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STABILITY INDICATING RP HPLC METHOD FOR THE DETERMINATION OF CANDISARTAN IN PURE AND PHARMACEUTICAL FORMULATION.

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Abstract

An accurate and precise HPLC method was developed for the determination of candesartan. Separation of the drug was achieved on a reverse phase C18 column using a mobile phase consisting of phosphate buffer and acetonitrile in the ratio of 30:70v/v. The flow rate was 1.2ml/min and the detection wavelength was 255 nm. The linearity was observed in the range of 20-60µg/ml with a correlation coefficient of 1.000. The method was successfully validated in accordance to ICH guidelines acceptance criteria for specificity, linearity, precision, recovery, ruggedness and robustness. The drug undergoes degradation under acidic, basic, Peroxide and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Key words: Candesartan, RP-HPLC, Degradation studies.

Introduction

Candesartan Cilexetil, (±)-1-[(Cyclohexyloxy) carbonyl]oxy ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate, is a novel, potent, highly selective non peptide angiotensin II type 1 (AT1) receptor blocker which is administered orally as Candesartan cilexetil, which is rapidly and completely hydrolyzed to Candesartan, the active moiety, during absorption from the gastrointestinal tract. The objective of this work was to develop an analytical HPLC procedure, which would serve as stability indicating assay method for candesartan. A thorough literature survey revealed that the reported analytical procedures describing a stability indicating HPLC method for candesartan were more economical. The Objective of this study was to develop the method with less economical, precise, simple and sensitive and determination of candesartan in the presence of its degradation products.

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Here direct use of the mobile phase as diluent for formulations in quantitative analysis minimizes errors that occur during tedious extraction procedures. From the best of our knowledge via literature search, this is the first known RP-HPLC method that can separate all the related compounds of candesartan from each other and from candesartan with less economical and is therefore suitable to conduct stability studies of candesartan.

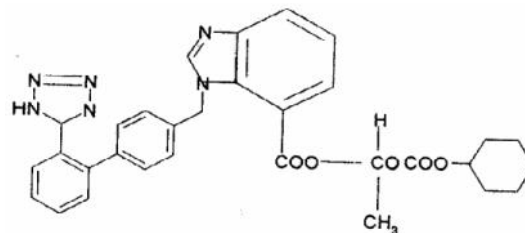


Fig 1: Structure of Candesartan Cilexetil

Material and Methods

Chromatographic conditions: The analysis of the drug was carried out on a Waters HPLC system equipped with a reverse phase Xterra C18 column (100 mmx4.6mm; 3.5µm), a 2695 binary pump, a 20 µl

injection loop and a 2487 dual absorbance detector and running on Waters Empower software.

Chemicals and Solvents: The reference sample of candesartan was supplied by Sun Pharmaceutical Industries Ltd., Baroda. HPLC grade water and acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai.

Preparation of phosphate buffer: Seven grams of KH_2PO_4 was weighed into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. 2 ml of Triethyl amine was added and pH adjusted to 3.0 with orthophosphoric acid.

Preparation of mobile phase and diluents 350 ml of the phosphate buffer was mixed with 650 ml of acetonitrile. The solution was degassed in an ultrasonic water bath for 5 minutes and filtered through 0.45 μ filter under vacuum.

Standard Solution Preparation:

Accurately weigh and transfer 10mg of Candesartan Cilexetil Working standard into 10 ml volumetric flasks add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Sample Solution Preparation:

Weigh 5 Candesartan Cilexetil Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Candesartan Cilexetil into a 10 ml volumetric flask. Add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Procedure:

A mixture of buffer and acetonitrile in the ratio of 30:70v/v was found to be the most suitable mobile phase for ideal separation of candesartan. The solvent mixture was filtered through a 0.45 μ membrane filter and sonicated before use. It was pumped through the column at a flow rate of 1.2ml/min. The column was maintained at ambient temperature. The pump pressure was set at 800 psi. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 255 nm. The run time was set at 6 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 3.089min. A typical chromatogram showing the separation of the drug is given in Figure 2.

Preparation of calibration graph:

Preparation of stock solution: Accurately weigh and transfer 10mg of Candesartan Cilexetil API sample into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20, 30, 40, 50, 60, μ g/ml):

0.2ml, 0.3, 0.4, 0.5, 0.6ml of stock solution has taken in 10 ml of volumetric flask diluted up to the mark with diluent.

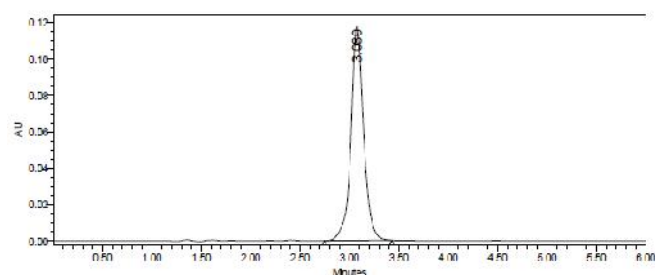


Fig 2: Typical chromatogram of candesartan

Procedure:

Injected each level into the chromatographic system and measured the peak area. Plotted a graph of peak area versus concentration and calculated found to be linear in the concentration range of 20-60 μ g/ml of the drug.

The relevant data are furnished in Table-1. The regression equation of this curve was computed. The regression equation was later used to estimate the amount of candesartan in tablet dosage forms.

Table 1: calibration data of the method

Concentration ($\mu\text{g/ml}$)	Mean peak area (n=5)
20	521185
30	771352
40	1034493
50	1277669
60	1541985

Validation of the proposed method:

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of candesartan. Solution containing $40\mu\text{g/ml}$ of candesartan was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2.

The accuracy of the HPLC method was assessed by analyzing solutions of candesartan at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Table 2: Precision of the proposed HPLC method

Concentration of Candesartan($40\mu\text{g/ml}$)	Peak area	
	Intra day	Inter day
Injection-1	1050994	1069436
Injection-2	1050910	1067920
Injection-3	1062555	1072559
Injection-4	1065327	1075473
Injection-5	1065412	1076588
Average	1059040	1072395
Standard Deviation	7471.9	3737.1
%RSD	0.71	0.35

Estimation of candesartan in tablet dosage forms:

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate candesartan in tablet formulations twenty tablets were weighed and powdered. An accurately

weighed portion of this powder equivalent to 25 mg of candesartan was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 30:70v/v mixture of phosphate buffer and acetonitrile. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluents was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluents and the solution was filtered through a 0.45μ membrane filter. This solution containing $15\mu\text{g/ml}$ of candesartan was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug.

Table 3: Accuracy studies

Concentration	Amount added(mg)	Amount found (mg)	% Recovery	% Mean recovery
50%	5.0	5.01	100.20	
100%	10.0	9.87	98.76	99.20
150%	14.8	14.60	98.66	

Forced degradation studies

Acid Degradation:

Accurately weigh and transfer 10mg of candesartan working standard into a 10mL volumetric flask add about 3 mL of 0.5N HCl and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. Neutralized the sample solution using 0.5N NaOH and diluted up to the mark with diluents. (Stock solution). Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through $0.45\mu\text{m}$ filter.

Base Degradation:

Accurately weigh and transfer 10mg of candesartan working standard into a 10mL volumetric flask add about 3mL of 0.5N NaOH and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. Neutralized the sample solution using 0.5N HCl and diluted up to the mark with diluents. (Stock solution). Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through $0.45\mu\text{m}$ filter.

Thermo Degradation:

Accurately weigh and transfer 10mg of candesartan working standard into a 10mL volumetric flask and oven under heat at 105 degrees for 12 hours. (Stock solution).

Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Peroxide Degradation:

Accurately weigh and transfer 10mg of candesartan e Working standard into a 10mL volumetric flask add about 1 mL of 30% Hydrozen Peroxide (H₂O₂) and sonicated for 5minutes and Refluxed under heat at 60 degrees in a heating mantle for 2 hours. Further pipette 0.3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Results and Discussion:

In the proposed method, the retention time of candesartan was found to be 2.269 min. Quantification was linear in the concentration range of 20-60 μ g/ml. The regression equation of the linearity plot of concentration of candesartan over its peak area was found to be $Y=10170+25479X$ ($r^2=1.000$), where X is the concentration of candesartan (μ g/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 2190, which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.05 μ g/ml and 0.09 μ g/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and acetonitrile in the ratio of 30:70v/v resulted in peak with good shape and resolution.

Stability indicating property:

The chromatogram of no stress treatment sample (as control) showed no additional peak (Fig: 3). The retention time (RT) of standard and sample were 3.626min .The chromatogram of acid degraded sample showed no additional peaks (fig: 4). The chromatogram of alkali degraded sample showed no additional peaks (fig: 5). The chromatogram of thermal degraded sample showed no additional peaks (fig: 6). The chromatogram of hydrogen peroxide degraded

sample showed additional peak at RT of 2.90 min (Fig: 7), and the values were shown in Table 4.

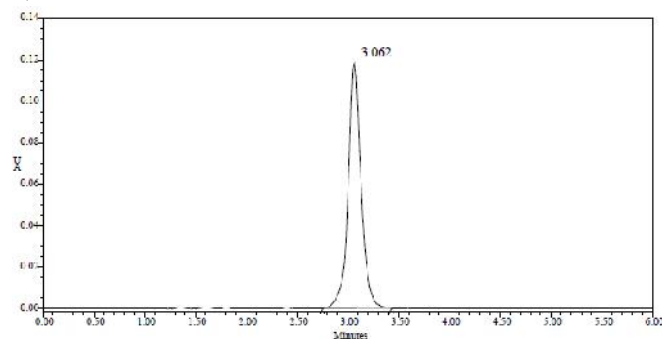


Fig 3:
The chromatogram of no stress treatment sample

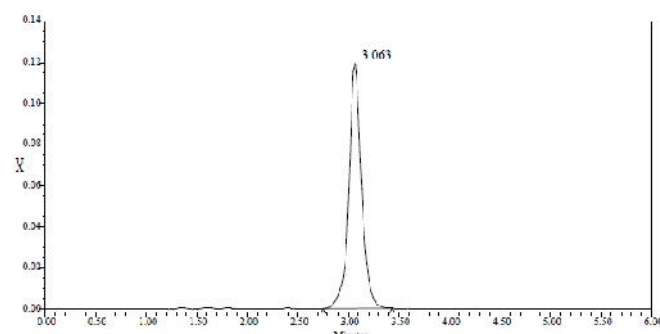


Fig. 4: The chromatogram of acid degraded sample

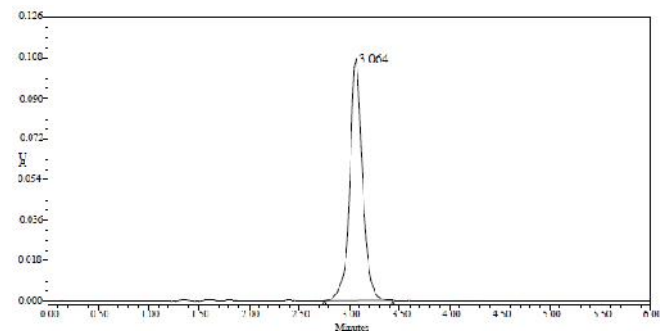


Fig. 5: The chromatogram of alkali degraded sample

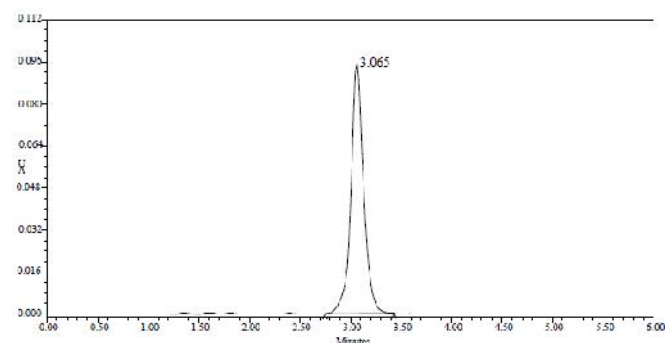


Fig. 6:
The chromatogram of thermal degraded sample

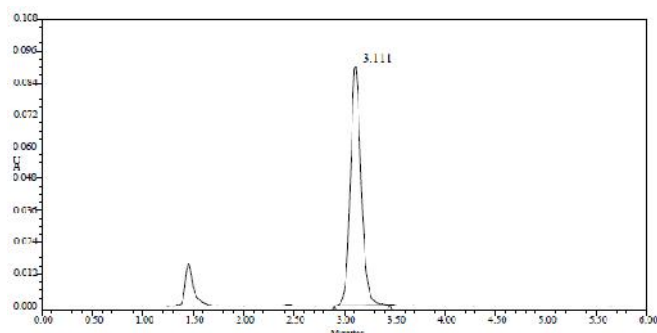


Fig. 7: The simple chromatogram of Hydrogen Peroxide degraded sample.

Conclusion

The developed HPLC technique is precise, specific, accurate and stability-indicating. Statistical analysis proves that the method is suitable for the analysis of candesartan as bulk drug and in Pharmaceutical formulation without any interference from the excipients. This study is a typical example of a stability-indicating assay, established following the

recommendations of ICH guidelines. The method can be used to determine the purity of drug available from various sources by detecting any related impurities. The method has been found to be better than previously reported methods, because of use of a less economical and readily available mobile phase, lack of extraction procedures, no internal standard, and use of the same mobile phase for washing of the column. All these factors make this method suitable for quantification of candesartan in bulk drugs and in pharmaceutical dosage forms. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range

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Table 4: Stressed study data of candesartan

S. No	Condition	Time(hrs)	% assay of candesartan	Retention time of candesartan	%degradation
1	No stress treatment	-	99.4	3,602	Nil
2	Acid	2	-	-	Nil
3	Alkali	2	-	-	Nil
4	H ₂ O ₂	2	100.4	3.11	1.00
5	Thermal	12	-	-	Nil

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