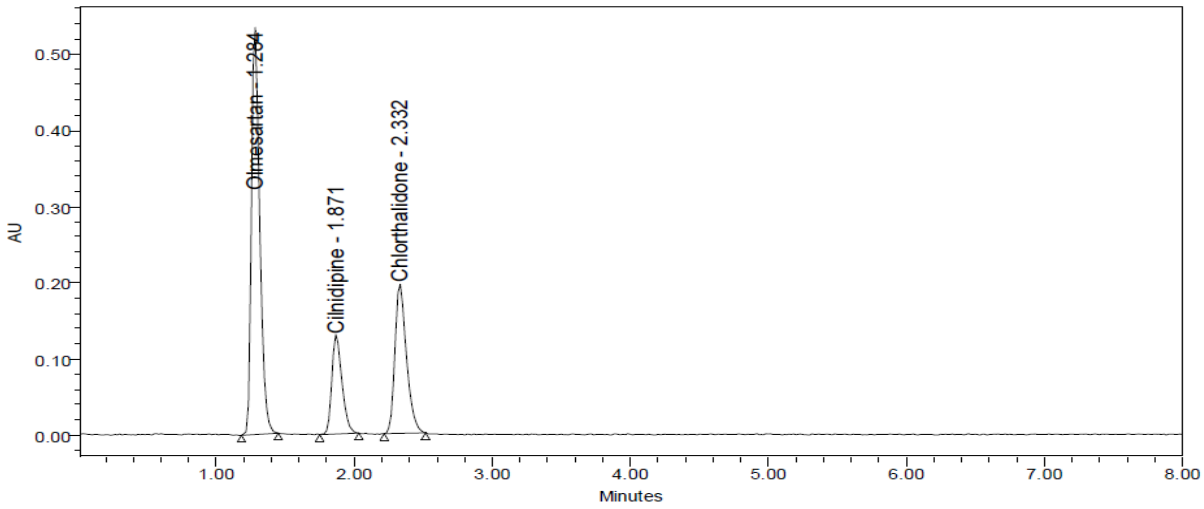

Figure-13: Water Degradation chromatogram

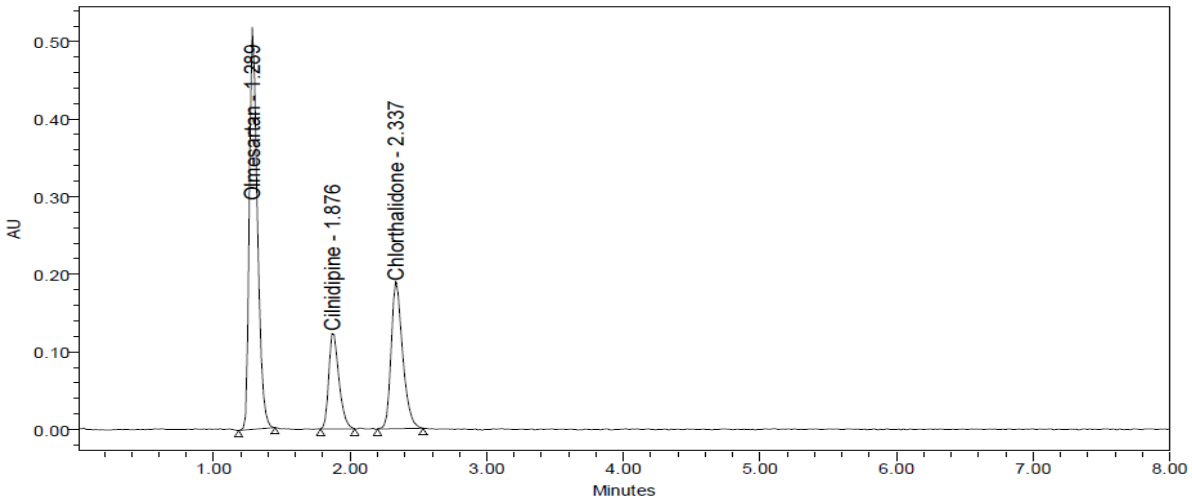

Figure-14: UV/ Visible Degradation chromatogram

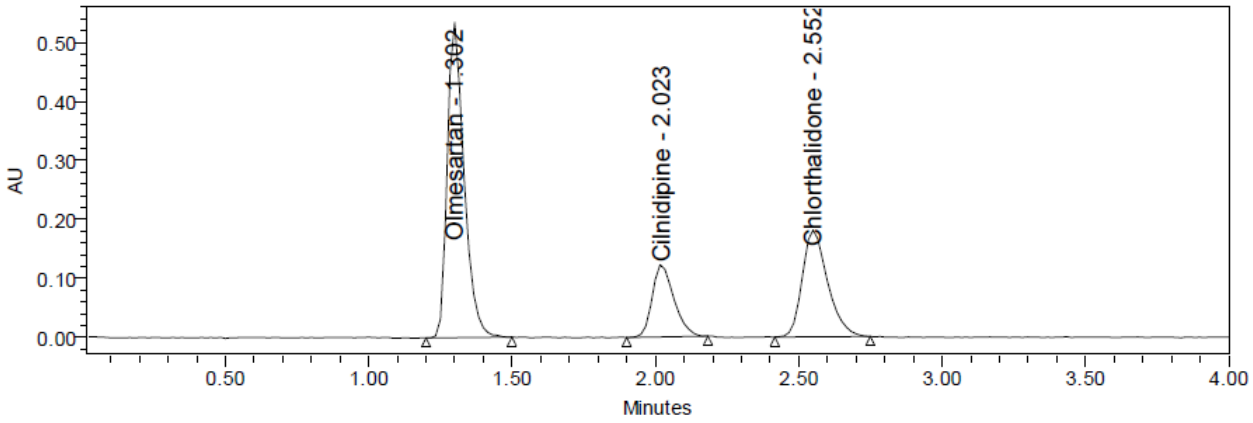

Figure-15: Humidity degradation chromatogram

Olmesartan degradation results				
S.No.	**Name of Stress and condition**	**% assay**	**% degradation**	**Peak purity**
1.	Acid stress/2N HCl-60°C/30 min	95.66	4.92	PASS
2.	Base Stress/1N NaOH- 60°C/ 30min	97.08	4.07	PASS
3.	Peroxide stress/2%- 2-8°C/30min	90.89	9.11	PASS
4.	Thermal (105°C for 6 hrs)	95.53	1.85	PASS
5.	UV/visible light / 7 days	97.81	0.48	PASS
6.	Water stress-60°C/2 hrs	98.65	0.28	PASS
7.	Humidity / 75%RH, 40°C 2days	98.52	1.48	PASS
Cilnidipine degradation results				
1.	Acid stress/2N HCl-60°C/30 min	94.78	5.22	PASS
2.	Base Stress/1N NaOH- 60°C/ 30min	92.08	3.91	PASS
3.	Peroxide stress/2%- 2-8°C/30min	94.04	2.69	PASS
4.	Thermal (105°C for 6 hrs)	96.19	0.89	PASS
5.	UV/visible light / 7 days	98.18	1.82	PASS
6.	Water stress-60°C/2 hrs	99.18	0.82	PASS
7.	Humidity / 75%RH, 40°C 2days	99.16	0.84	PASS
Chlorthalidone degradation results				
1.	Acid stress/2N HCl-60°C/30 min	93.92	3.25	PASS
2.	Base Stress/1N NaOH- 60°C/ 30min	96.80	2.32	PASS
3.	Peroxide stress/2%- 2-8°C/30min	90.66	1.80	PASS
4.	Thermal (105°C for 6 hrs)	94.8	1.00	PASS
5.	UV/visible light / 7 days	97.21	0.52	PASS
6.	Water stress-60°C/2 hrs	98.34	0.35	PASS
7.	Humidity / 75%RH, 40°C 2days	98.69	1.31	PASS

Table-3: Specificity results

Linearity:

Linearity was validated with six different concentration levels such as 25%, 50%, 75%, 100%, 125% and 150%. Three replicates were injected for each linearity level and average values of each linearity levels were calculated. Concentration vs linearity levels correlation coefficient values were calculated. Linearity chromatograms were represented in figure-16 to 21. Linearity results were tabulated in table-4. Linearity results were satisfactory.

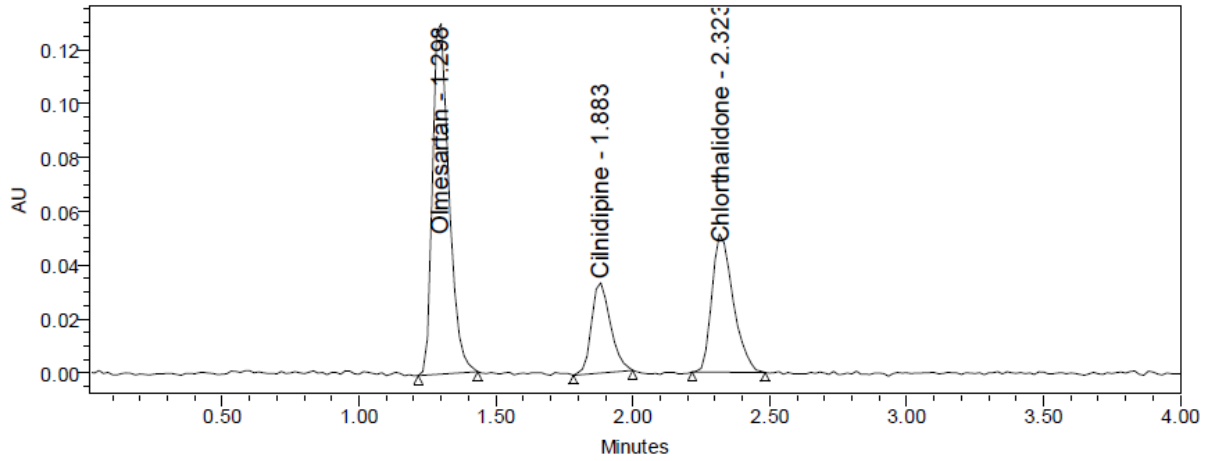

Figure-16: Linearity 25% level chromatogram

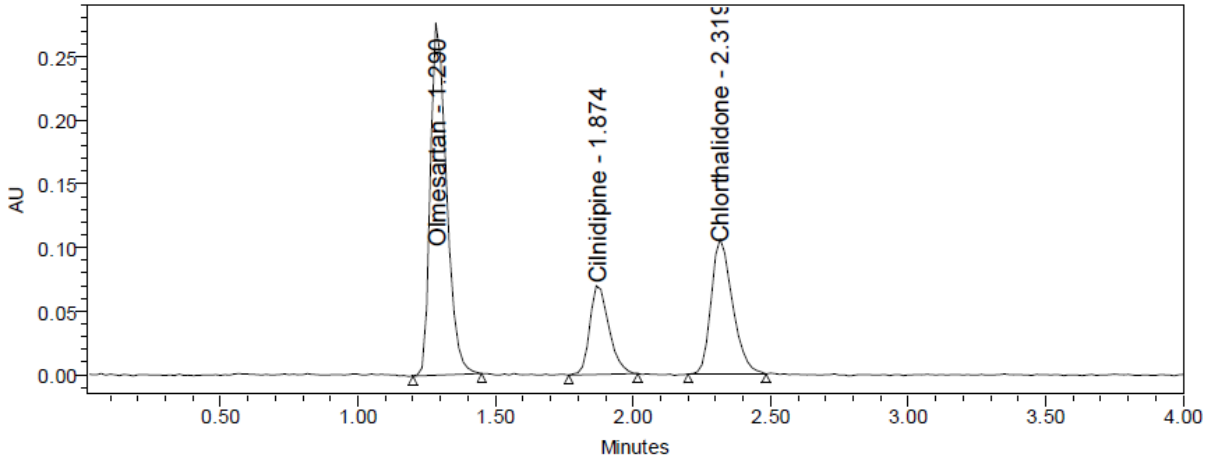

Figure-17: Linearity 50% level chromatogram

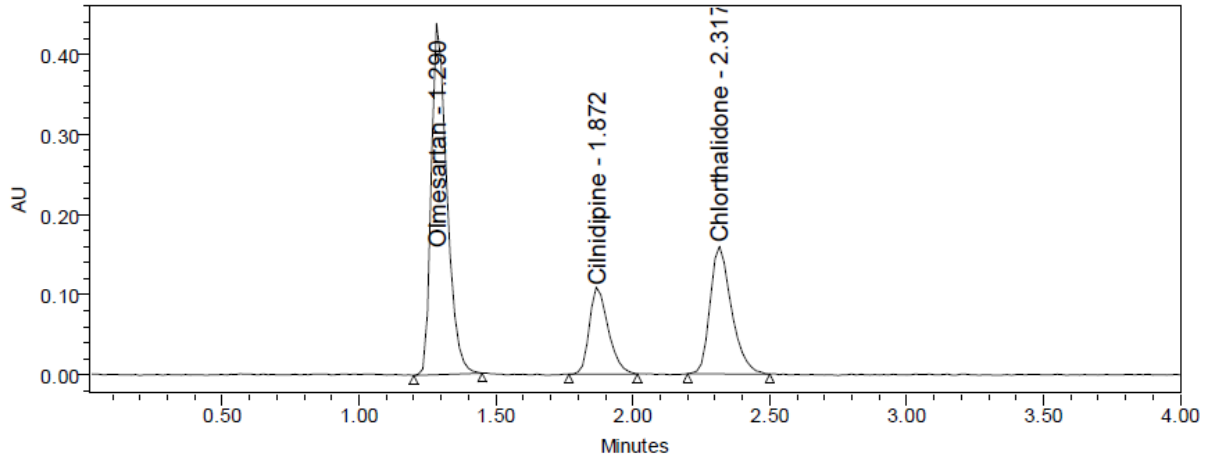

Figure-18: Linearity 75% level chromatogram

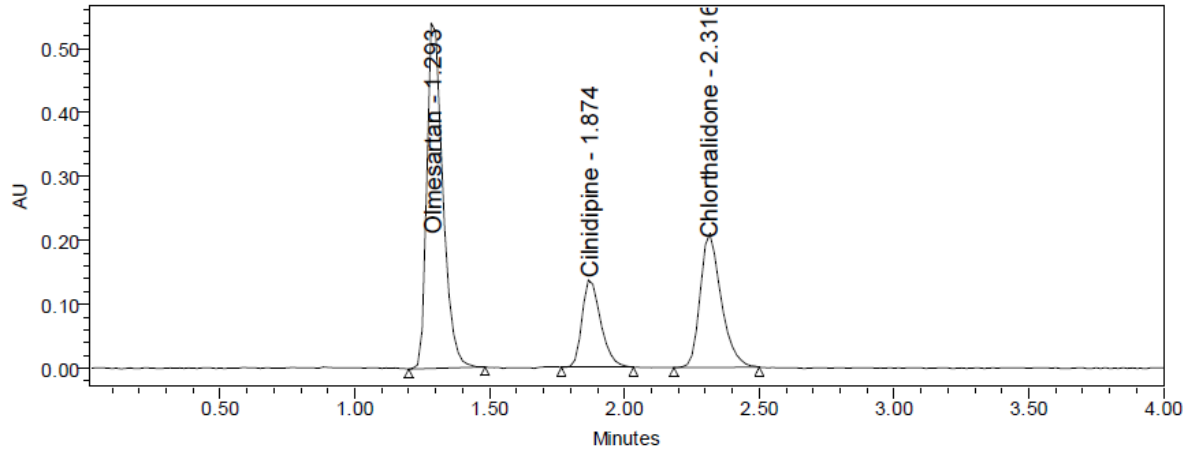

Figure-19: Linearity 100% level chromatogram

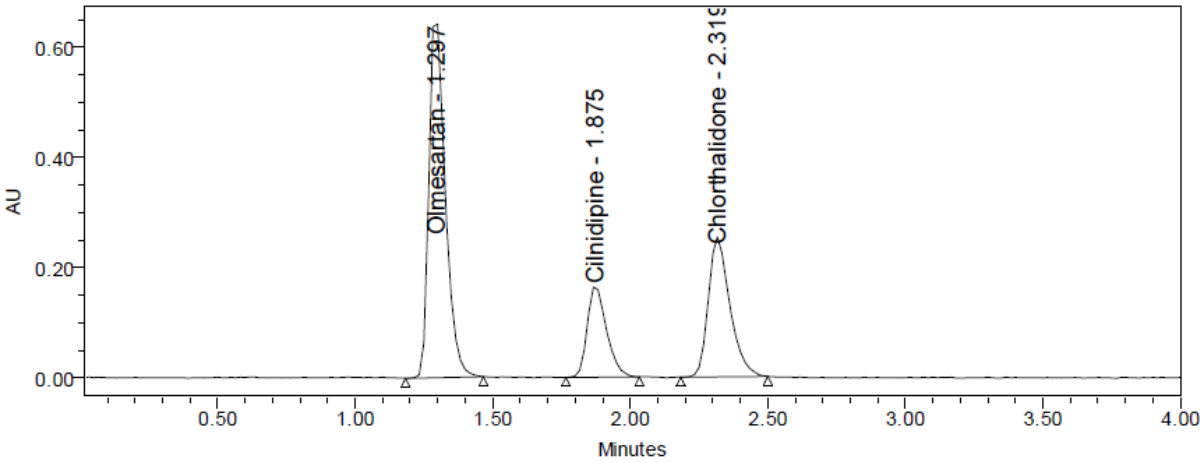

Figure-20: Linearity 125% level chromatogram

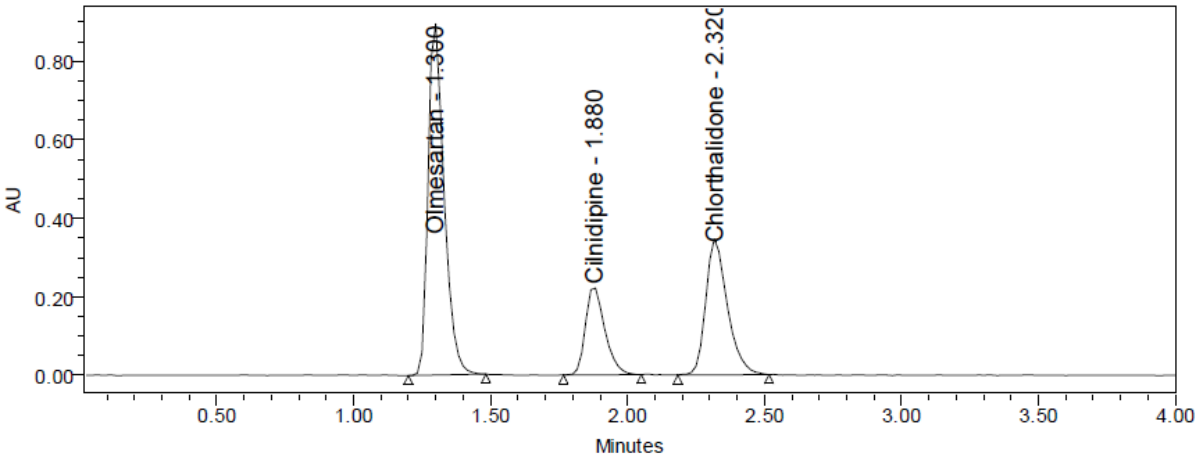

Figure-21: Linearity 150% level chromatogram

	Olmesartan		Cilnidipine		Chlorthalidone	
Linearity level	**Conc.**	**Area**	**Conc.**	**Area**	**Conc.**	**Area**
25%	25	541715	12.5	161781	15.625	284985
50%	50	1088512	25	334691	31.25	568913
75%	75	1646945	37.5	504659	46.875	847626
100%	100	2168566	50	658022	62.5	1125726
125%	125	2699357	62.5	813767	78.125	1393383
150%	150	3273416	75	983295	93.75	1678474
Corr.Coe.	0.9999		0.9997		0.9999	

Table-4: Linearity results

Accuracy:

Accuracy of the method was performed with 50% level, 100% level and 150% level. These three accuracy level concentrations were prepared as per the test concentration. Placebo stock solutions were spiked to test solution to achieve the target concentrations. Accuracy recovery values were calculated against the concentrations added. Recovery results found within the acceptable limit 97% to 103%. Recovery results were tabulated in table-4.

Olmesartan accuracy results									
Level	50%			100%			150%		
Recovery (%)	99.47	98.91	99.51	99.93	99.88	101.95	100.60	100.95	100.39
Mean (%)	99.29			100.59			100.64		
Cilnidipine accuracy results									
Level	50%			100%			150%		
Recovery (%)	101.30	100.86	99.14	101.27	99.15	99.69	100.54	100.44	99.84
Mean (%)	100.43			100.04			100.27		
Chlorthalidone accuracy results									
Level	50%			100%			150%		
Recovery (%)	100.00	99.16	99.95	100.53	101.0	100.10	100.81	100.73	100.08
Mean (%)	99.70			100.54			100.54		

Table-4: Accuracy results

Ruggedness:

Ruggedness of the method was evaluated with bench top and refrigerator storage stability studies. Precision samples were used for ruggedness studies. Both samples were kept on bench top and refrigerator and analysed after day-1 and day 3 for bench top storage samples, day 3 and day-5 for refrigerator storage conditions. Results were tabulated in table-5 and ruggedness results were satisfactory.

Olmesartan ruggedness results							
Time in day	Bench top stability test solution				Tailing factor	%RSD	Bench top stability standard solution
	Test-1	Test-2	Difference				Similarity factor
			Test-1	Test-2			
Initial	101.87	100.59	NA	NA	1.2	0.60	0.99
Day-1	101.20	100.25	0.67	0.34	1.4	0.25	1.00
Day-3	101.13	100.10	0.74	0.49	1.3	0.31	0.98
	Refrigerator stability test solution						Refrigerator stability standard solution
Initial	101.87	100.59	NA	NA	1.3	0.56	0.99
Day-3	101.28	100.29	0.59	0.30	1.5	0.29	0.98
Day-5	101.03	100.21	0.84	0.38	1.2	0.39	0.99
Cilnidipine ruggedness results							
Time in day	Bench top stability test solution				Tailing factor	%RSD	Bench top stability standard solution
	Test-1	Test-2	Difference				Similarity factor
			Test-1	Test-2			
Initial	101.47	101.04	NA	NA	1.2	0.56	0.99
Day-1	101.21	100.29	0.26	0.75	1.6	0.36	1.00
Day-3	101.30	100.26	0.17	0.78	1.3	0.52	0.99
	Refrigerator stability test solution						Refrigerator stability standard solution
Initial	101.47	101.04	NA	NA	1.3	0.54	1.00
Day-3	101.21	100.61	0.26	0.43	1.5	0.53	0.98
Day-5	101.31	100.68	0.16	0.36	1.3	0.58	0.99
Chlorthalidone ruggedness results							
Time in day	Bench top stability test solution				Tailing factor	%RSD	Bench top stability standard solution
	Test-1	Test-2	Difference				Similarity factor
			Test-1	Test-2			
Initial	100.02	100.02	NA	NA	1.4	0.51	0.99
Day-1	100.25	100.15	0.23	0.13	1.2	0.56	0.98
Day-3	100.69	100.1	0.67	0.08	1.3	0.49	1.00
	Refrigerator stability test solution						Refrigerator stability standard solution
Initial	101.47	101.04	NA	NA	1.3	0.43	1.00
Day-3	101.21	100.80	0.26	0.24	1.6	0.38	0.99
Day-5	101.52	100.29	0.05	0.75	1.4	0.29	0.98

Table-5: Ruggedness results

Robustness:

Method robustness was validated with chromatographic conditions variations and filter validation. Chromatographic variations flow rate, column oven temperature and mobile phase organic solvent ratio variations. Robustness results were tabulated in table-6 and 7. Results confirmed the method robustness and meeting the acceptable limits.

Variation condition		**Flow rate ml/min**			**Column temperature**		
Variation changes		0.8	1.0	1.2	25°C	30°C	35°C
Olmesartan	Tailing factor	1.3	1.4	1.2	1.4	1.3	1.6
	% RSD	0.3	0.5	0.4	0.3	0.5	0.4
Cilnidipine	Tailing factor	1.2	1.5	1.3	1.5	1.3	1.4
	% RSD	0.6	0.4	0.3	0.4	0.6	0.4
Chlorthalidone	Tailing factor	1.4	1.3	1.6	1.3	1.4	1.2
	% RSD	0.41	0.3	0.4	0.6	0.5	0.6
Variation condition		**M.P organic solvent ratio**					
Variation changes		55:45	60:40	65:35			
Olmesartan	Tailing factor	1.5	1.4	1.3			
	% RSD	0.6	0.4	0.6			
Cilnidipine	Tailing factor	1.3	1.5	1.3			
	% RSD	0.5	0.6	0.3			
Chlorthalidone	Tailing factor	1.4	1.5	1.2			
	% RSD	0.5	0.4	0.3			

Table-6: Results of Effect of variations

Olmesartan filter validation (% assay)									
Centrifuged		**Nylon filter**				**PVDF filter**			
% assay		% assay		% Difference		% assay		% Difference	
Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2	Spl-1	Spl-2
99.98	100.21	100.31	99.99	0.33	0.22	100.10	100.16	0.12	0.05
Cilnidipine filter validation									
100.21	100.32	100.20	100.51	0.01	0.19	100.25	100.31	0.04	0.01
Chlorthalidone filter validation									
100.31	100.25	100.21	100.69	0.10	0.44	100.61	100.28	0.30	0.03

Table-7: Filter Variability results

Conclusion:

Olmesartan, Cilnidipine and chlorthalidone are available in solid dosage form. This combination product can be used to treat Calcium channel blocker, angiotensin receptor blocker, treat diuretic medication and high blood pressure. Optimized method was validated with precision, ruggedness, robustness, accuracy, linearity and specificity. UPLC method confirmed the method intended capability and specificity. Method can be applied for regular evaluation of this three drug product combinations.

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