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A Stability Indicating RP-HPLC Method Development and Validation for the Determination of Combined Tablet Formulation of Amlodipine & Candesartan.

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ABSTRACT

A stability indicating high Performance Liquid Chromatographic (HPLC) method was developed and validated for the determination of combined tablet formulation of Amlodipine & Candesartan. Chromatographic separation was optimized by RP-HPLC on a Grace C18 (250mm x 4.6ID, Particle size: 5 micron) Software was HPLC Workstation utilizing a mobile phase consisting a Methanol: P. Buffer (pH-3, Adjusted with 0.1% OPA) 80:20 % v/v at a flow rate of 0.8ml/min with UV-3000-M at 244nm. The retention time of Amlodipine & Candesartan was 4.2min and 6.3 min respectively.

Good linearity obtained over the range of 5µg/ml to 25µg/ml & 8µg/ml to 40µg/ml for Amlodipine & Candesartan. Correlation coefficient was found to be 0.999&0.999 for Amlodipine & Candesartan respectively. The % RSD of precision Amlodipine & Candesartan was found to be 0.54 and 0.60 respectively. The % mean recovery was found to 98.93-99% for Amlodipine and 99.75-99.87%for Candesartan. The results obtained for accuracy, precision, LOD, LOQ and Ruggedness were within the limits. Thus the validated economical method was applied for forced degradation study of Amlodipine & Candesartan tablet.

KEYWORDS: Amlodipine & Candesartan, stress study, HPLC method

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INTRODUCTION

Hypertension is another name for high blood pressure. It can lead to severe complication and increases the risk of heart disease, stroke and death. Blood Pressure is the force exerted by the blood against the walls of the blood vessels. The pressure depends on the work being done by the heart and the resistance of the blood vessels. Medical guidelines define hypertension as a blood pressure higher than 130 over 80 mm of Hg, according to guidelines issued by the American Heart Association (AHA) in November 2017. Around 85 million people in the United States have high blood pressure. Hypertension and heart disease is global health concerns. The World Health Organization (WHO) suggests that the growth of the processed food industry has impacted the amount of salt in diets worldwide and this plays a role in hypertension. From the literature survey it is clear that UV,UPLC,HPLC&HPTLC single drug as well as in combination of Amlodipine & Candesartan Methods are developed

MATERIALS AND METHODS

Materials

1. Drug sample

Amlodipine Besylate, and Candesartan Cilexetil was kindly supplied as gift samples by Glenmark Pharmaceuticals Ltd., Mumbai. The procured drug standards were standardized by measurement of physical properties like Melting Point, Infrared spectrum and UV absorption spectrum and comparing with the data reported in literature.

2. Chemicals and Reagents

Solvents Methanol used for chromatographic analysis was of HPLC grade purchased from S.D. Fine Chemicals, Mumbai. The water used for HPLC was double distilled assembly (BOROSIL) and passed through a 0.45 µm filter.

All other chemicals and solvents were of AR grade and purchased from S.D. fine chemicals, Mumbai, India. The 0.45 µ Nylon filter papers were purchased from India Pvt. Ltd., Mumbai, India.

RESULTS AND DISCUSSION

Chromatographic Conditions:

Binary Gradient System HPLC on a Grace C18 (250mm x 4.6ID, Particle size: 5 micron) Software was HPLC Workstation utilizing a mobile phase consisting a Methanol: P. Buffer (pH-3, Adjusted with 0.1% OPA) 80:20 % v/v at a flow rate of 0.8ml/min with UV-3000-M at 244nm.

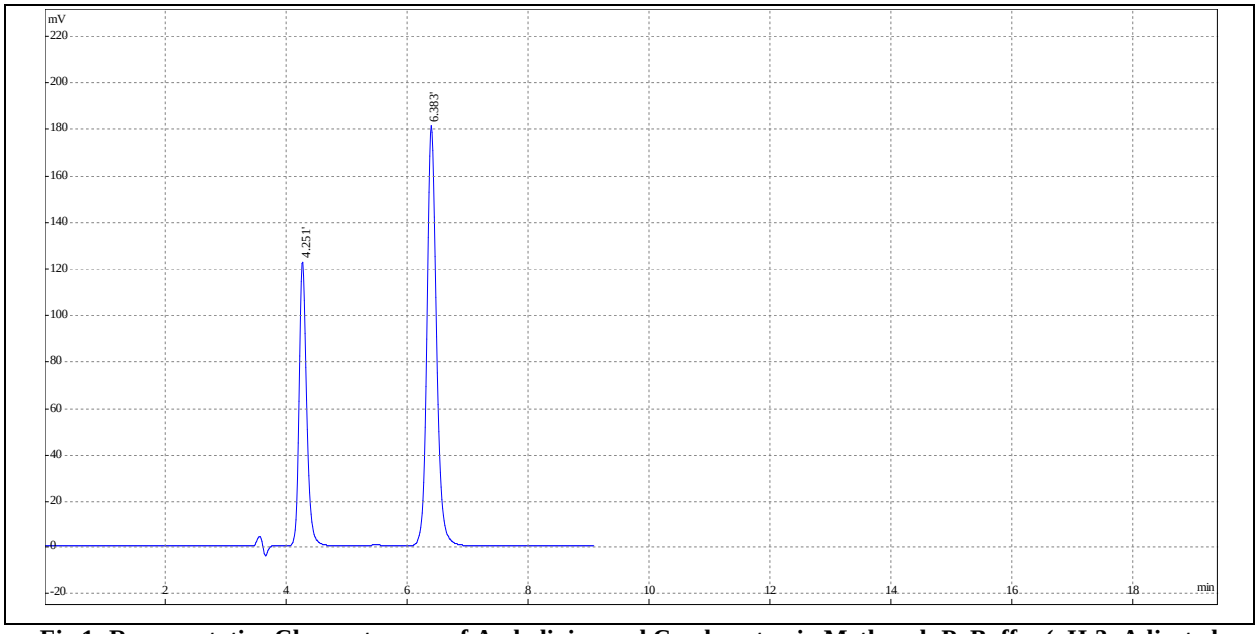

Fig 1: Representative Chromatogram of Amlodipine and Candesartan in Methanol: P. Buffer (pH-3, Adjusted with 0.1% OPA) 80:20 % v/v

METHOD VALIDATION:

The developed Method was validated for linearity, precision, accuracy, ruggedness and is applied for forced degradation studies as per the ICH guidelines. [9-10]

Linearity:

Linear concentrations of both drugs were prepared and the best fit line was calculated. Wide range calibration was determined by solutions containing 5µg/ml to 15µg/ml for Amlodipine and 8µg/ml to 40µg/ml Candesartan.

Correlation coefficient was found to be 0.999&0.999 for Amlodipine & Candesartan respectively (fig 2&3)

Table 1: Linearity data for Amlodipine & Candesartan

Sr. No.	Candesartan Cilexetil		Amlodipine Besylate	
	Concentration (µg/ml)	Area	Concentration (µg/mL)	Area
1	8	333909	5	161153
2	16	677818	10	332306
3	24	1001727	15	493459
4	32	1335636	20	634612
5	40	1669545	25	805765
Equation	**y = 41614x +5000**		**y = 31831x +8000**	
Regression	**R^2 = 0.999**		**R^2 = 0.999**	

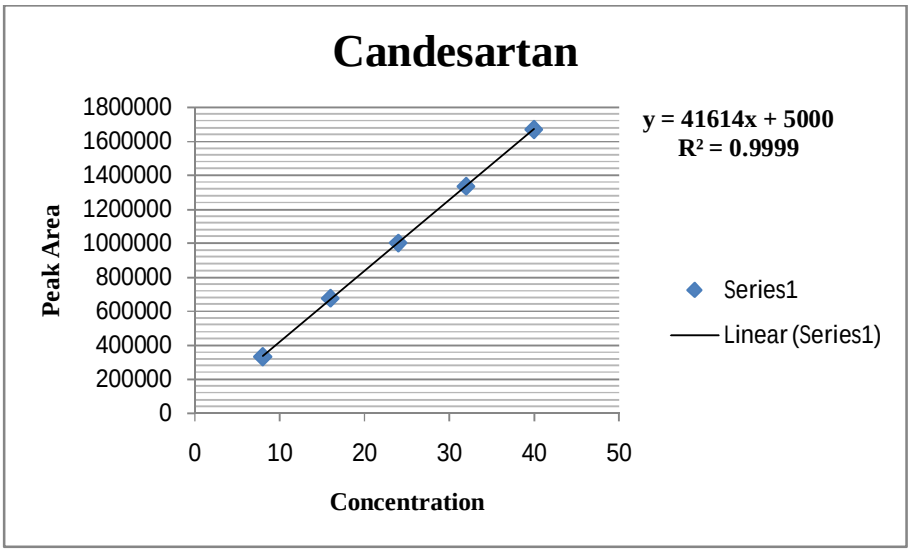

Fig 2: Linearity graph for Accuracy and Precision of Candesartan Cilexetil

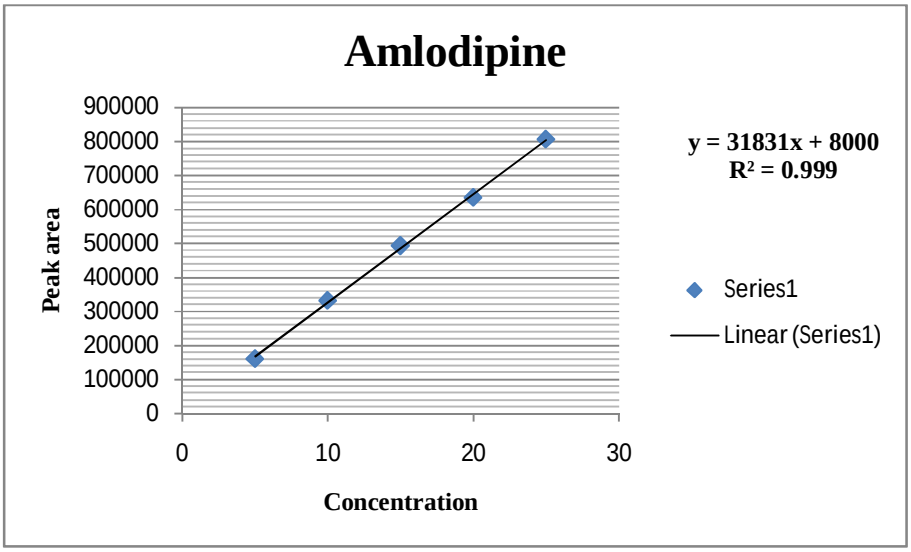

Fig 3: Linearity graph for Accuracy and Precision and Amlodipine Besylate

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD is calculated using the formula 3.3 times σ/s where "σ" is standard deviation of the intercept obtained for calibration curve and "s" is the slope of the calibration curve. Similarly LOQ is calculated using the formula 10 times σ/s. The calculated LOD and LOQ are shown in table 2 &3

Table 2: Showing LOD & LOQ Results of Amlodipine

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg Area
5	161153	162264	160042	161153
10	332306	336196	337293	335265
15	493459	497756	492249	494488
20	634612	634413	634511	634512
25	805765	806876	804654	805765
Intercept	8000	11269	9817.2	9695.4
slope	9695.4	9695.4	9695.4	31769.67
Intercept Standard Deviation				1337.34
LOD (µg/ml)				0.138913
LOQ(µg/ml)				0.420949

Table 3: Showing LOD & LOQ Results of Candesartan

Conc (µg/ml)	Area 1	Area 2	Area 3	Avg Area
8	333909	336721	332964	334531.3
16	677818	676717	678726	677753.7
24	1001727	1010522	1011626	1007958
32	1335636	1354633	1324633	1338301
40	1669545	1678545	1657544	1668545
Intercept	5000	2958.4	12579	6845.8
slope	41614	42020	41188	41607.33
Intercept Standard Deviation				4138.77
LOD (µg/ml)				0.328259
LOQ(µg/ml)				0.994723

PRECISION:

The intraday precision was demonstrated by injecting standard solutions of Amlodipine and Candesartan with 10µg/ml and 16µg/ml respectively as per the test procedure (Table 4) & recording the chromatograms of six standard solutions. The % RSD of Amlodipine and Candesartan was found to be 0.97 and 0.67 respectively.

Table 4: Method Precision data of Amlodipine and Candesartan

Amlodipine (10µg/ml)		Candesartan (16µg/ml)
Sr.No	**Area**	**Area**
1	332306	677961
2	336196	675427
3	337293	673224
4	329598	665243
5	339422	676332
6	336543	668376
Mean	335226.3	672760.5
SD	3285.196	4525.161
%RSD	0.979993	0.672626

Intermediate Precision:

Intermediate precision of the analytical method was determined by performing method precision on in three successive days by different analysts under same experimental condition by injecting six replicate standards preparations was determined and the mean % RSD of Amlodipine and Candesartan with 10µg/ml and 16µg/ml was found to be 0.54 and 0.60 respectively (Table 5).

Table 5: Precision Data for Amlodipine and Candesartan

Amlodipine Area for 10µg/ml					Candesartan (16µg/ml)			
S.No	**Day 1**	**Day 2**	**Day 3**	**Avg**	**Day 1**	**Day 2**	**Day 3**	**Avg**
1	332306	332405	323349	329353.3	677961	677818	682412	679397
2	336196	337193	335432	336273.7	675427	676717	668748	673630.7
3	337293	337293	336287	336957.7	673224	678726	672241	674730.3
4	329598	339597	331234	333476.3	665243	669213	669878	668111.3
5	339422	339422	332242	337028.7	676332	685622	674545	678833
6	336543	336543	335344	336143.3	668376	664311	678543	670410
Mean	335226.3	337075.5	332314.7	334872.2	672760.5	675401.2	674394.5	674185.4
SD	3285.196	2381.255	4401.332	2738.179	4525.161	6884.495	4803.337	4092.953
%RSD	0.979993	0.706446	0.731575	0.540174	0.672626	1.019319	0.712244	0.607096

ACCURACY:

Accuracy of the method was established by performing recovery studies according to the ICH guidelines. Spiked samples were prepared by spiking pre-analyzed sample solutions with pure drug at three different concentration levels each in triplicate. Mean percentage recovery values at three different concentrations of the two drugs was calculated. The % mean recovery of Amlodipine (98.93-99%) & Candesartan (99.75-99.87%) at each level was within the limits of 98% and 102% (Table 6)

Table-6: Accuracy of Amlodipine & Candesartan

Accuracy of Amlodipine						
S.No.	**Conc.**	**Calculated Conc.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1.	5	4.89	97.8	99	0.864099	0.872827
2.	5	4.97	99.4			
3.	5	4.99	99.8			
4.	10	10.1	101	98.93	1.463633	1.479414
5.	10	9.8	98			
6.	10	9.78	97.8			
7.	15	14.88	99.2	99.97	0.65403	0.654183
8.	15	15.12	100.8			
9.	15	14.99	99.93			
Accuracy of Candesartan						
S.No.	**Conc.**	**Calculated Conc.**	**%Recovery**	**Mean Recovery**	**SD**	**%RSD**
1.	8	7.98	99.75	99.875	1.177037	1.17851
2.	8	8.11	101.375			
3.	8	7.88	98.5			
4.	16	15.9	99.375	99.77	0.603895	0.605277
5.	16	15.89	99.315			
6.	16	16.10	100.625			
7.	24	24.01	100.04	99.8	0.278209	0.278766
8.	24	23.86	99.41			
9.	24	23.99	99.95			

Ruggedness:

The ruggedness of method for Amlodipine and (15µg/ml) and Candesartan(24µg/ml) was calculated with six injections of in two batches using two different columns. The % RSD of

ruggedness for Amlodipine was 0.79 with column-1 and 1.0 with column-2 and the % RSD of ruggedness for Candesartan was 0.34 with column-1 and 0.38with column-2 (Table-7), which is within acceptance limits

Table 7: Showing the results of Ruggedness

	Amlodipine 15µg/ml		Candesartan (24µg/ml)	
S.NO	**Column 1**	**Column 2**	**Column 1**	**Column 2**
1	14.88	15.2	24.01	24.12
2	15.12	15.00	23.86	24.01
3	14.99	14.88	23.99	24.01
4	15.01	15.12	23.89	23.86
5	15.10	14.99	24.11	23.99
6	14.78	14.74	23.94	23.86
Mean	14.98	14.98833	23.96667	23.975
± SD	0.119024	0.150379	0.082597	0.091424
%RSD	0.794551	1.003308	0.344632	0.38133
% Accuracy	99.86	99.86	99.83	99.89

Results of Stress Degradation Studies:

Stress degradation studies were performed as per the ICH guidelinesQ1A (R2) Stability Testing of New Drug Substances and Products, using the proposed validated analytical method.(Table 8&9)

Acid Degradation studies:

To 1ml of stock solution Amlodipine and Candesartan, 1ml of Acid 0.1N HCL 60°C (Refluxed for 30 min) from the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. Comparison of the peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 14.92% & 6.42%degradation respectively. (Figure 4)

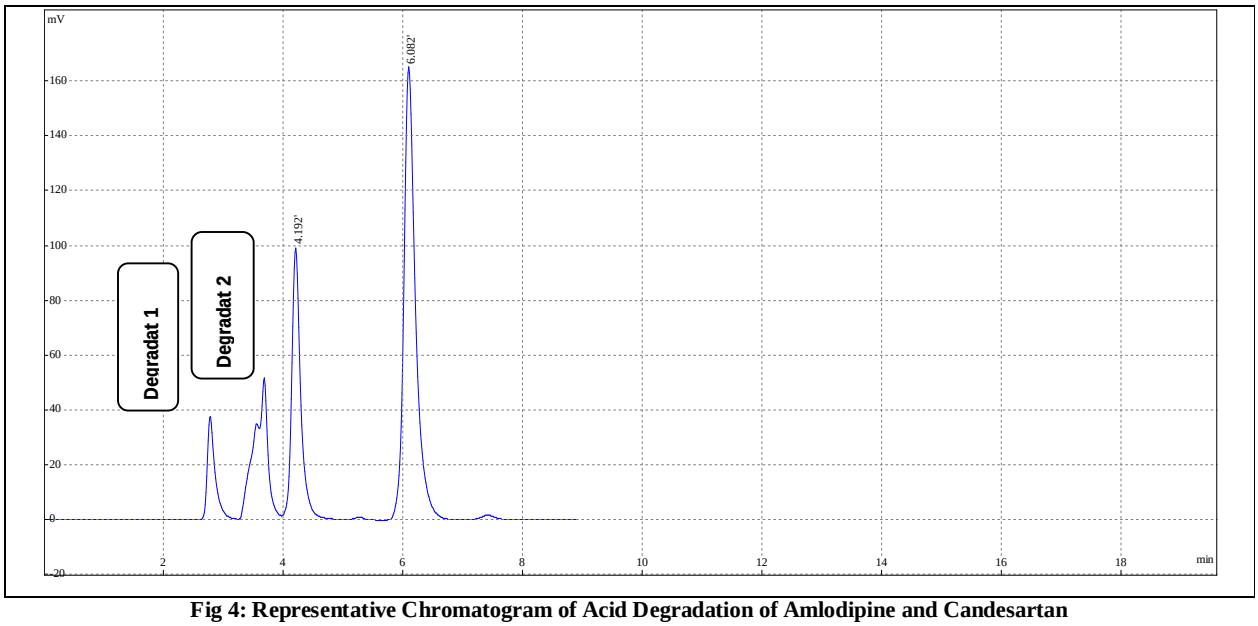

Fig 4: Representative Chromatogram of Acid Degradation of Amlodipine and Candesartan

Alkali Degradation Studies:

To 1ml of stock solution of of standard drug and sample Amlodipine and Candesartan, 1ml of Alkali 0.1N NaOH 60°C (Refluxed for 30 min). From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. Comparison of the peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 14.22% & 8.90% degradation respectively.(Figure 5)

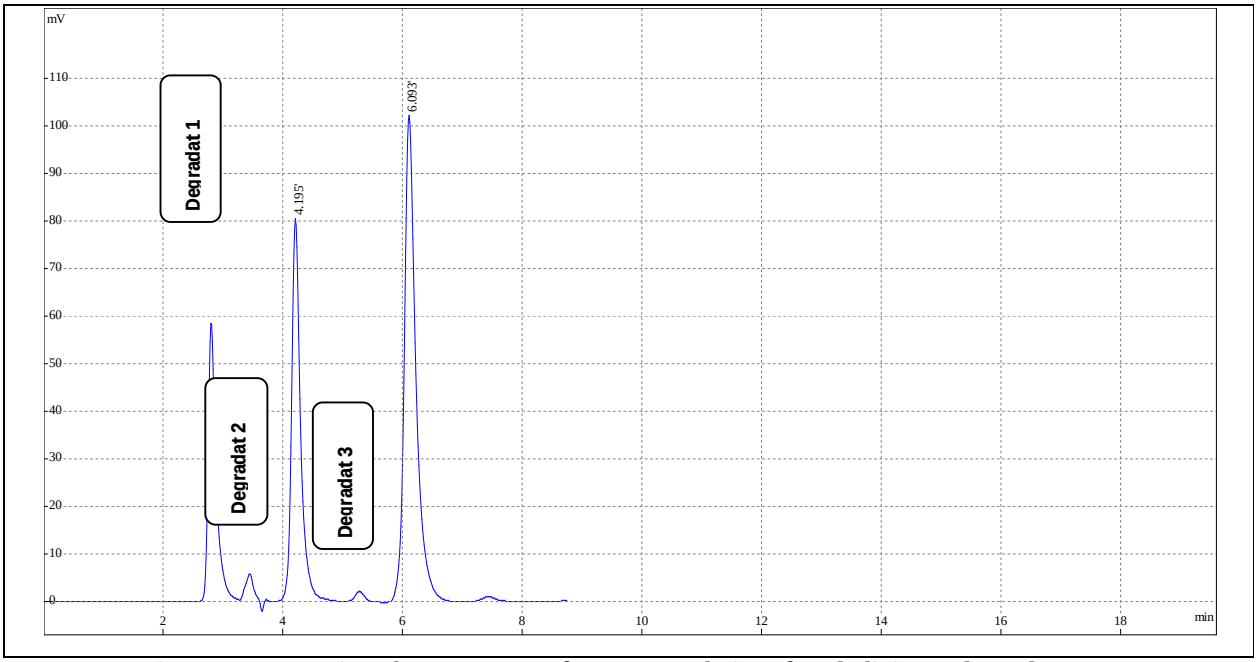

Fig 5: Representative Chromatogram of Base Degradation of Amlodipine and Candesartan

Oxidative Degradation:

To 1ml of stock solution of standard drug and sample of Amlodipine and Candesartan, 1ml of 3.0%v/v H_2O_2(room temperature for 24hrs. From the above solution10 µl was injected into the system and the chromatograms were recorded to detect the stability of sample. Comparison of the peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 13.25% & 8.70%degradation & retention time 3.78min & 501 min.respectively. (Figure 6)

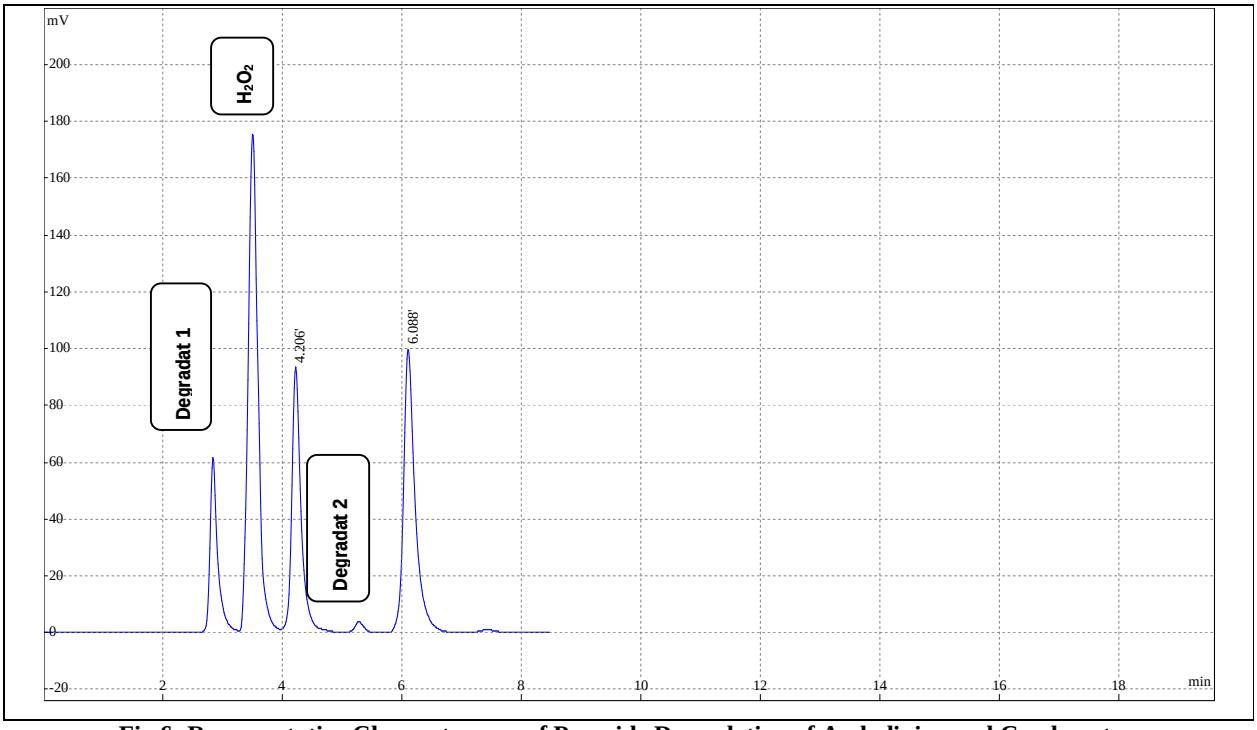

Fig 6: Representative Chromatogram of Peroxide Degradation of Amlodipine and Candesartan

Photo Stability Studies:

The drug was dissolved in methanol exposed to sunlight for 8hrs. When the stressed sample was analyzed, no degradation was found and hence the exposure time was extended for 24hrs and 48hrs. When stressed sample was analyzed, there was no additional peak found. There were no additional peaks at the same retention time when blank, zero and stressed blank samples analyzed and confirming the formation of no degradation product. Hence it was concluded that the drug was stable under the conditions tested. (Figure 7)

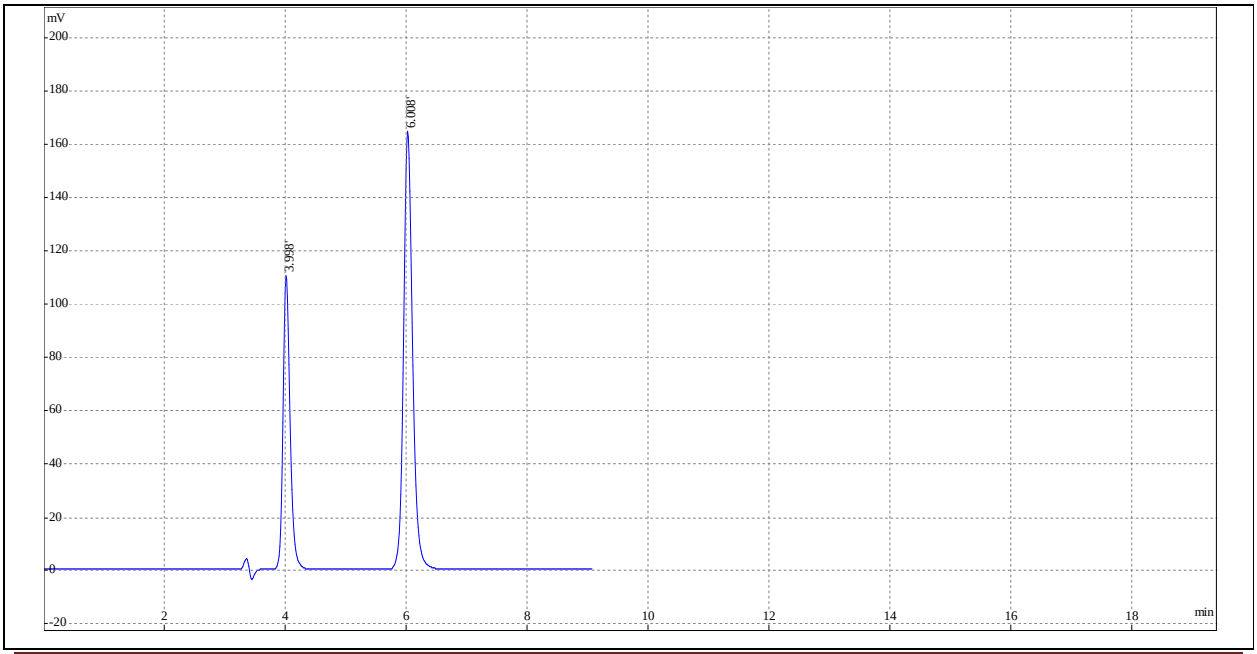

Fig 7: Representative Chromatogram of Photolytic Degradation of Amlodipine and Candesartan

Wet heat degradation

There was degradation found when the drug was refluxed for 30 min with water at 80°C.When stressed sample was analyzed, there were two additional peaks at the retention time 2.492 min.& 5.88min When blank, zero and stressed blank samples analyzed and confirming the formation of two degradation product. Comparison of the peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 2.85% &1.2%degradation respectively.(Figure: 8)

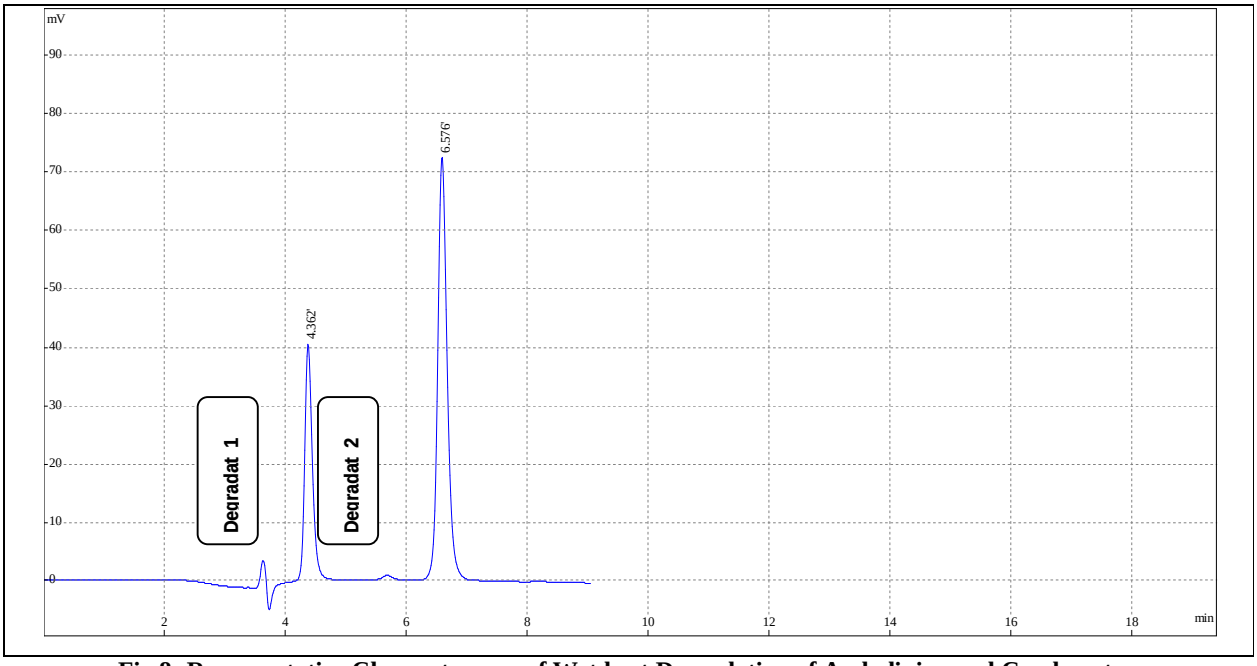

Fig 8: Representative Chromatogram of Wet heat Degradation of Amlodipine and Candesartan

Dry heat degradation

Stability of Amlodipine and Candesartan in dry heat was studied by keeping it for 1 hr at 50 °C. When the stressed sample was analyzed, no degradation was found and hence it was decided to extended the heating time for 3hrs, 5hrs, 24hrs, 48hrs with increased in temperature 70°C.When the stressed sample was analyzed, there was no additional peak found. Also the comparison between the peak areas of stressed sample of Amlodipine and Candesartan with that of zero time sample showed no difference, indicating that there was no degradation. Hence it was concluded that the drug was stable under the conditions tested.(Figure: 9)

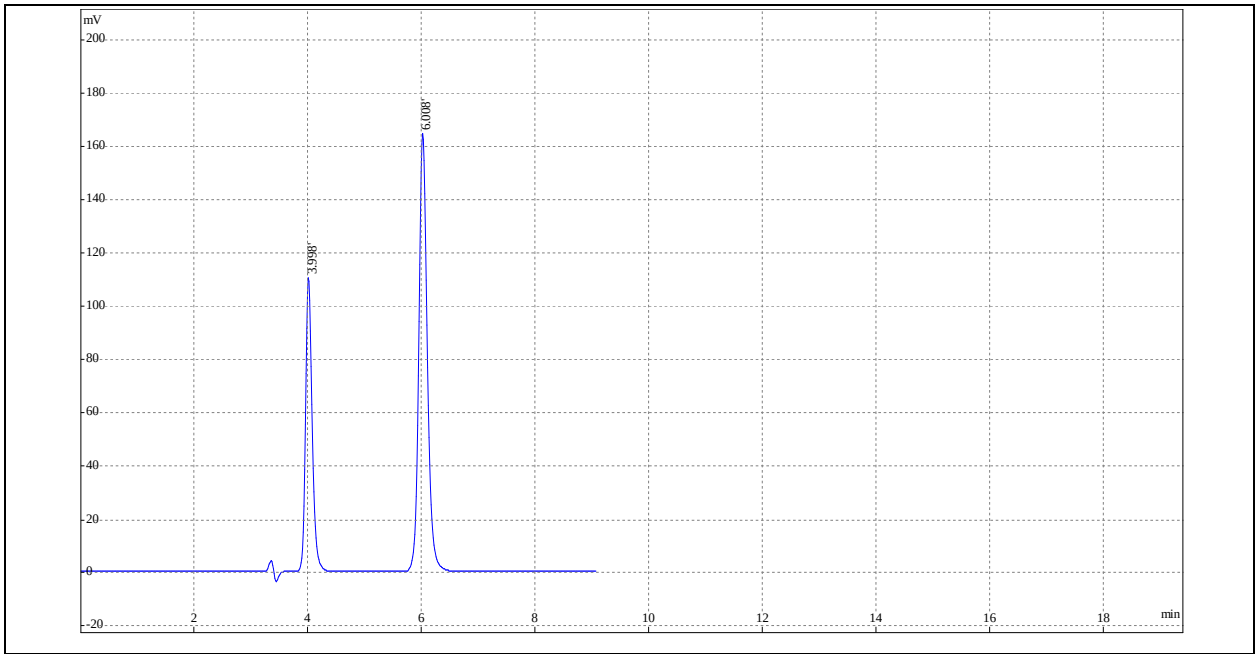

Fig 9: Representative Chromatogram of Dry heat Degradation of Amlodipine and Candesartan

Table 8. Results of stress degradation studies of Amlodipine

Sr. No.	Stress Condition	Drug peak area at zero time sample (mcV.sec)	Drug peak area of stressed sample (mc.V.sec)	Retention time(s) of degradation products (min)	% Degradation
1	Acid 0.1N HCL 60°C (Refluxed for 30 min)	1381133	1174963	2.883	14.92%
2	Alkali 0.1N NaOH 60°C (Refluxed for 30 min)	1198700	1028215	2.617,3.52	14.22%
3	Wet heat 80°C for 30min	1198764	1164511	2.492	2.85%
4	Oxidative 3.0%v/v H_2O_2(room temperature for 24hrs	1198623	1039727	3.78	13.25%
5	Dry heat 70°C(kept in oven for 30min)	1384411	1384423	No Degradation	No Degradation
6	Photolytic (exposed to sunlight for 24 hrs)	1342256	1355155	No Degradation	No Degradation

Table 9. Results of stress degradation studies of Candesartan

Sr. No.	Stress Condition	Drug peak area at zero time sample (mcV.sec)	Drug peak area of stressed sample (mc.V.sec)	Retention time(s) of degradation products (min)	% Degradation
1	Acid 0.1N HCL 60°C (Refluxed for 30 min)	2433541	2277226	3.87	6.42 %
2	Alkali 0.1N NaOH 60°C (Refluxed for 30 min)	21884582	1948282	3.24	8.90%
3	Wet heat 80°C for 30min	21753542	21482822	5.88	1.2%
4	Oxidative 3.0%v/v H_2O_2(room temperature for 24hrs	21773471	1895025	5.1	8.70%
5	Dry heat 70°C(kept in oven for 30min)	23672453	23753542	No Degradation	No Degradation
6	Photolytic (exposed to sunlight for 24 hrs)	2363782	2372793	No Degradation	No Degradation

Amlodipine and Candesartan undergoes significant degradation in acidic, oxidation, Wet heat and alkaline comparatively

More degradation was found with acid &alkali for Amlodipine and Candesartan. As per ICH guidelines peak purity angle should be less than peak purity threshold. One thing it is observed that wet heat degradation gives the 2.85% for Amlodipine &1.2%for Candesartan where as no degradation in dry heat degradation.

Hence, method of the analysis of Amlodipine and Candesartan in tablet dosage form shows that the degradation product doesn't interfere with the analytical determination. Hence the proposed analytical method is also useful for the determination of Amlodipine and Candesartan stability in sample of pharmaceutical dosage form.

CONCLUSION

A simple, precise, accurate, robust & cost-effective method was developed for the routine analysis. The method was successfully validated in terms of linearity, precision, accuracy as per ICH guidelines. The method provides a linear response across a wide range of concentrations. Present method is giving the future scope for researchers that to identified degradation to develop method for impurity profiling. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results & found to be suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing these drugs either individually or in combination.

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CONFLICT OF INTEREST:-No

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