INTERNATIONAL JOURNAL
OF
PHARMACEUTICAL SCIENCES
AND
RESEARCH

STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CANDESARTAN IN PHARMACEUTICAL DOSAGES FORM

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ABSTRACT: Candesartan was degraded together under different stress test conditions prescribed by International Conference on Harmonization. The samples so generated were used to develop a stability-indicating high performance liquid chromatographic (HPLC) method for the candesartan. The drug was well separated from degradation products using a reversed-phase (C-18) column and a mobile phase comprising of Acetonitrile: Buffer (80:20 v/v) at pH- 3. Other HPLC parameters were: flow rate, 1 mL/min; detection wavelength, 225 nm; and injection volume 20 µl. The method was validated for linearity, precision, accuracy etc. Results obtained after validation study, indicating that the proposed single method allowed analysis of Candesartan, in the presence of their degradation products formed under a variety of stress conditions. The developed procedure was also applicable to the determination of stability of the drug in commercial pharmaceutical dosage form.

INTRODUCTION: Candesartan is an angiotensin II receptor antagonist [1] used mainly for the treatment of hypertension. Chemically Candesartan is, 2-Ethoxy-1-[[2I-(1H-tetrazol-5-yl)[1,1I-biphenyl)-4-yl]methyl]-1H-benzimidazole-7-carbox-ylate, 1-[[(cyclohexyloxy) carbonyl] oxy] ethylester. The structure of Candesartan is shown in **figure 1.**
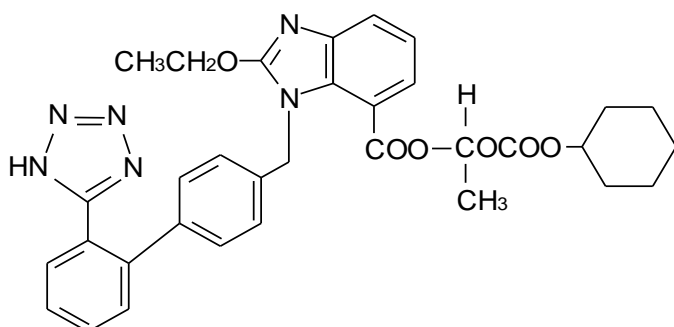

FIGURE 1: STRUCTURE OF CANDESARTAN

There are few analytical methods are available for its estimation in biological fluids includes HPLC with fluorometric detection and with UV detection [2, 3].

The aim of the present study was to develop an accurate, precise, specific, reproducible and stability indicating HPLC method for the estimation Candesartan. The Candesartan was purposely degraded by acid, base, oxide, dry heat, wet heat and UV light treatment to check the stability and to develop stability indicating assay method [4-6]. The developed analytical method was validated by means of linearity, accuracy, precision, LOD, LOQ, ruggedness and robustness as per International Conference on Harmonization (ICH) guidelines [7-8].

MATERIALS AND METHODS: Candesartan was supplied by Dr. Reddy's Laboratory, Hyderabad. All the chemicals used during the study were of AR / HPLC / GC grades and procured from Merck, Mumbai,

Qualigens, Mumbai, Rankem, New Delhi, etc. Millipore membrane filters (0.45µ) were used for filtration of mobile phase and working solutions. Double distilled water and the volumetric glasswares of class 'A' grade were used throughout the experimental work.

Shimadzu HPLC system consists of following components: Shimadzu HPLC pump LC-10ATvp, On Line Degasser DGU-14A, Low pressure Gradient flow control unit FCV-10ALvp, Rheodyne injector 7725 I with 20 µl loop, Column oven CTO-10ASvp, UV-VIS detector SPD-10AVp with SHIMADZU class CSW software, Column Thermo Hypersil ODS 5 µ C18 (150 x 4.6mm)

Forced Degradation of Candesartan

1. **Acid induced degradation**: The 50 mg of Candesartan was dissolved in 20 mL of methanol; 5 mL of 1N Hydrochloric acid (HCl) was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature acid was neutralized with 1 N NaOH then volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

2. **Base induced degradation**: The 50 mg of Candesartan was dissolved in 20 mL of methanol; 5 mL of 1N NaOH was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature base was neutralized with 1 N HCl then volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

3. **Hydrogen peroxide induced degradation**: The 50 mg of Candesartan was dissolved in 20mL of methanol; 5 mL of 30 % Hydrogen peroxide was added. The solution was kept for 6.0 h in dark at room temperature then the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL. 6.0 h in dark at room temperature then the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

4. **Wet heat degradation**: The 50 mg of Candesartan was dissolved in 20 mL of methanol; 5 mL of water was added. The solution was refluxed for 6.0 h at 96-98°C on boiling water bath. After cooling at room temperature the volume was completed up to 50 mL with methanol to obtain concentration 1.0 mg/mL.

5. **Dry heat degradation**: The Candesartan was kept in oven at 110 ºC for 12 h, 50mg of drug was weighed and dissolved in methanol to obtain concentration 1.0 mg/mL to study dry heat degradation.

6. **Photochemical degradation**: The Candesartan drug was kept in sun light for 30 h (cumulative), 50mg of drug was weighed and dissolved in methanol to obtain concentration 1.0 mg/mL to study photochemical degradation.

All six degraded samples solutions of concentration of 1mg/mL from the solution 5mL was pipette out and diluted up to 10 mL with methanol to obtain 500µg/mL, from the solution 5mL was pipette out and diluted up to 25 mL with mobile phase to obtain concentration 100µg/mL which was used for degradation study.

Development of HPLC method: HPLC studies were carried out on all the reaction solutions individually, and on a mixture of the solutions in which decomposition was observed. The separations were achieved by gradient elution using Acetonitrile: Buffer (80:20 v/v)[Buffer pH- 3 (Buffer Preparation - 4.0 mL Ortho-Phosphoric Acid was mixed in 1000 mL of water and pH -3 adjusted with Triethylamine)] as the mobile phase. It was filtered through 0.45 µ membrane filter and degassed before use. The injection volume was 20 µL.

The working concentration was 100 µg/mL and mobile phase flow rate was 1 mL/min with column oven temperature 30°C. The detection was carried out at 225 nm. Chromatogram of Candesartan is shown in **figure 2**.
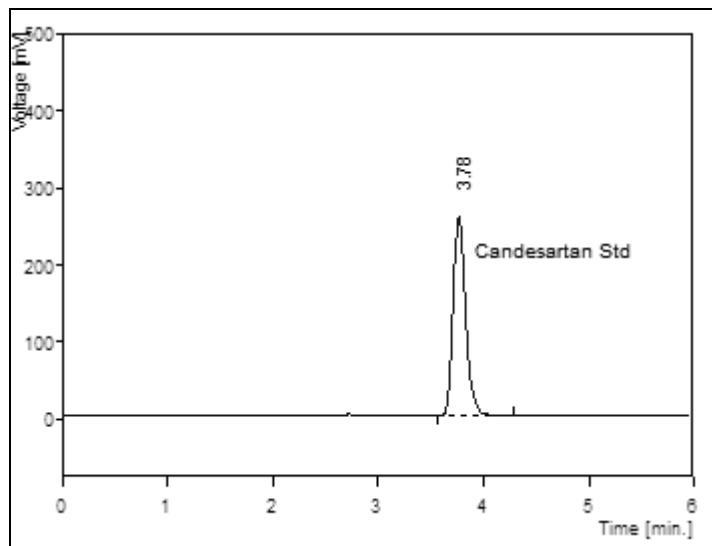
FIGURE 2: CHROMATOGRAM OF CANDESARTAN STANDARD

Validation Parameters

1. **Accuracy**: Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method. In the study standard was added to fixed quantity of tablet powder at different levels of labeled claim (i.e. 60 to 140 % of labeled claim).

2. **Precision**: Precision of any analytical method was ascertained by replicate estimation of drug in sample of homogeneous tablet powder by different analyst, intraday and interday. The results are expressed as SD and % RSD of series of measurements.

3. **Limit of Quantitation (LOQ)**: The quantitation limit was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

4. **Linearity and range**: The linearity of analytical method was determined by injecting 50 to 200 µg/mL of Candesartan and peaks were recorded and plotted graph, concentration versus area response of Candesartan. The range of analytical method was established with a suitable level of precision, accuracy and linearity.

Application of developed method on Marketed Formulation:

Standard Preparation: Accurately weighed quantity of Candesartan (25 mg) was dissolved in the methanol and volume was completed up to the 50 mL to obtain conc 500µg/mL, 5mL of the solution was diluted up to 25 mL with mobile phase to obtain conc 100µg/mL.

Sample Preparation: Twenty tablets were weighed and finely powdered. An accurately weighed quantity of tablet powder equivalent to 25 mg of Candesartan was taken into 50 mL volumetric flask and dissolved in the methanol. It was then sonicated for 10 min and volume was completed to the mark with the methanol. The solution was filtered through 0.45µ membrane filter. 5 mL of the filtered solution diluted to 25 mL with mobile phase to obtain conc 100 µg/mL of Candesartan. The percent label claim was calculated against the standard.

RESULT AND DISCUSSION: HPLC studies of samples obtained on stress testing of Candesartan under different conditions using Acetonitrile: Buffer (80:20 v/v) as the mobile solvent system suggested the following degradation behavior.

a. **Acid induced degradation product**: The chromatogram of the acid degraded sample for Candesartan showed additional peak at RT 1.77 min and RT 2.73 min (**figure 3**) indicating that Candesartan undergoes degradation under acidic condition.
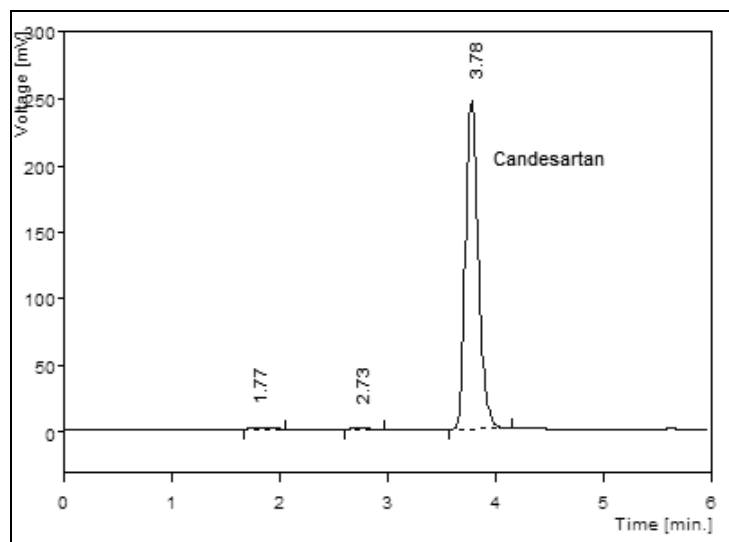

FIGURE 3: CHROMATOGRAM OF CANDESARTAN AND ITS ACID DEGRADATION PRODUCTS

b. **Base induced degradation product:** The chromatogram of the base degraded sample for Candesartan showed additional peak at RT 1.77 min, RT 2.73 min and RT 5.64 min (**figure 4**) indicating that Candesartan undergoes degradation under basic condition.
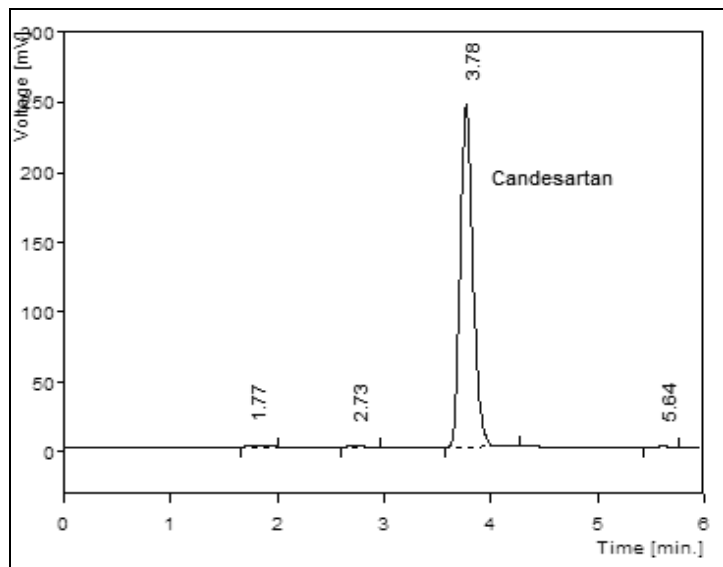

FIGURE 4: CHROMATOGRAM OF CANDESARTAN AND ITS BASE DEGRADATION PRODUCTS

c. **Hydrogen peroxide induced degradation product:** The chromatogram of the Hydrogen peroxide degraded sample for Candesartan showed additional peak at RT 1.77 min and RT 2.73 min (**figure 5**) indicating that Candesartan undergoes degradation under hydrogen peroxide condition.
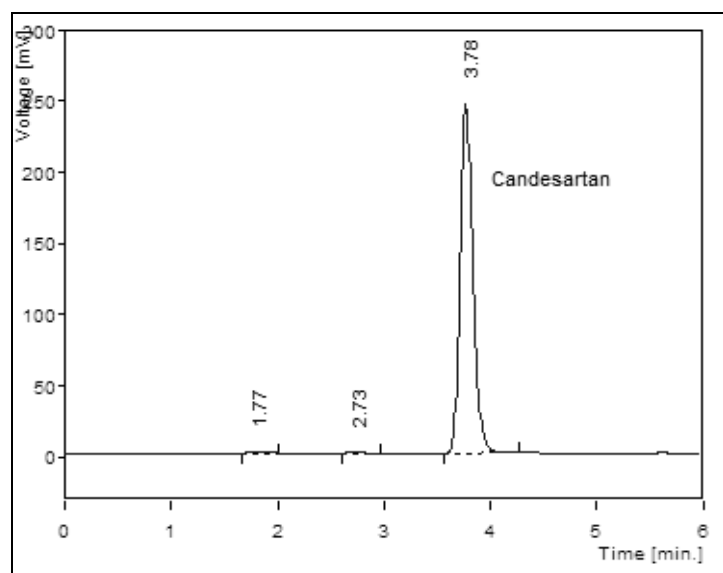

FIGURE 5: CHROMATOGRAM OF CANDESARTAN AND ITS OXIDE DEGRADATION PRODUCTS

d. **Wet heat degradation product:** The chromatogram of the wet heat degraded sample for Candesartan showed additional peak at RT 1.77 min and RT 2.73 min (**figure 6**) indicating that Candesartan undergoes degradation under wet heat condition.
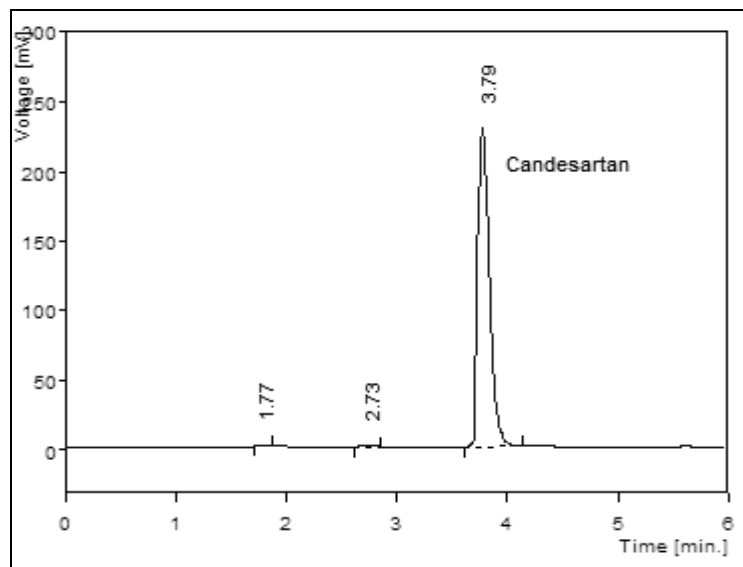

FIGURE 6: CHROMATOGRAM OF CANDESARTAN AND ITS WET HEAT DEGRADATION PRODUCTS

e. **Dry heat degradation product:** The chromatogram of the dry heat degraded sample for Candesartan showed additional peak at RT 1.77 min (**figure 7**) indicating that Candesartan undergoes degradation under dry heat condition.
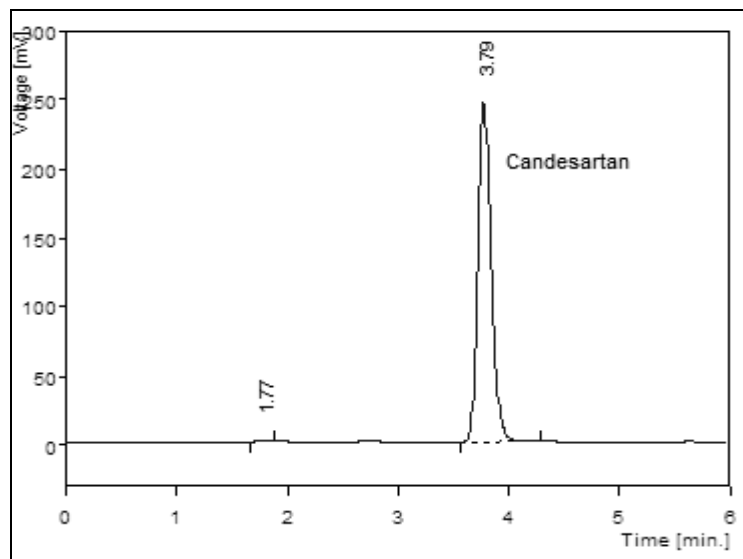

FIGURE 7: CHROMATOGRAM OF CANDESARTAN AND ITS DRY HEAT DEGRADATION PRODUCTS

f. **Photochemical degradation product**: The chromatogram of the photochemical degraded sample for Candesartan showed additional peak at RT 1.78 min and RT 2.73 min (**figure 8**) indicating that Candesartan undergoes degradation under photochemical exposure.
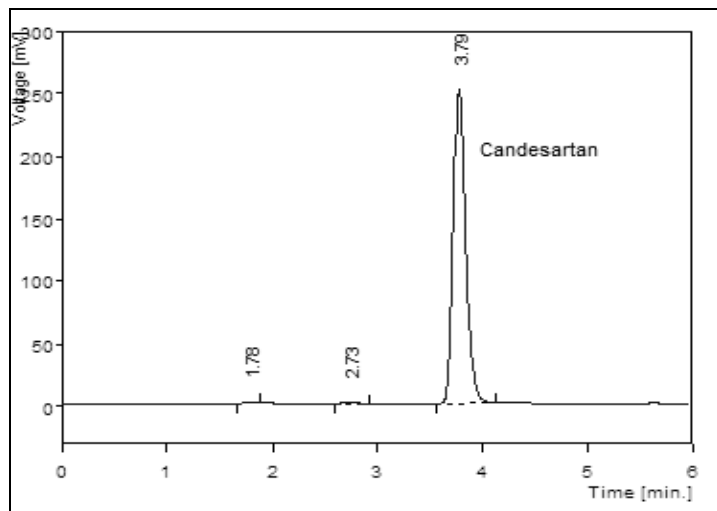

FIGURE 8: CHROMATOGRAM OF CANDESARTAN AND ITS PHOTO DEGRADATION PRODUCTS

The results of forced degradation study are shown in **Table 1**.

Method Validation:

1. **Accuracy**: The HPLC area responses for accuracy determination are depicted in Table 2. Good recoveries (100.41–101.46%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate.

2. **Precision:** Results of precision study are shown in **Table 3 and 4**. The values of SD or RSD under different conditions are within the prescribed limit of 2 %, showing high precision of the method.

TABLE 1: RESULTS OF DEGRADATION STUDY

Sr. No.	Treatment	Time (h)	% of Candesartan* Assay	% of Candesartan* ±SD	% of Degradation*
1.	Refluxed with 5 mL of 1N HCl on boiling water bath	6	94.75	0.5462	5.25
2.	Refluxed with 5 mL of 1N NaOH on boiling water bath	6	92.78	0.4562	7.22
3.	Stored with 5 mL of 30% H_2O_2 in dark at room temperature	6	91.45	0.8526	8.55
4.	Refluxed with 5 mL water on boiling water bath	6	97.15	0.4754	2.85
5.	Dry Heat 120°C	12	98.45	0.7565	1.55
6.	Photo degradation	30	97.15	0.2548	2.85

* Each value is mean of five observations

TABLE 2: RESULTS OF RECOVERY STUDY

Candelong Tablet (Avg Wt 280.52 mg for 16 mg of Candesartan)					
Sr. No.	Wt. of Tablet (mg)	Amount of Pure drug added (mg)	Peak area of standard	Peak area of sample	% Recovery*
1	219.46	2.5	2060.39	1245.52	100.89
2	218.56	7.5	2060.39	1659.54	100.41
3	218.45	12.5	2060.39	2085.56	100.89
4	219.48	17.5	2060.39	2498.85	101.21
5	220.45	22.5	2060.39	2909.45	101.46
				Mean	100.97
				±S.D.	0.3962
				R.S.D.	0.3924

* Each value is mean of five observations

TABLE 3: RESULTS OF PRECISION STUDY (DIFFERENT ANALYST)

Sr. No.	Different analyst	% Drug estimation* Candelong
1	I	99.25
2	II	101.36
3	III	100.96
	Mean	100.52
	±S.D.	1.1207
	R.S.D.	1.1149

* Each value is mean of five observations

TABLE 4: RESULTS OF PRECISION STUDIES (INTRADAY AND INTERDAY)

Sr. No.	Particular	% Drug Estimation* Candelong	
		Intraday	Interday
1	I	99.56	100.12
2	II	98.99	100.85
	Mean	99.28	100.49
	±S.D.	0.4031	0.5162
	R.S.D.	0.4060	0.5378

* Each value is mean of five observations

3. **Limit of quantitation**: Developed method stability indicating method was able to determine 15µg/mL minimum level concentration of candesartan with high accuracy and precision.

4. **Linearity and range**: The response for the drug was linear ($r2 = 0.9990$) in the concentration range between 60-140 µg/mL.

Application of developed method on marketed formulation: The developed HPLC method is suitable for estimation of Candesartan as it extracts the drug from formulation and determines the percent content with good %RSD. The results of the application of developed method are shown in **Table 5**.

TABLE 5: RESULTS OF ESTIMATION OF CANDESARTAN IN TABLETS

Candelong (Avg Wt 280.52 mg for 16 mg of Candesartan)				
Sr. No.	Weight of Sample (mg)	Peak area of standard	Peak area of sample	% Drug Estimation*
1	438.64	2060.39	2055.56	99.69
2	445.65	2060.39	2102.22	100.35
3	441.25	2060.39	2056.36	99.14
4	440.25	2060.39	2045.45	98.84
5	442.56	2060.39	2105.54	101.21
			Mean	99.85
			±S.D.	0.9559
			R.S.D.	0.9574

* Each value is mean of five observations

CONCLUSION: The developed HPLC technique is precise, specific, accurate and stability indicating. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is repeatable and selective for the analysis of Candesartan as bulk drug and in pharmaceutical formulations. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities.

It may be extended to study the degradation kinetics of Candesartan and for estimation of pure and its metabolites in plasma and other biological fluids.

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How to cite this article:
Bhagwate S, Gaikwad NJ and Tarte P: Stability indicating HPLC method for the determination of Candesartan in Pharmaceutical Dosages Form. *Int J Pharm Sci Res* 2013; 4(3); 1079-1085.