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DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR THE DETERMINATION OF THE CANDESARTAN CILEXETIL IN TABLET DOSAGE FORMS

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ABSTRACT

A rapid and reproducible reverse phase high performance liquid chromatographic method has been developed for the determination of candesartan cilexetil from its dosage forms. The separation was effected on a C_{18} Kromasil column (150 x 4.6 mm; 5 µ) using a mobile phase consisting of water, acetonitrile and trifluoro acetic acid in the ratio of 48 : 52 : 0.1 v/v at a flow rate of 1.5 mL/min. The retention time of the drug was found to be 5.54 min. The method produced linear responses in the concentration range of 25-200 µg/mL of the drug. The proposed method was validated as per the ICH guidelines. The method is accurate and precise and is found to be suitable for the quantitative analysis of the drug in its tablet dosage forms.

Key words: Candesartan cilexetil, Determination, Tablets, RP-HPLC

INTRODUCTION

Candesartan cilexetil[1-4] is a novel antihypertensive drug approved by the U.S. FDA. It is an angiotensin II type 1 receptor antagonist. It is chemically, 2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl) [1, 1′-biphenyl]-4-yl] methyl]-1*H*-benzimidazole-7-carboxylic acid. So far, very few HPLC[4,5] and HPTLC[6] methods were reported for the estimation of candesartan cilexetil in dosage forms and in human plasma in therapeutic drug monitoring studies. The authors now propose a rapid, sensitive and validated[8] HPLC method for the estimation of candesartan cilexetil in tablet dosage forms.

EXPERIMENTAL

Chemicals, reagents and solutions: A reference standard sample of candesartan

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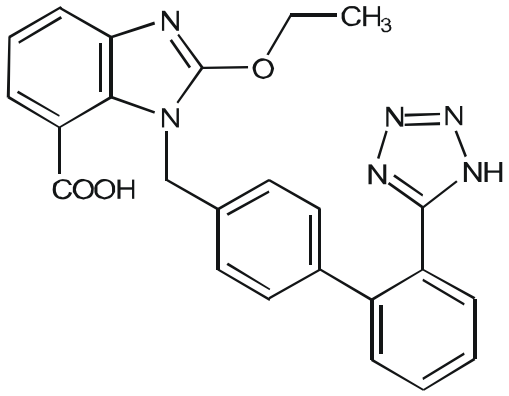
cilexetil was obtained from Matrix Laboratories, Hyderabad, India. A commercial sample of tablets of candesar 32 (containing 32 mg of candesartan) of Asia Pharmaceuticals was purchased from the local market. HPLC grade acetonitrile, trifluoroacetic acid and water were procured from Merck Fine Chemicals, Mumbai. ExcelaR grade sodium hydroxide, hydrochloric acid and hydrogen peroxide were purchased from Qualigens, Mumbai. A mixture of acetonitrile and water in the ratio of 60 : 40 v/v was used as the diluent for the preparation of the standard and sample solutions of candansartan cilexetil.

Chemical structure of candesartan cilexetil



Chromatographic conditions

Chromatography was performed on a Kromasil C_{18} column (250 x 4.6 mm; 5µ) using an Agilent 1100 HPLC instrument equipped with a quaternary pump, a degasser and a photodiode-array detector. The system was controlled by Chemstation software. A column temperature of 30^0C was maintained in the study. A mobile phase mixture of 48 : 52 : 0.1 v/v of water, acetonitrile and trifluoroacetic acid was pumped through the column at a rate of 1.5 mL/min. The mobile phase was filtered and degassed in an ultrasonic bath prior to use. The injection volume was 10 µL and detection was done at 240 nm. Peak homogeneity was expressed as peak purity and was obtained directly from the spectral analysis report.

Preparation of standard stock solution

About 40 mg of candesartan cilexetil was weighed accurately and transferred into a 50 mL volumetric flask containing 30 mL of the diluent. The solution was sonicated for about 20 min and then the volume was made up with further quantity of the diluent to get a 0.8 mg/mL solution. The solution was filtered through a 0.45 µm PVDF filter. This solution

was suitably diluted with the diluent to get a working standard solution of 80 µg/mL of candesartan cilexetil

Sample solution

Five tablets of candesartan cilexetil (32 mg) were powdered in a mortar and the powder was transferred into a 200 mL volumetric flask containing 140 mL of the diluent. The solution was sonicated for about 20 min and then the volume made up with further quantity of the diluent to get a 0.16 mg/mL solution. The solution was filtered through a 0.45 µm PVDF filter. This solution was suitably diluted with the diluent to get a sample solution containing 125 µg/mL of candesartan cilexetil.

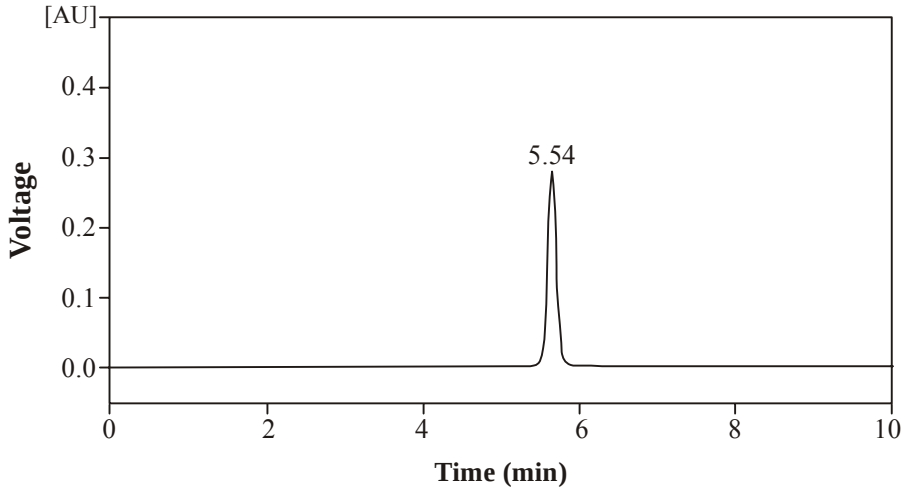


Fig. 1: A representative chromatogram of candesartan cilexetil from the tablet solution

Optimization of the chromatographic conditions

To develop a new method, optimization was carried out on different stationary phases, like C_{18} (Zorbax, BDS) and CN (ALLTIME) columns. In order to effect ideal separation of the drug under isocratic conditions, mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested. The objective was to achieve a peak tailing factor of < 2 and a retention time between 3 to 10 min for the drug. A sharp peak of candesartan cilexetil was achieved on a Kromasil C_{18} column (250 x 4.6 mm; 5µ). A mobile phase mixture of water, acetonitrile and trifluoro acetic acid in the ratio of 48 : 52 : 0.1 (v/v) at a flow rate of 1.5 mL/ min was found to be

suitable for good base line separation and resolution. A column temperature of 30^0C was maintained in the study. Under these conditions, the retention time obtained for candesartan cilexetil was 5.6 min.

Method Validation

Specificity

A study was conducted to establish the non-interference of the placebo contents with the drug. Samples were prepared in triplicate by taking the placebo equivalent to the weight in portion to the test preparation. A chromatogram of the placebo solution did not show any extra peaks. This indicates that the excipients used in the formulation do not interfere in the estimation of candensartan cilexetil.

Calibration curve and linearity

Linearity was tested using solutions containing 10 – 300 % of the targeted level of the assay concentration (100 µg/mL). Solutions for assessment of linearity were injected in triplicate. The calibration curve was plotted between the amounts added and the amounts found. The regression equation of the calibration plot was $y = 0.992x + 0.273$. The plot was linear in the concentration range 10-300 µg/mL with a correlation coefficient of 1.

Table 1: Linearity data

% Spike level	Amount taken (mg)	Amount obtained (mg)
10	16.4	16.28
25	40.5	40.69
50	80.27	79.81
75	120.27	119.91
100	160.27	159.61
150	240.17	238.74
250	400.23	397.57
300	480	476.42

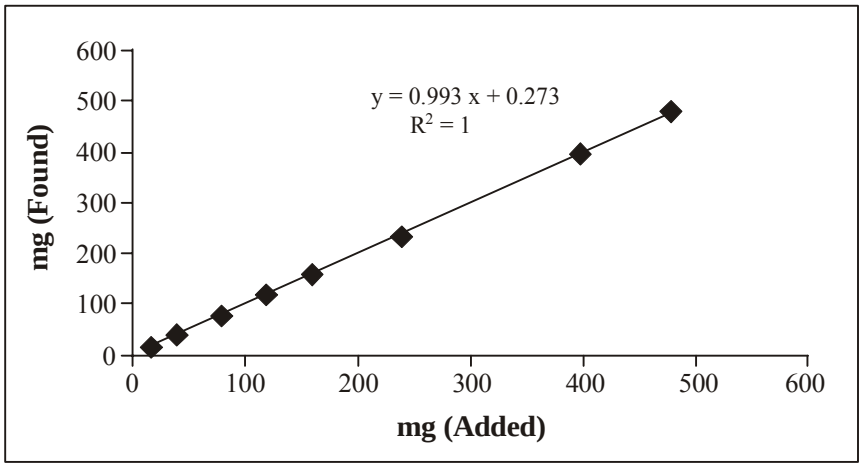


Fig 1: Linearity plot of candesartan

Precision

Precision was assessed at two levels i.e. repeatability and intermediate precision. The repeatability was determined as intra-day variation whereas intermediate precision was determined by measuring inter-day variation in the assay of the drug in six replicate runs (n = 6). The assay results for repeatability and intermediate precision are 101.8 and 101.5 percent, respectively.

Table 2: **Method precision (Repeatability)**

S. No	% Assay for 16 mg strength
1.	101.1
2.	101.8
3.	102.1
4.	101.6
5.	102.0
6.	102.3
Mean	**101.8**
% RSD	**0.4**

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions of method. To determine the robustness of the method, the experimental conditions were deliberately altered. The method conditions such as flow rate (± 10%), column oven temperature (± 5^0C), wave length of detection (± 5 nm), organic content in mobile phase (± 2%) and pH of buffer in mobile phase (± 0.2) were altered and the influence of these changes on the assay, peak tailing, theoretical plate number and peak area was studied.

Table 3: Robustness study

S. No	Assay (mg/tablet)					
	Set-I	**Set-II**	**Set-III**	**Set-IV**	**Set-V**	**Set-VI**
1	32.1	32.2	32.0	32.1	32.1	32.1
2	32.2	32.2	32.1	32.1	32.2	32.2
3	32.0	32.1	32.0	32.2	32.2	32.2
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
Overall mean	32.12	32.14	32.10	32.13	32.14	32.14
Overall SD	0.109	0.101	0.112	0.100	0.101	0.101
Overall RSD (%)	0.340	0.315	0.348	0.311	0.315	0.315

Set I & II = Variation in flow rate (± 10%)
Set III = Column oven temperature (35°C)
Set IV & V = Variation in wavelength (280 ± 5 nm)
Set VI = Variation in acetonitrile content in mobile phase (53 %)

Accuracy

A study of the accuracy of the method was conducted by injecting three samples spiked at 50, 100 and 150% levels of drug in the placebo in triplicate. The results of the individual recovery are in between 99.3 and 99.8. The % RSD for recovery at each level was not more than 0.2.

Table 4: Accuracy data

% Spike level	Added (mg)	Found (mg)	% Recovery	Mean % recovery	% RSD
50	80.10	79.67	99.5	99.4	0.1
50	80.40	79.85	99.3		
50	80.30	79.90	99.5		
100	160.20	159.18	99.4	99.6	0.2
100	160.20	159.60	99.6		
100	160.40	160.06	99.8		
150	240.10	238.80	99.5	99.4	0.1
150	240.20	238.44	99.3		
150	240.20	238.97	99.5		

Solution stability

The stability of the working standard solution of the drug (80 µg/mL) was tested at 12 and 26 hrs. The stability of solutions was determined by comparing percent area and peak purity of the drug. The difference in assay values was within 0.5 percent after 26 hrs. This indicates that the solution is stable for 26 hrs. at ambient temperature because of the absence of impurity peaks in the chromatogram.

Table 5: Bench top stability for standard and test solutions

Time	% Assay		
	Standard	Formulation	
		Trial– 1	Trial – 2
0 hrs.	98.8	101.1	101.8
At 26 hrs.	99.2	100.9	101.4
Difference	0.4	0.2	0.4

CONCLUSION

The proposed HPLC method is rapid, sufficiently sensitive and reproducible for the determination of candensartan cilexetil from its tablet dosage forms and thus, it can be used for the routine quality control analysis with short run time

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