RP-HPLC method for simultaneous estimation of Candesartan and Amlodipine in bulk and pharmaceutical dosage forms

Raja B[1], Lakshmana Rao A[2*]

1. Anurag Pharmacy College, Ananthagiri- 508 206, A.P., India.
2. V.V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, A.P., India.

[*]Corresponding Author: E-mail: dralrao@gmail.com

ABSTRACT

A simple, rapid, accurate and precise isocratic reversed phase high performance liquid chromatographic method has been developed and validated for simultaneous estimation of Candesartan and Amlodipine in tablet dosage forms. The chromatographic separation was carried out on C18 analytical column (150 mm x 4.6 mm I.D., 5 µm particle size) with a mixture of phosphate buffer pH 6.8 was adjusted with potassium hydroxide and acetonitrile in the ratio of 35:65 V/V as a mobile phase at a flow rate of 1.0 mL/min. UV detection was performed at 238 nm. The retention times were 3.610 minutes and 4.773 minutes for Candesartan and Amlodipine respectively. Calibration plots were linear (r^2=0.999 for Candesartan and 1 for Amlodipine) over the concentration range of 4-24 µg/mL for Candesartan and 2.5-15 µg/mL for Amlodipine. The method was validated for linearity, precision, accuracy, ruggedness and robustness. The proposed method was successfully used for simultaneous estimation of Candesartan and Amlodipine in tablet dosage forms. Validation studies revealed that method is specific, rapid, reliable and reproducible. The high % recovery and low % RSD confirms the suitability of the proposed method for routine quality control analysis of Candesartan and Amlodipine in bulk and tablet dosage forms.

Key words: Candesartan, Amlodipine, Estimation, HPLC.

INTRODUCTION

Candesartan cilexetil is a nonpeptide prodrug, is hydrolyzed Candesartan during absoption forms the gastrointestinal tract. Candesartan is a selective AT1 subtype angiotensin II receptor antagonist (Sweetman, 2011). Chemically it is (±) -1- hydroxyethyl 2-ethoxy-1- [p- (o-1 *H* – tetrazol – 5 ylphenyl) benzyl] – 7-benzimidazolecarboxylate, cyclohexyl carbonate (ester) (Fig.1). Candesartan blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland (Neil, 2006).

Amlodipine besylate is a potent long-acting calcium channel blocker used for the treatment of hypertension, congestive heart failure and angina pectoris (Indian Pharmacopoeia, 2014). Chemically it is 3-ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)- l, 4 – dihydro – 6 – methyl -3, 5pyridinedicarboxylate, monobenzenesulphonate (Fig. 2). Amlodipine inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Candesartan cilexetil is an effective drug when used in combination with Amlodipine in the treatment of moderate-to-severe essential hypertension (Kloner, 2001; Koyanagi, 2013).

Literature survey reveals that only UV spectrophototmetric (Kotecha, 2014) method was reported for simultaneous estimation of Candesartan and Amlodipine in pharmaceutical formulations and no HPLC method was reported for simultaneous estimation of Candesartan and Amlodipine. Therefore, an attempt has been made to develop a novel, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Candesartan and Amlodipine in tablet dosage form and validated in accordance with ICH (ICH, Q2 (R1), 2005) guidelines.

MATERIALS AND METHODS

Instrumentation: To develop a high performance liquid chromatographic method for simultaneous estimation of Candesartan and Amlodipine using Waters 2695 HPLC system on C18 (150 mm x 4.6 mm I.D., 5 µm particle size) column was used. The instrument is equipped with an auto sampler and UV-Vis detector. A 20 µL rheodyne injector port was used for injecting the samples. Data was analyzed by using Empower 2 software. A Systronics-361 pH meter was used for pH measurements.

Chemicals and solvents: The working standards of Candesartan cilexetil and Amlodipine besylate was provided as gift samples from Chandra Labs, Hyderabad, India. The marketed formulation of Candesartan cilexetil and Amlodipine besylate tablets (Candesartan cilexetil 8 mg and Amlodipine besylate 5 mg) were procured from local market. HPLC grade water and acetonitrile were purchased from E.Merck (India) Ltd., Mumbai, India. Potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate and potassium hydroxide of AR grade were obtained from S.D. Fine Chemicals Ltd., Mumbai, India.

Chromatographic conditions: Phosphate buffer pH 6.8 adjusted with potassium hydroxide and acetonitrile in the ratio of 35:65 V/V was found to be the most suitable mobile phase for ideal chromatographic separation for simultaneous estimation of Candesartan cilexetil and Amlodipine besylate. The solvent mixture was filtered through a 0.45 µm membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.0 mL/min. Injection volume was 20 µL and the column was maintained at a temperature of 35^0C. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solution. The detection of the drug was monitored at 238 nm. The run time was set as 10 minutes.

Preparation of phosphate buffer pH 6.8: 1.36 grams of potassium dihydrogen orthophosphate and 0.6 gms dipotassium hydrogen phosphate was weighed accurately, transferred into a 1000 mL beaker and dissolved in 500 mL of HPLC grade water. The solution was sonicated for 30 minutes, degassed and then made to total volume with water. The pH of the resulting solution was adjusted to 6.8 with potassium hydroxide and filtered through 0.45 µm membrane filter.

Preparation of mobile phase and diluent: The mobile phase was prepared by mixing 350 mL of phosphate buffer pH 6.8 with 650 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 µm filter under vacuum. The same mobile phase was used as diluent.

Preparation of standard solution: Accurately weighed, transferred 8 mg of Candesartan and 5 mg of Amlodipine working standards into 50 mL volumetric flask and was dissolved in 30 mL of diluent. Sonicated the solution for few minutes and dissolved the drugs completely. Then it was filtered through 0.45 µm filter and the volume is made up to 50 mL with diluent. From this stock solution 10 mL was transferred into 100 mL volumetric flask and diluted up to the mark with diluent.

Preparation of sample preparation: Twenty commercial tablets were weighed, powdered and weighed accurately the tablet powder equivalent to 8 mg of Candesartan and 5 mg of Amlodipine, transferred in to 50 mL volumetric flask and was dissolved in 30 mL of the diluent. Sonicated the solution for few minutes and dissolved the drugs completely. Then it was filtered through 0.45 µm filter and the volume is made up to 50 mL with diluent. From this stock solution 10 mL was transferred into 100 mL volumetric flask and diluted up to the mark with diluent.

Procedure: The column was maintained at a temperature of 35^0C. The run time was set at 10 minutes. The column was equilibrated by pumping the mobile phase through the column for at least 30 minutes prior to the injection of the drug solutions. Inject 20 µL of the standard and sample solutions six times into the chromatographic system at a flow rate of 1.0 mL/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed.

Method validation:

Linearity: Several aliquots of standard solutions of Candesartan and Amlodipine were taken in different 100 mL volumetric flasks and diluted up to the mark with diluent such that the final concentrations were in the range of 4-24 µg/mL for Candesartan and 2.5-15 µg/mL for Amlodipine. Different linearity levels was prepared and injected into the HPLC system keeping the injection volume constant. The drugs were eluted with UV detector at 238 nm, peak areas was recorded for all the peaks. The linearity curves were constructed by plotting concentration of the drugs against peak areas. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of drugs in tablet dosage forms.

Precision: Precision for Candesartan and Amlodipine was determined in terms of system precision and method precision. Every sample was injected six times. The measurements for peak areas were expressed in terms of % RSD.

Accuracy: The accuracy of the method was assessed by recovery studies of Candesartan and Amlodipine at three concentration levels 50%, 100% and 150%. Fixed amount of pre-analyzed sample was spiked with known amount of Candesartan and Amlodipine. Each level was repeated three times. The % recovery of Candesartan and Amlodipine were calculated.

System suitability: The system suitability parameters like retention time, theoretical plates and tailing factor were evaluated by six replicate analysis of Candesartan and Amlodipine and compared with standard values. The acceptance criteria are % RSD of peak areas not more than 2%, theoretical plates numbers (N) at least 3000 per each peak and tailing factors not more than 2.0 for Candesartan and Amlodipine.

Limit of detection and limit of quantification: The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions of Candesartan and Amlodipine using the developed HPLC method. LOD and LOQ were estimated from signal-to-noise ratio. LOD and LOQ were calculated using 3.3 σ/s and 10 σ/s formulae, respectively. Where, σ is the standard deviation of the peak areas and S is the slope of the corresponding calibration curve.

Ruggedness and robustness: The ruggedness of the method was determined by carrying out the experiment on different instruments by different operators using different columns of similar types. The robustness of the method was determined by making slight changes in the few parameters such as variation of flow rate, buffer composition and percent composition of the mobile phase.

Assay: 20 µL of sample solution was injected and from the peak areas of Candesartan and Amlodipine, amount of each drug in the sample were computed. The results were compared with the label claim of Candesartan and Amlodipine in tablet dosage forms.

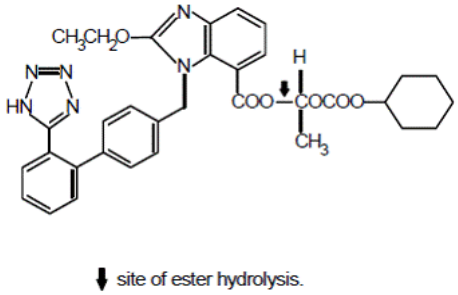

Figure.1. Molecular structure of Candesartan cilexetil

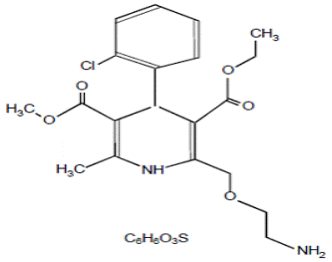

Figure.2. Molecular structure of Amlodipine besylate

RESULTS AND DISCUSSION

The HPLC procedure was optimized with a view to develop an accurate, precise and reproducible method in tablet dosage form using C18 analytical column (150 x 4.6 mm; 5 µm)) in isocratic mode with mobile phase composition of phosphate buffer pH adjusted to 6.8 with potassium hydroxide and acetonitrile in ratio 35:65 V/V. The use of phosphate buffer and acetonitrile in the ratio of 35:65 V/V resulted in peak with maximum separation, good shape and resolution. Flow rates between 0.8 to 1.2 mL/min were studied. A flow rate of 1.0 mL/min gave an optimum signal-to-noise ratio with reasonable separation time, the retention times for Candesartan and Amlodipine were found to be 3.610 minutes and 4.773 minutes respectively. Total run time was 10 minutes. The drug components were measured with UV detector at 238 nm. The results of optimized chromatographic conditions were shown in Table 1.

Linearity was obtained in the range of 4-24 µg/mL for Candesartan and 2.5-15 µg/mL for Amlodipine. The correlation coefficient (r^2) was found to be 0.999 for Candesartan and 1 for Amlodipine. The regression equation of the linearity plot of concentration of Candesartan over its peak area was found to be y=72646x+6140, where x is the concentration of Candesartan (µg/mL) and y is the corresponding peak area. The regression equation of the linearity plot of concentration of Amlodipine over its peak area was found to be y=10047x+4514, where x is the concentration of Amlodipine (µg/mL) and y is the corresponding peak area. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated. The linearity results were shown in Table 2 and Table 3 and the calibration curves were shown in Fig. 3 and Fig. 4.

The % RSD for system precision and method precision for Candesartan were found to be 0.19% and 0.56% respectively (limit % RSD<2.0%). The % RSD for system precision and method precision for Amlodipine were found to be 0.12% and 0.41% respectively (limit % RSD<2.0%) and hence the method is precise. The precision data of Candesartan and Amlodipine were furnished in Table 4 and Table 5.

The mean recovery of the drugs Candesartan and Amlodipine was 99.77% and 99.84% respectively and the high percentage of recovery of Candesartan and Amlodipine indicates that the proposed method is highly accurate. The results of accuracy studies of Candesartan and Amlodipine were shown in Table 6 and Table 7.

The retention times for the drugs Candesartan and Amlodipine was 3.610 minutes and 4.773 minutes

respectively. The number of theoretical plates calculated for Candesartan and Amlodipine was 4509 and 5666 respectively. The tailing factor for Candesartan and Amlodipine was 1.12 and 1.09 respectively, which indicates efficient performance of the column. The limit of detection (LOD) and limit of quantification (LOQ) for Candesartan and Amlodipine were found to be 0.13 µg/mL and 0.40 µg/mL; 0.08 µg/mL and 0.24 µg/mL for Candesartan and Amlodipine respectively, which indicate the sensitivity of the method. The summary of system suitability parameters and validation parameters were shown in Table 8.

The robustness studies indicated that no considerable effect on the determination of the drugs. Therefore the test method is robust for the quantification of the drugs. In all deliberately varied conditions, the % RSD for replicate injections of Candesartan and Amlodipine were found to be within the acceptable limits.

Validated method was applied for the simultaneous estimation of Candesartan and Amlodipine in commercial tablet dosage forms. The % Assay of Candesartan and Amlodipine were found to be 99.76% and 99.98% respectively. The results for the drugs assay showed good agreement with label claims. No interfering peaks were found in the chromatogram of the tablet formulation within the run time indicating that excipients used in tablet formulation did not interfere with the simultaneous estimation of the drugs Candesartan and Amlodipine by the proposed HPLC method. The assay results are shown in Table 9.

The chromatograms were checked for appearance of any extra peaks under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with standard and were found to be within limits. As shown in chromatogram, two analytes are eluted by forming symmetrical peaks. The typical chromatogram of Candesartan and Amlodipine standard were shown in Fig. 5.

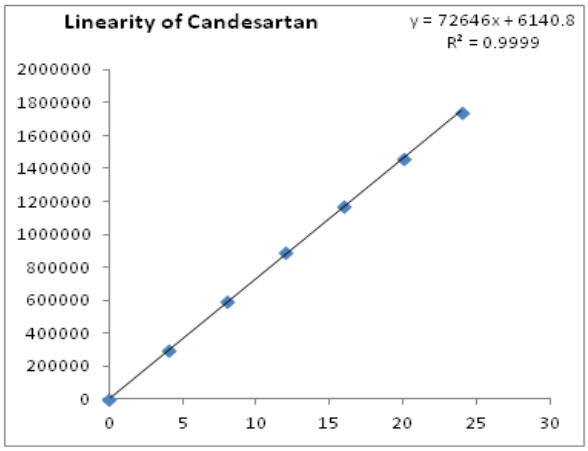

Figure.3. Calibration curve of Candesartan

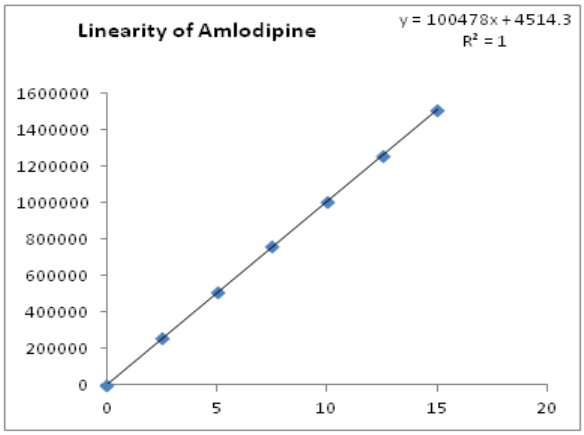

Figure.4. Calibration curve of Amlodipine

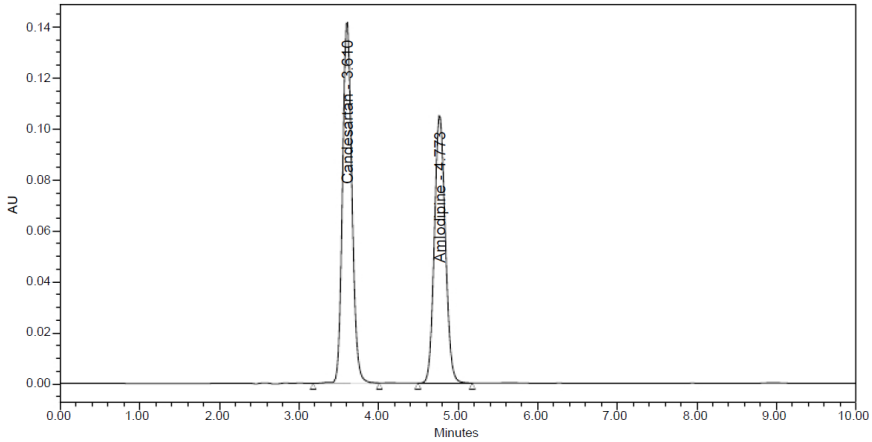

Figure.5. Typical chromatogram of standard for Candesartan and Amlodipine

Table.1. Optimized chromatographic conditions

Parameter	Condition
Mobile phase	Phosphate buffer:acetonitrile (35:65 V/V)
pH	6.8
Diluent	Mobile phase
Column	C18 (150 mm x 4.6 mm, 5 µm)
Column temperature	35^0C
Wave length	238 nm
Injection volume	20 µL
Flow rate	1.0 mL/min.
Run time	10 min.

Table.2. Linearity results of Candesartan

Concentration (µg/mL)	Mean peak area
4	296509
8	588808
12	888304
16	1168636
20	1459445
24	1743553

Table.3. Linearity results of Amlodipine

Concentration (µg/mL)	Mean peak area
2.5	255984
5	508172
7.5	764971
10	1009620
12.5	1259852
15	1508089

Table.4. Precision data of Candesartan

S. No.	Peak area	
	System precision	Method precision
Injection-1	1156423	1121095
Injection-2	1155048	1130265
Injection-3	1159240	1133324
Injection-4	1159890	1129454
Injection-5	1160014	1136579
Injection-6	1160020	1139022
Mean	1158439	1131623
SD	2158.255	6318.038
% RSD	0.19%	0.56%

Table.5. Precision data of Amlodipine

S. No.	Peak area	
	System precision	Method precision
Injection-1	1008541	1004530
Injection-2	1009124	1005564
Injection-3	1008992	1006029
Injection-4	1008998	1005436
Injection-5	1009004	1006012
Injection-6	1006021	1005426
Mean	1008447	1007166
SD	1205.281	4083.133
% RSD	0.12%	0.41%

Table.6. Accuracy studies of Candesartan

% Concentration level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	% Mean recovery
50 %	8	7.984	99.80%	99.77%
100%	16	16.046	100.29%	
150%	24	23.817	99.24%	

Table.7. Accuracy studies of Amlodipine

% Concentration level	Concentration added (µg/mL)	Concentration found (µg/mL)	% Recovery	% Mean recovery
50 %	5	4.993	99.86%	99.84%
100%	10	10.017	100.17%	
150%	15	14.925	99.50%	

Table.8. System suitability parameters of proposed method

Parameters	Candesartan	Amlodipine
Linearity (µg/mL)	4-24	2.5-15
Correlation coefficient	0.999	1
Retention time (min.)	3.610	4.773
Resolution	--	4.88
Tailing factor	1.12	1.09
Theoretical plates (N)	4509	5666
LOD (µg/mL)	0.13	0.08
LOQ (µg/mL)	0.40	0.24

Table.9. Assay results of marketed formulations

Formulation		Label claim	Amount found	% Assay
Formulation-1	Candisartan	8 mg	7.981 mg	99.76%
	Amlodipine	5 mg	4.999 mg	99.98%

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the simultaneous estimation of Candesartan and Amlodipine and can be reliably adopted for routine quality control analysis of Candesartan and Amlodipine in its tablet dosage forms.

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