A stability indicating RP-HPLC method development and validation for the estimation of combined tablet formulation of Amlodipine&Candesartan.

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- ***INTRODUCTION***
- Hypertension is one more name for high blood pressure. 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. 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. So our aim was to develop a simple, precise, accurate, robust & cost-effective stability indicating HPLC method which can be used in routine analysis. [1-8, 11-18]
- **MATERIALS AND METHODS**
- 1. Drug sample
- Amlodipine Besylate, and Candesartan Cilexetil was kindly supplied as gift samples by Glenmark Pharmaceuticals Ltd., Mumbai.
- 2. Chemicals and Reagents
- All solvents used for chromatographic analysis was of HPLC grade purchased from S.D. Fine Chemicals, Mumbai.
- ***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.
- ***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:***
- Correlation coefficient was found to be 0.999&0.999 for Amlodipine & Candesartan respectively.
- ***Limit of Detection (LOD) and Limit of Quantification (LOQ):***
- As per references procedure mentioned LOD and LOQ was calculated.
- ***Precision:***
- The % RSD of Amlodipine and Candesartan was found to be 0.97 and 0.67 correspondingly.
- ***Intermediate Precision:***
- The mean % RSD of Amlodipine and Candesartan with 10µg/ml and 16µg/ml was found to be 0.54 and 0.60 correspondingly
- ***Accuracy:***
- The % mean recovery of Amlodipine (98.93-99%) & Candesartan (99.75-99.87%) at each level was within the limit.
- ***Ruggedness:***
- 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 , which is within acceptance limits.
- ***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. [9-10]
- ***Acid Degradation studies:***
- 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.[9-10,14]
- ***Alkali Degradation Studies:***
- The peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 14.22% & 8.90% degradation correspondingly.
- ***Oxidative Degradation:***
- 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 & 5.01 min.respectively.
- ***Photo Stability Studies:***
- 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. [9-10,14]
- **Wet heat degradation**
- When stressed sample was analyzed, there were two additional peaks at the retention time 2.492 min.& 5.88min.The peak area of Amlodipine and Candesartan in stressed condition with that of the zero time samples gave 2.85% &1.2%degradation respectively.
- **Dry heat degradation**
- 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. 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.
- **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.

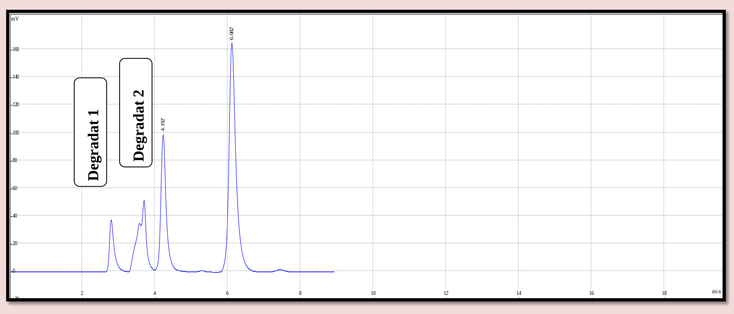

Fig 1: Representative Chromatogram of Acid Degradation of Amlodipine and Candesartan

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)	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 1. 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)	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

Table 2. Results of stress degradation studies of Candesartan

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