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Analytical method development and validation with degradation protocol for amlodipine and cilazapril

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Abstract
Objective: To develop and validate RP-HPLC method that will be helpful in the estimation of drugs in the dosage form in an economic way and on a routine basis for Amlodipine and Cilazapril.
Method: Chromatographic separation was achieved with a Shimadzu's high-performance liquid chromatography C18 column (150X4.6 mm, 5mm) with a mobile phase Acetonitrile: potassium dihydrogen phosphate buffer: Methanol in a ration 50:20:25 v/v/v with a buffer having pH 6.8. The flow rate was set at 1ml/min and the detection wavelength was 365 nm for Amlodipine and 254 nm for Cilazapril. This developed chromatographic method gave well-resolved symmetric peaks.
Results: The retention time of Amlodipine was found to be 2.81min and that of Cilazapril was around 4.77 min. The plate count of Amlodipine was in range and was found to be 4744 and Cilazapril has 4477 with tailing factor of 1.26 and 1.23 for Amlodipine and Cilazapril respectively. The method was linear in a range of 5 to 25µg/ ml for Amlodipine and 2 to 10 µg/ ml for Cilazapril through UV spectroscopy with regression 0.9918 and 0.996, respectively.
Conclusion: Based on standard calibration curve, the LOD and LOQ values were calculated. The LOD and LOQ for Amlodipine were found to be 0.1711 μg/mL and 0.4998 μg/mL and for Cilazapril it was found to be 0.4891 μg/mL and 0.4049 μg/Ml. The sample recoveries were in good agreement with the respective formulation, which suggested non-interference from formulation additives in the estimation.

Keywords: analytical method development, rp-hplc, uv-spectroscopy, amlodipine besylate, cilazapril

Introduction

Amlodipine is (2-[(2-Aminoethoxy) methyl]-4-(2-chlorophenyl)-3- ethoxy carbonyl- 5-methoxycarbonyl-6-methyl-1, 4-dihydropyridine benzene sulfonate. Amlodipine, initially approved by the FDA in 1987, is a popular antihypertensive drug belonging to the group of drugs called di hydro pyridine calcium channel blockers. Due to their selectivity for the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other calcium channel blockers.[1] Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has antioxidant properties and an ability to enhance the production of nitric oxide (NO), an important vasodilator that decreases blood pressure. Cilazapril is (1S,9S)-9-{[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl] amino}-10-oxo-octahydro-1H-pyridazino[1,2-a] [1, 2] diazepine-1-carboxylic acid having molecular Formula $C_{22}H_{31}N_3O_5$. It is a ACE Inhibitor, Anti-Hypertensive. It is a prodrug that is hydrolyzed to cilazapril at which is the main metabolite. Literature survey also reveals Spectrophotometric Methods, HPTLC, RP-HPLC, LCMS, UPLC, for determination. The literature survey reveals that, there have been several publications describing analytical methods for the determination of AMD and Cilizapril [2, 23].

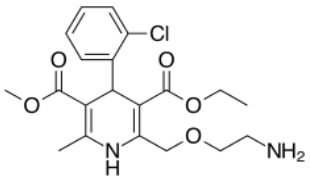

Fig 1: Amlodipine

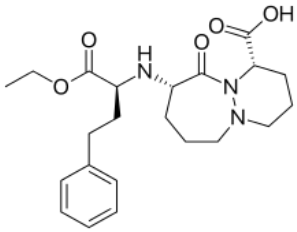

Fig 2: Cilazapril

Material and Methods

Preparation of standard stock solution

Amlodipine Besylate: Weigh accurately 10 mg of Amlodipine besylate in 10 ml volumetric flask and was dissolved in 1ml methanol and 9 ml buffer solution (For standard stock solution). 1ml of the above standard solution was taken in volumetric flask (100 ml) and the volume was made to 100 ml with buffer solution to get 10 µg/mL of concentration of the solution.

Cilazapril: *Weigh accurately 10mg of Cilazapril in 10 ml volumetric flask and dissolve in 1ml methanol and 9ml of buffer was prepared in a 10ml volumetric flask. (For standard stock solution), 1ml of this was taken and dissolved in 99ml of buffer to make 10 µg/mL of solution in 100ml volumetric flask.*

For Absorbance Maxima Using a solution of a concentration of 10 µg/mL of Amlodipine and 10 µg/mL of Cilazapril, λ max was calculated.

Calibration Curve Determination

The prepared standard solution of Amlodipine and Cilazapril was diluted to a concentration of 5-25µg/mL and 2-10 µg/mL respectively using pH 6.8 buffer. The absorbance of the individual solution was obtained at 365nm and 254nm using a buffer pH of 6.8 as blank. The graph later was prepared as concentration Vs Absorbance at a wavelength selected for both the drugs.

Estimation of Amlodipine and Cilazapril simultaneously

A known concentration of both solutions was mixed thoroughly and absorbance was measured at 365nm and 254nm for Amlodipine and Cilazapril respectively.

Selection of mobile phase and λmax

The mobile phase selected for the process was Acetonitrile: potassium dihydrogen phosphate buffer: Methanol in a ration 50:20:25 v/v/v with a buffer having pH 6.8. The mobile phase every day was prepared freshly.

Chromatographic condition-optimized

The parameters used analysis of Amlodipine and Cilazapril using RP-HPLC:

- **Mode of operation** – Isocratic
- **Stationary phase** – C18 column (150X4.6 mm, 5mm)
- **Mobile phase –** Acetonitrile: potassium dihydrogen phosphate buffer: Methanol
- **Ratio –** 50:20:25 v/v/v
- **Flow rate –** 1 mL/ min
- **Run time-**10 min.
- **Detection wavelength** –365nm and 254nm
- **Column Temperature –** Ambient

Standard stock solution preparation: 10mg of Amlodipine and 10mg of Cilazapril was weighed accurately and transferred in a volumetric flask (10ml) and volume was made upto mark using the mobile phase, to get a solution of 1000µg/mL of Amlodipine and for Cilazapril 1000 µg/ml. The solution so made was sonicated to get proper mixing for 5-7 mins.

Preparation of Sample solution: 20µg/mL of a solution of the standard stock was prepared using the mobile phase.

Result and Discussion

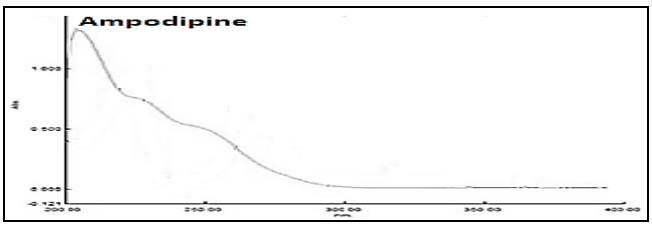

Fig 3: U.V. Spectra- Amlodipine

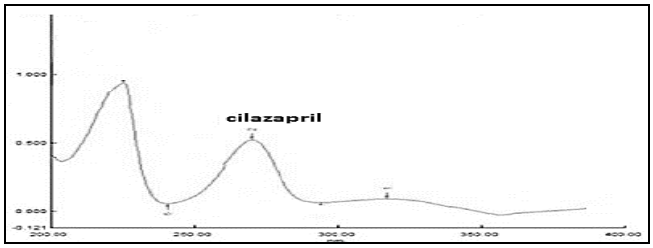

Fig 4: U.V. Spectra- cilazapril

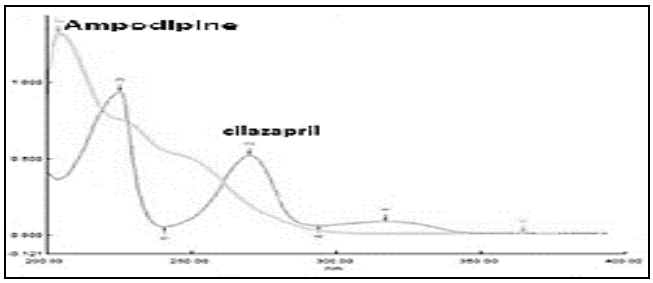

Fig 5: Overlay spectra of Amlodipine and cilazapril

Linearity

The linearity graph of both the drugs was obtained in a range of 5 to 25µg/ ml for Amlodipine and 2 to 10 µg/ ml for Cilazapril.

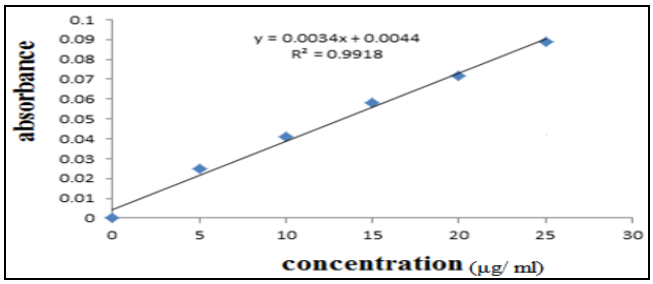

Fig 6: Calibration curve—Amlodipine

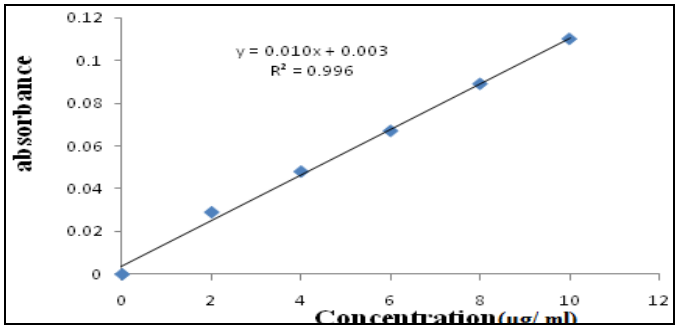

Fig 7: Calibration curve-Cilazapril

Table 1: Parameters of UV for Amlodipine and Cilazapril

Parameters	Amlodipine	Cilazapril
Wavelength (nm)	365	254
Beer's Law limit (µg/ mL)	5-25	2-10
Molar absorptivity (L mol–1 cm–1)	6487	5716
Co-relation coefficient (r)	0.9918	0.996
Regression Equation (y = mx + c)	y = 0.0034x + 0.0044	y = 0.0107x + 0.0037
Slope (m)	0.0033	0.0107
Intercept (c)	0.0374	0.0298
LOD (µg/ mL)	0.897	0.12
LOQ (µg/ mL)	2.72	1.11
%RSD	0.1210	0.3938

Precision studies

The precision studies of Amlodipine as well as Cilazapril gave percentage RSD within range for both inter and intraday absorbance.

Table 2: inter-day and intraday study of Amlodipine

Concentration µg/ mL	Intra-day absorbance			Inter-day Absorbance		
	Mean absorbance	± SD	%RSD	Mean absorbance	± SD	%RSD
5	0.698	0.0035	1.8311	0.675	0.0065	0.1972
10	0.131	0.0014	1.2321	0.134	0.0027	0.1823
15	0.195	0.0031	1.0354	0.189	0.0046	0.1695
20	0.198	0.0035	1.8211	0.190	0.0041	0.1691
25	0.139	0.0039	1.0324	0.191	0.0050	0.1771

Table 3: inter-day and intraday study of Cilazapril

Concentration µg/ mL	Intra-day absorbance			Inter-day Absorbance		
	Mean absorbance	± SD	%RSD	Mean absorbance	± SD	%RSD
2	0.436	0.0035	0.1872	0.441	0.0017	0.1725
4	0.945	0.0018	0.2112	0.935	0.0023	0.2314
6	1.207	0.0029	0.3315	1.210	0.0026	0.3152
8	1.209	0.0017	0.3312	1.211	0.0028	0.3151
10	1.211	0.0031	0.3211	1.219	0.0030	0.3152

Percentage recovery, LOD and LOQ

The optical properties of the drugs were studied and the results found are:

The limit of quantification and detection were also determined using linearity studies which were done few times and calculated using slope and standard deviation response. The percent recovery studies were studied in order to evaluate the accuracy of the method. The drug of the known amount was added to a pre-analyzed solution containing formulation and the mixture was analyzed thereby calculating the percent recovery. It was found that percentage RSD is less than 2%.

Table 4: Percent recovery Amlodipine and Cilazapril

Drug	Percent-age	Amount present	Amount added	Amount recovered	percent Recovery	S. D	% RSD
Amlodipine	60	6	4	3.9699	99.2475	0.0031	0.1535
	80	6	5	4.9888	99.776	0.0027	0.0187
	100	6	6	6.0260	100.433	0.0013	0.1909
Cilazapril	60	8	6.4	6.4207	100.33	0.6638	0.6609
	80	8	9.6	9.5663	99.64	0.0251	0.0252
	100	8	8	7.9944	99.94	0.4951	0.4954

Ruggedness Study

With the help of different analysts, the ruggedness studies were confirmed along with calculation of percentage RSD.

Table 5: Ruggedness studies of Amlodipine and Cilazapril

Drug	Condition	Average percentage obtained	S. D	% RSD
Amlodipine	Analyst-1	100.28	0.0455	1.035
	Analyst-2	99.37		
Cilazapril	Analyst-1	100.24	0.1174	0.98
	Analyst-2	98.32		

Amlodipine and Cilazapril

Method development

After trying many mixtures of mobile phases with different ratios, a typical chromatogram was obtained with Acetonitrile, potassium dihydrogen phosphate buffer and Methanol in ratio of 50:20:25 v/v. The pH of the mobile phase was adjusted to 6.8 using Orthophosphoric acid at a flow rate of 1ml/min. The chromatographic separation was performed on C-18 Column (150 × 4.6 mm, 5 μ) by injecting 20μg/ml of sample.

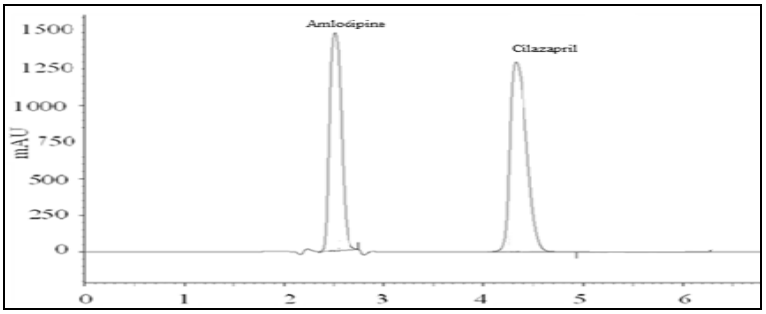

Fig 8: Chromatogram of Amlodipine and cilazapril

The retention time of Amlodipine was found to be 2.81min and that of Cilazapril was around 4.77 min. The plate count of Amlodipine was in range and was found to be 4744 and Cilazapril has 4477 with tailing factor of 1.26 and 1.23 for

Amlodipine and Cilazapril respectively.

Method Validation

The validation studies were performed according to ICH guidelines. Various parameters like system suitability, precision, accuracy, linearity, robustness, LOD and LOQ.

System Suitability

This was performed in order to verify the acceptability of the resolution and repeatability of the current method. System suitability was carried out by injecting six replicate injections of the standard solution. The parameters evaluated were peak area, USP tailing, theoretical plates, retention time and peak asymmetry, with these parameters, percent RSD was also evaluated and found to be within the limits. The results are shown in table below.

Table 6: System Suitability parameters of Amlodipine and Cilazapril

Parameters	Amlodipine	Cilazapril
Retention time (min)	2.81	4.77
USP plate count	4744	4477
USP tailing	1.26	1.23

Accuracy

The accuracy was determined and evaluated by calculation of recovery studies of the drug sample at three different level concentration (50%, 100%, and 150%) using standard addition method. A known amount of the two drugs were added to pre-analyzed and pre-quantified sample solutions and three replicates of each concentration were injected in the chromatogram.

Mean percent recovery for Amlodipine and Cilazapril was obtained, varying between the range of 99.95 to 103.87% which indicated that the method developed was accurate. The results of percent recovery in given in table below:

Table 7: Percent recovery parameters of Amlodipine and Cilazapril

Spiked level	Percentage recovery		Mean percent recovery	%RSD	
	Amlodipine	Cilazapril		Amlodipine	Cilazapril
50%	101.92	99.43	100.56	0.898	0.479
	100.09	100.01			
	100.47	100.33			
100%	99.95	100.22	100.82	0.434	0.212
	100.67	103.82			
	99.99	99.73			
150%	99.76	100.49	99.82	0.569	0.312
	100.11	99.31			
	99.91	99.67			

Linearity

The linearity was performed and obtained at different levels of concentration ranging between 1 to 5μg/mL of Amlodipine and 1 to 5 μg/mL of Cilazapril. The samples were injected in the system and the linearity curve was constructed. From the results, the present work was found to be linear with regression coefficient (r)=0.9975 for Amlodipine and 0.9935 for Cilazapril.

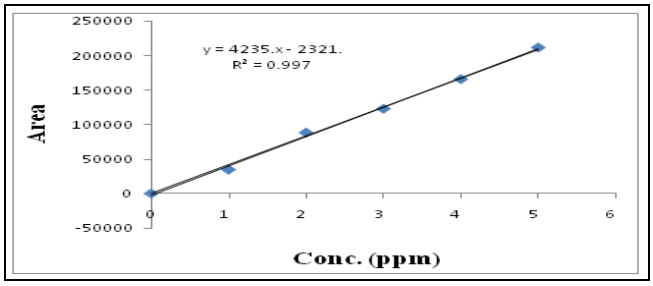

Fig 9: Calibration curve of Amlodipine

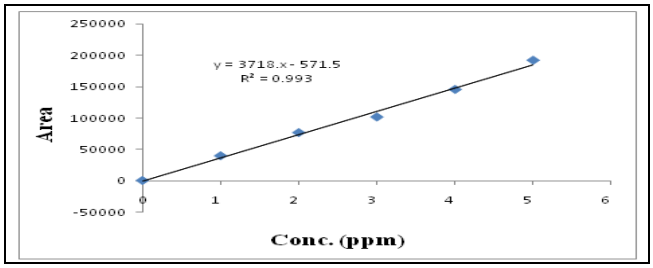

Fig 10: Calibration curve of Cilazapril

LOD and LOQ

Based on standard calibration curve, the LOD and LOQ values were calculated. The LOD and LOQ for Amlodipine was found to be 0.1711 μg/mL and 0.4998 μg/mL and for Cilazapril it was found to be 0.4891 μg/mL and 0.4049 μg/Ml

Robustness

By doing deliberate changes in the system, the robustness of the method was evaluated. The changes were made in mobile phase composition, flow rate, pH of mobile phase and temperature and it was observed and confirmed that no change in parameter altered the method to a great extent. The percentage RSD was found to be within the expected range and the method was considered as robust. The results are shown in the following table.

Table 8: Robustness Studies of Amlodipine and Cilazapril

Sr. no	Parameters	Amlodipine			Cilazapril		
		RT	USPC	TF	RT	USPC	TF
1	Flow rate 0.8ml	2.81	4742	0.19	4.52	4465	0.07
	Flow rate1.2ml	2.82	4687	0.21	4.96	4341	0.19
2.	Temperature 25^{0}C	2.83	4598	0.01	4.77	4298	0.21
	Temperature 35^{0}C	2.85	4119	0.09	4.75	4278	0.14
3.	Mobile Phase -5%	2.87	4768	0.20	4.78	4449	0.12
	Mobile Phase +5%	2.90	4739	0.19	4.81	4468	0.22

PA: peak area; RT: retention time (min); USPC-USP plate count; TF: tailing factor

Force degradation studies

From the peak area obtained after degradation, the percentage assay of degradation was calculated and was compared with the assay of non-degraded conditions of both pairs of the drug. Types of degradations are

a. Acid and Alkaline degradation
b. Oxidative degradation
c. Thermal degradation
d. Photo degradation

Conclusion

Since the methods included, use of chemicals that are economic, the method can be used in industries as well as research area for routine analysis of sample. In method development and validation, the robustness studies of drugs proved that developed method can and will give proper results wherever performed and the ruggedness studies, which were performed with help of different analysts proved the method's precision along with its accuracy. Method development through reverse phase chromatography technique, also gave proper results with all the method validation parameters like limit of quantitation and detection, precision, linearity, system suitability along with robustness and ruggedness. Hence, both the method development whether UV spectroscopy or reverse phase chromatography are feasible and simple for the estimation of the selected drugs and also the chemicals used in the whole process were easily available and were economic. Apart from the method development, forced degradation studies were also performed so as to understand, the purity content of the drugs that remains intact even when they undergo degradation process be it due to shelf life or environmental conditions. By making the drugs undergo all sorts of degradation tests namely, acidic, photolytic, alkaline, thermal and oxidation, from the obtained results, it is concluded that; the drugs retained their basic structure which proves that the properties also remains the same and the degraded material did not have any effect over the original drug material. As a result, it can be concluded that the present work is feasible to do at any laboratory or research with ease by any analyst anywhere and has a good future scope.

Conflict of Interests

Declared none

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