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RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF CANDESARTAN AND AMLODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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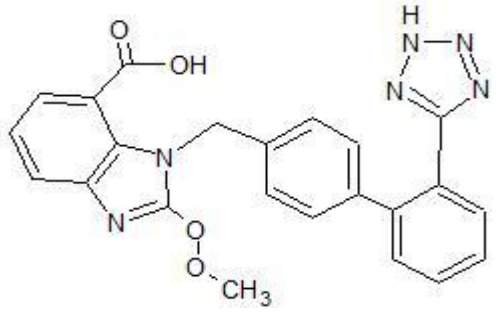
ABSTRACT
The main objective of this study is to develop a reverse phase HPLC method for the simultaneous determination of candesartan and amlodipine bulk and pharmaceutical dosage form with a simple, rapid, specific, validated and sensitive method. An isocratic separation is achieved using C18(150 x 4.6mm, 5µ) with mobile phase comprises of water and methanol in the ratio of 10:90 v/v. candesartan shows a retention time 3.5min and amlodipine shows 1.17min at 1ml/min flow rate and the wavelength was detected at 355nm. Robustness, specificity, precision, accuracy, linearity, LOD and LOQ was validated using this method. The LOD and LOQ are 0.48 and 1.5 for amlodipine and 0.75 and 2.3 for candesartan respectively. The calibration curve in the concentration range of 4-24 mcg/ml for both AMLO and CANDE are linear with the coefficient of correlation 0.997 and 0.998 respectively. The % of recovery of candesartan is 99.5% and amlodipine is 100.3% and the % od RSD is <2%. This method is successfully applied for quantitative determination of candesartan and amlodipine bulk as well as the formulation.

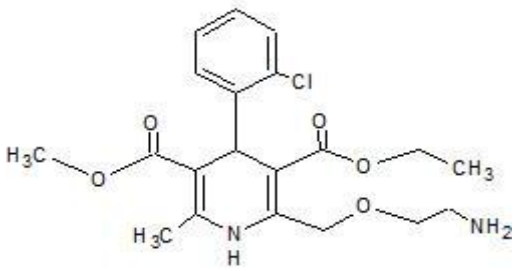
KEYWORD: method development, validation, candesartan, amlodipine, ICH.

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). 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.



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.



Literature survey reveals that only UV-Spectrophotometric method was reported for simultaneous estimation of Candesartan and Amlodipine in pharmaceutical formulations. 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 METHOD

Chemical and apparatus

HPLC grade methanol, HPLC grade water, orthophosphoric acid, water 1525 binary HPLC pump, water 2487 dual λabsorbance detector, digital ultrasonic

cleaner (INDOSATI), volumetric flask, measuring cylinder, injector, Vacuum filter, syringe filter, micro pipette, Whatman filter paper.

HPLC instrumentation and condition

The analysis was carried out on a HPLC system equipped with UV detector, pressure control by prominent pump and operated by empower3 software. C 18 column was used consisting diameter of 150 x 4.6 mm, 5µm particle size was used for separation. Mobile phase used for separation was a mixture of two components i.e. methanol and water in the ration of 90;10 adjusting the pH to 3.2 with orthophosphoric acid. The flow rate was kept 1.0 ml/min, system was carried out in a room temperature and eluents were detected by UV detector at the wavelength 355nm, the injection volume was 20µl.

Selection of solvents

The solubility of both drugs is determined in verity of solvents as per pharmacopoeia standard. Solubility test was carried out in different solvents like acetonitrile HPLC grade, methanol HPLC grade, water HPLC grade, ethanol. From this, studies it was found that methanol and water which is a good solvent for both drugs.

Preparation of standard stock solution

An accurately weighed quantity of 10 mg was taken in a 10 ml of volumetric flask. Small amount of mobile phase was added to it for dissolve and sonicated for 10 min and made the volume up to the mark (1000 mcg/ml). From that solution 4 ml was taken in a 100 ml of volumetric flask and 80 ml of solvent was added to it. This was again sonicated for 10 mins and volume was diluted up-to the mark with solvent to get the concentration 40 mcg/ml (working standard).

Preparation of mobile phase

mobile phase was prepared by using 90 volume of methanol with 10 volume of water HPLC grade was mixed and was adjusted the pH 3.2 with orthophosphoric acid. Sonicated the solvent for 10-15 mins. The mobile phase was then ultrasonicated, filtered through 0.45 µm membrane filter paper with the help of vaccum filtration, degassed the content again.

METHOD VALIDATION

System suitability: it was performed by injecting the blank solution once 100% test concentration standard solution for six times into HPLC system. The system suitability test was evaluated from the chromatograms obtained.[11]

Specificity: it was determined by comparison between standard drug with sample. Fixed concentration of working standard of both standard drug and sample test solution were injected to HPLC system for six time were analysed. % of RSD was calculated from their peak area.[12]

Linearity: linearity was demonstrated over the range of 4-24 µg/ml of test concentration. The solution at six level of the concentration was prepared and 20 µl of each solution were injected into the HPLC system to get the chromatograms. By plotting concentration against the peak area, the linearity curve was constructed and the regression equation was calculated by the method of least squares, the correlation coefficient, y-intercept and slope of the regression line were reported.[9]

Accuracy: the closeness of agreement of between a test result and true value.

Accuracy (%) = 100 x test value /reference value

Method was established by performing recovery studies, recovery studies was performed by spiking sample solution with pure standard drug at three different concentration level. Mean recovery of the drug was calculated.

Precision Intraday and interday precision of the method were demonstrated by taking one of the test concentrations. The concentration injected in triplicate in to HPLC system to obtained the chromatogram and peak areas were recorded from the obtained peaks. The average and the standard deviation of the peak areas at each concentration level were calculated.[10]

% of RSD can be calculated by 100 x SD/X

SD= standard deviation of "n" responses

X= mean of "n" responses

Limit of detection: it can be defined as the lowest amount of the analyte in a sample which can be detected but not necessarily quantitated as an extract value, using a specific method under the required experimental conditions.[13]

$LOD = 3\sigma/S$

where σ = standard deviation of the response

S = slope of the calibration curve

Limit of quantification: it can be defined as the smallest concentration of analyte which gives a response that can be accurately quantified. It can be calculated by

$LOD = 10\sigma/S$

where σ = standard deviation of response

S = slope of the calibration curve

Robustness: For the determination of method's robustness, deliberate change in flow rate, mobile phase composition, pH, temperature was made to evaluate the impact of this variation on the method.[14]

RESULT AND DISCUSSION

Optimization of chromatographic condition: To develop a suitable RP-HPLC method for simultaneous estimation of amlodipine and candesartan, different chromatographic conditions were applied and optimized chromatographic conditions were developed (see figure 4).

Optimized chromatographic conditions are as follows:
Instrument: water 1525 binary HPLC pump, water 2487 dual λabsorbance detector and empower3 software.
Mobile phase: methanol and water along with orthophosphoric acid to adjust pH 3.2 (90:10 v/v)
Injection volume: 20 µl
Flow rate: 1ml/min
Detection wavelength: 355 nm
Runtime: 5 min
Temperature: room temperature

VALIDATION

Specificity

Table 1: specificity data.

	Avg standard area	Avg sample area	SD	RSD	interference
AMLO	59241	59107	174.5	0.29	RSD found to be <2
CANDE	17534	17241	187.8	1.05	

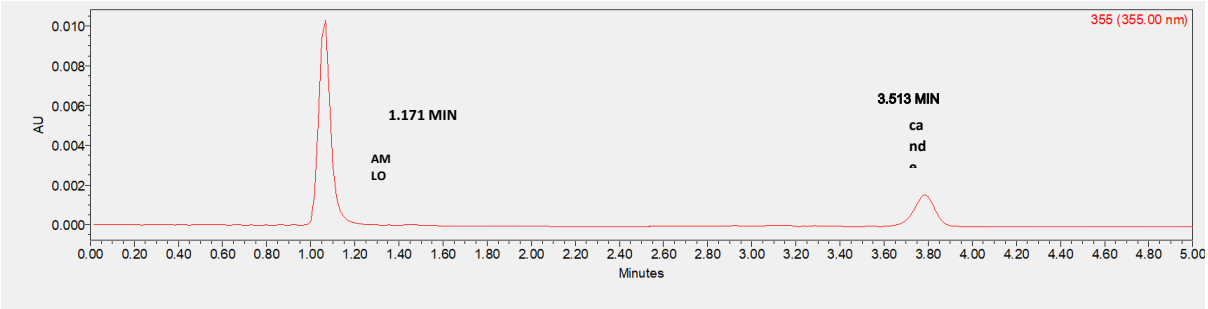


Figure 4: optimized chromatogram of amlodipine and candesartan.

Linearity: the calibration curve was constructed with concentration on X-axis and peak area on Y-axis to establish the linearity of drug. From the calibration curve, it was observed that the method was linear over the concentration range of 4-24 mcg/ml for both drugs. (see figure 5 & table 2)

Table2: peak areas of linearity standard solution of amlodipine and candesartan.

Sl. no	concentration	Area of AMLO	Area of cande
1	4	45041	7310
2	8	49113	10473
3	12	54479	13950
4	16	59793	17411
5	20	64360	20495
6	24	68215	23254

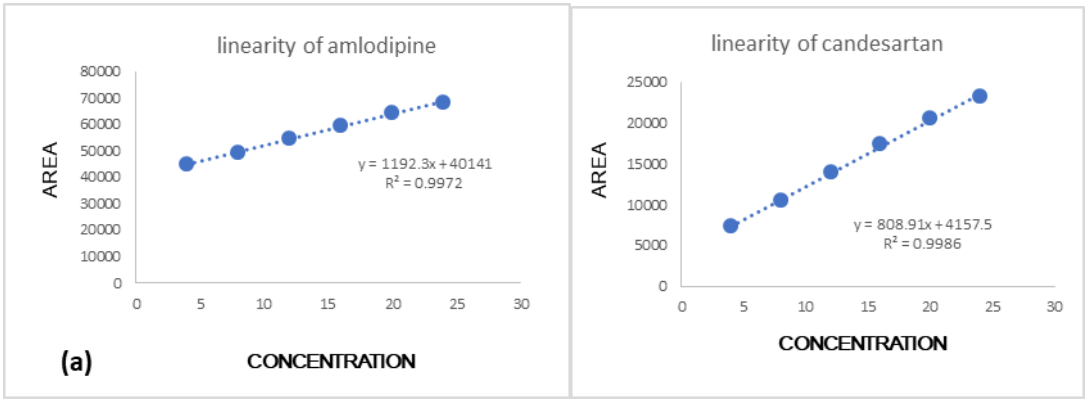


Figure 5: linearity plot of amlodipine(a) and candesartan(b) (4-24mcg/ml) standard solution.

Accuracy: The recovery studieswere performed to check the accuracy of the method at three levels 50%,100% and150%. The mean recovery of amlodipine was found to be 100.3% and candesartan was 99.5%. (see table 4 and 3)

Table3: recovery studies of CANDESARTAN.

Level	Added amount (µg/ml)	Found amount	Recovery	Average % recovery
50%	4	3.94	98.5	99.5
100%	12	11.93	99.4	
150%	20	20.15	100.4	

Table4: recovery studies of amlodipine.

Level	Added amount (µg/ml)	Found amount (µg/ml)	% Recovery	Average % recovery
50%	4	3.99	99.8	100.3
100%	12	11.91	98.2	
150%	20	20.36	101.8	

Precision: precision of the method was studied by making repeated injections of the mixture of drugs. The coefficient variation CV was after six determination was 0.29% & 0.1% for amlodipine and candesartan at 16mcg/ml respectively. The % of RSD of peak area of chromatograms of AMLO and CANDE is <2 for intraday as well as interday precision respectively (see table no 5 and 6).

Table 5: intraday precision.

injection (16mcg/ml)	Area amlodipine	Area valsartan	Interference
1	59125	17495	% RSD was found to be <2
2	59325	17385	
3	59010	17425	
4	59450	17397	
5	59397	17652	
6	59139	17852	
Mean	59241	17534.6	
Standard deviation	174.5	184.7	
CV	0.29	0.1	
RSD	0.3	1.05	

Table 6: interday precision.

AMLO						
	8 mcg/ml	12 mcg/ml	16 mcg/ml	20 mcg/ml	24 mcg/ml	28 mcg/ml
Day 1	44825	49173	54479	59724	64365	68217
Day 2	44752	49124	54782	59545	64954	68425
Day 3	44321	49554	54254	59442	64382	68124
Mean	44632.67	49283.67	54505	59570.33	64567	68255.33
STD DEV	272.368	235.394	264.9585	142.6966	335.2596	154.1179
RSD	0.610244	0.477631	0.486118	0.239543	0.519243	0.225796
Cande						
day1	7325	10472	13952	17415	20493	23259
day2	7365	10482	13949	17454	20886	23257
day3	7345	10751	14175	17548	20471	23954
mean	7345	10568.33	14025.33	17472.33	20616.67	23490
sd	20	158.273	129.6238	68.3691	233.5087	401.837
Interference: % RSD was found to be <2						

Limit of detection and limit of quantification: (LOD &LOQ) of amlodipine and candesartan were determined by calibration curve method. Solution of both were prepared in the range of 4-24 mcg/ml and injected triplicate (see table no 7)

Formulas: LOD = 3.3 x SD/SLOPE
LOQ = 10 X SD/SLOPE

Table 7: result of LOD AND LOQ of amlodipine and candesartan.

	AMLO	CANDE
SD	174.5	184.8
SLOPE	1192.3	808.3
LOD	0.48	0.75
LOQ	1.5	2.3

Robustness

The standard chromatogram of the drugs was within limit for variation in flow rate (0.2 ml) and flow rate within the range of 0.8-1.0ml was allowed and variation in wavelength (2nm). The % RSD values is <2.0%, hence the method is proved to be robust.

Table 8: result of robustness.

Parameters	Retention time AMLO	AMLO %RSD	Retention time CANDE	CANDE %RSD	Interference
Flow rate					
1.0 ml/min	1.57 min	0.7	3.59	0.8	%RSD was found to be <2
0.8 ml/min	1.7 min	0.4	3.71	0.5	
Wavelength					
355 nm	1.56 min	0.6	3.56	0.78	%RSD was found to be <2
350 nm	1.53 min	0.45	3.53	0.52	

System suitability: SD, %RSD were calculated by performing the system suitability test the retention time of 1.5 min and 3.5 min were exhibited by the chromatograms. From the system suitability studies, it was observed that %RSD of peak area was to be 0.10 for amlodipine and 0.08 for candesartan standard drug. See table no 9.

Table 9: data of system suitability.

Injection	Retention time (AMLO)	Peak area	Retention time (CANDE)	Peak area
1	1.53	59125	3.51	17495
2	1.51	59325	3.59	17385
3	1.58	59010	3.54	17425
4	1.49	59450	3.49	17397
5	1.57	59397	3.52	17652
6	1.49	59139	3.53	17852
SD		174.5		184.7
%RSD		0.3		1.05

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for the simultaneous determination of amlodipine and candesartan by RP-HPLC. The method was validated for the parameter like linearity, specificity, accuracy, precision, lod, loq and system suitability value was found to be within the limits. The method has significant advantages of shorter time of analysis. The validation study indicates that the method can be carried for suitable routine analysis of amlodipine and candesartan.

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