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R E S E A R C H A R T I C L E

Analytical Method Development and Validation for Candesartan and Amlodipine in combined Dosage Form by RP-HPLC

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A B S T R A C T

A new method was established for simultaneous estimation of Candesartan and Amlodipine by RP-HPLC method. The chromatographic conditions were success fully developed for the separation of Candesartan and Amlodipine by using Symmetry C18 (4.0×250 mm) 5.0µm, flow rate was 1ml/min, mobile phase ratio was Methanol: Sodium acetate buffer (75: 35% v/v) pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 250nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, Empower-software version-2. The % purity of Candesartan and Amlodipine was found to be 101.27% and 99.97% respectively. The system suitability parameters for Candesartan and Amlodipine such as theoretical plates and tailing factor were found to be 4668, 1.3 and 6089 and 1.2, the resolution was found to be 6.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Candesartan and Amlodipine was found in concentration range of 5µg-25µg and 50µg-250µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 99.56% and 99.48%, %RSD for repeatability was 0.86 and 0.82, % RSD for intermediate precision was 0.44 and 0.19 respectively. The precision study was precise, robust, and repeatable.LOD value was 3.17 and 5.68, and LOQ value was 0.0172 and 0.2125 respectively.

Keywords: Symmetry C18 column, Candesartan and Amlodipine, RP-HPLC

A R T I C L E I N F O

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C O N T E N T S

1. Introduction

Candesartan is an angiotensin receptor blocker used mainly for the treatment of high blood pressure and congestive heart failure.

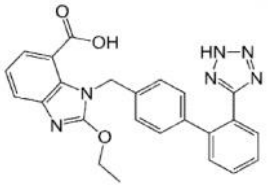

Fig 1: Structure of Candesartan

Amlodipine, sold under the brand name Norvasc among others, is a medication used to treat high blood pressure and coronary artery disease. While not typically recommended in heart failure, amlodipine may be used if other medications are not sufficient for treating high blood pressure or heart-related chest pain. It is taken by mouth and has an effect for at least a day.

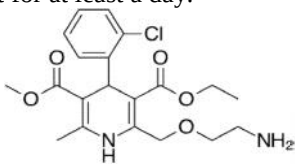

Fig 2: Structure of Amlodipine

2. Materials and Methods

Instrumentation:

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine.

Chemicals:

Candesartan and Amlodipine, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, K_2HPO_4.

Optimized chromatographic conditions

Column : Symmetry C18 (4.0×250 mm) 5.0µm
Mobile phase ratio: Methanol: Sodium acetate buffer (75: 35 % v/v)
Detection wavelength : 250 nm
Flow rate : 1ml/min
Injection volume : 10µl
Column temperature : Ambient
Auto sampler temperature : Ambient
Run time : 8min

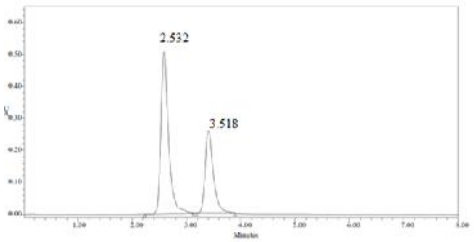

Fig 3: Chromatogram from optimized conditions

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Sample solution preparation:

1mg of Candesartan and 10 mg Amlodipine tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 2ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation:

1mg Candesartan and 10 mg Amlodipine working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 2ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Method Validation

- ✓ System Suitability
- ✓ Linearity
- ✓ Specificity
- ✓ Precision (Repeatability & Intermediate precision)
- ✓ Accuracy
- ✓ Limit of Detection and Limit of Quantification
- ✓ Robustness

3. Results and Discussion

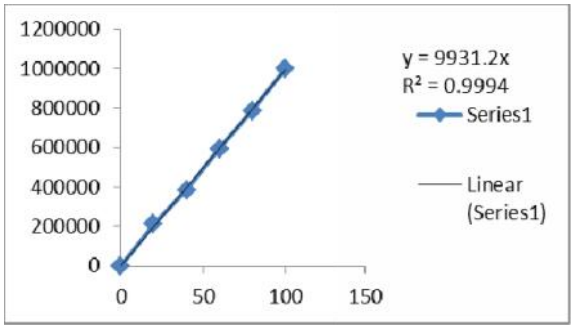

Fig 4: Showing calibration graph for Candesartan

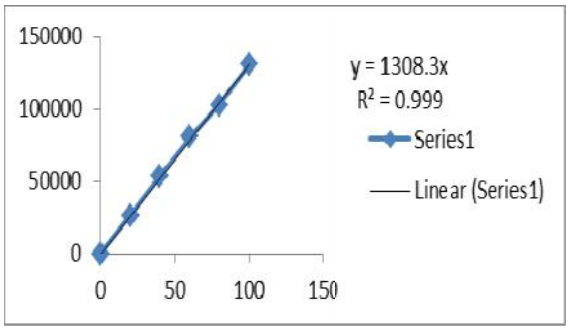

Fig 5: Showing calibration graph for Amlodipine

4. Conclusion

The method provides selective quantification of Candesartan and Amlodipine This developed RP-HPLC method for estimation of Candesartan and Amlodipine is

accurate, precise, robust and specific. The method has been found to be better than previously reported method, because of its, isocratic mode and use of an economical and readily available mobile phase, readily available column, UV detection and better resolution of peaks.

Table 1: Assay results

S.No	Name of compound	Amount taken	%purity
1	Candesartan	754.7	99.24
2	Amlodipine	735.6	101.04

Table 2: Linearity Results for Candesartan

S.No	Linearity Level	Concentration	Area
1	I	50 ppm	107359
2	II	100 ppm	221497
3	III	150 ppm	329389
4	IV	200 ppm	448105
5	V	250 ppm	570352
Correlation Coefficient			0.999

Table 3: Linearity Results for Amlodipine

S.No	Linearity Level	Concentration	Area
1	I	20ppm	26472
2	II	40 ppm	53841
3	III	60ppm	80655
4	IV	80ppm	102541
5	V	100ppm	130567
Correlation Coefficient			0.999

Table 4: Showing accuracy results for Candesartan

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	2630409	5	4.96	99.91%	99.56%
100%	5277055	10	9.98	99.18%	
150%	7514836	15	15.02	99.60%	

Table 5: Showing accuracy results for Amlodipine

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Table 6: Showing% RSD results for Candesartan

	Peak Name	RT	Aera	Height(V)
1	Candesartan	2.755	5223559	541538.3
2	Candesartan	2.687	5208511	485548.5
3	Candesartan	2.632	5323569	574440.4
4	Candesartan	2.612	5259147	557413.5
5	Candesartan	2.616	5273463	565020.1
Mean			5257650	
Std.Dev.			45206.4	
%RSD			0.86	

Table 7: Showing% RSD results for Amlodipine

	Peak Name	RT	Aera	Height(V)
1	Amlodipine	3.616	2742453	238643.4
2	Amlodipine	3.634	2762750	271543.5

3	Amlodipine	3.460	2797670	281711.6
4	Amlodipine	3.446	2793578	274499.8
5	Amlodipine	3.437	2778483	276713.0
Mean			2774987	
Std.Dev.			22806.9	
%RSD			0.82	

Table 7: Showing results for intermediate precision of Candesartan

	Peak Name	**RT**	**Aera**	**Height(V)**
1	Candesartan	2.756	5698542	539568.1
2	Candesartan	2.688	5682534	536985.4
3	Candesartan	2.633	5695846	539584.1
4	Candesartan	2.613	5689452	534569.8
5	Candesartan	2.617	5636591	534985.5
Mean			5600593	
Std.Dev.			203577.3	
%RSD			0.44	

Table 8: Showing results for intermediate precision of Amlodipine

	Peak Name	**RT**	**Aera**	**Height(V)**
1	Amlodipine	3.617	2624315	231325.6
2	Amlodipine	3.635	2623598	231315.4
3	Amlodipine	3.461	2623541	231250.1
4	Amlodipine	3.447	2624987	231342.6
5	Amlodipine	3.438	2635698	231765.2
Mean			2626428	
Std.Dev.			5215.78	
%RSD			0.19	

Table 9: Showing results for Limit of Detection

Drug name	**Standard deviation(σ)**	**Slope(s)**	**LOD(µg)**
Candesartan	373625.50	581075863	3.17
Amlodipine	5772.40	476579210	0.0172

Table 10: Showing results for Limit of Quantitation

Drug name	**Standard deviation(σ)**	**Slope(s)**	**LOQ(µg)**
Candesartan	372727.80	574265980	5.80
Amlodipine	5761.30	478828490	0.212

Table 11: Showing system suitability results for Candesartan

S. No	**Flow rate (ml/min)**	**System suitability results**	
		USP Plate Count	**USP Tailing**
1	0.8	5339	1.4
2	**1**	**4668**	**1.3**
3	1.2	5216	1.4

Table 12: Showing system suitability results for Amlodipine

S. No	**Flow rate (ml/min)**	**System suitability results**	
		USP Plate Count	**USP Tailing**
1	0.8	7036	1.3
2	**1**	**6089**	**1.2**
3	1.2	6998	1. 3

Table 13: Showing system suitability results for Candesartan

S. No	**Change in organic composition in the mobile phase**	**System suitability results**	
		USP Plate Count	**USP Tailing**
1	5 % less	6232	1.4

2	***Actual**	**4668**	**1.3**
3	5 % more	6387	1.4

Table 14: Showing system suitability results for Amlodipine

S. No	Change in organic composition in the mobile phase	System suitability results	
		USP Plate Count	USP Tailing
1	5 % less	5437	1.3
2	*Actual	6089	1.2
3	5 % more	4817	1.2

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