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RP- HPLC METHOD DEVELOPMENT AND VALIDATION OF CANDESARTAN CILEXETIL IN BULK AND THEIR PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

This paper describes the analytical method suitable for validation of Candesartan Cilexetil (CDN) by reversed Phase liquid chromatography (RP-LC) method. The method utilized RP-LC (Shimadzu LC-10AT with UV detector) model and Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) column was used for the separation. The mobile phase was consists of acetonitrile:0.05 M KH_2PO_4 buffer in the ratio of 65:35 at flow rate of 1.5 ml/ min. Validation experiments were performed to demonstrate System suitability, Specificity, Precision, Linearity, Accuracy study. The method was linear over the concentration range of 0.5-400 µg/ml. The method showed good recoveries (99.54-100.41%) and the recovery studies were carried out by adding different amounts (80%, 100% & 120%) of bulk samples of Candesartan cilexetil along with internal standard were 100.04%, 99.98% & 99.64% respectively. The proposed method is Precise, Accurate, Reproducible and rapid for the determination of Candesartan cilexetil in bulk and their pharmaceutical dosage forms.

INTRODUCTION: Candesartan Cilexetil, (±)-1-[[(Cyclohexyloxy) carbonyl] oxy] ethyl 2- ethoxy-1-[[2'- (1*H*- 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 (AT_1) 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.

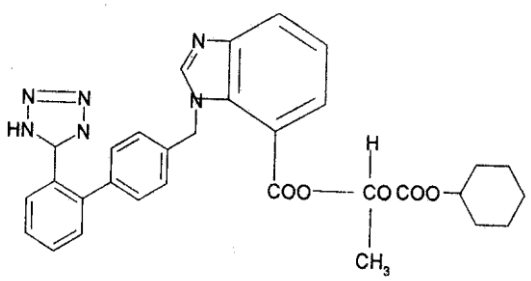

FIG. 1: STRUCTURE OF CANDESARTAN CILEXETIL

Candesartan has much greater affinity (> 10,000 folds) for the AT_1 receptor than for the AT_2 receptor blockade of the renin-angiotensin system with ACE inhibitors; it does not bind to or block other hormone receptors or ion channels known to be important in cardiovascular regulation. The absolute bioavailability of Candesartan is approximately 40% after an oral solution of Candesartan cilexetil. The relative bioavailability of the tablet formulation compared with the same oral solution is approximately 34% with very little variability. The mean peak serum concentration (C_{max}) is reached 3 to 4 hours following tablet intake (**table 1**).

TABLE 1: PHARMACOKINETIC PARAMETERS OF CANDESARTAN CILEXETIL

PARAMETER	VALUES
Elimination Half life	9.7 hrs
Oral Bioavailability	34-56%
Renal Clearance	0.37 ml. min^{-1}. Kg^{-1}
Volume of Distribution	0.13 l/kg
Peak Plasma Concentration	119±43 ng/ml
Peak Plasma Concentration Time	3-4 hrs

Hence a RP-LC method was developed and validated as per ICH guidelines. The literature reveals that various methods for the determination of Candesartan Cilexetil and pharmaceutical validations among these methods are LC-MS and LC-MS/MS, HPLC method for Candesartan. Spectrophotometery is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm). The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy measured by a galvanometer.

MATERIALS AND METHODS:

Apparatus: The analysis was performed by using the analytical balance G285 (Mettler Toledo), pH meter 2100 (Cyberscan), the HPLC used is of Shimadzu LC-10AT with UV detector. Column used in HPLC is of Hypersil ODS C-18 (250 x 4.6 mm, packed with 5 micron) with a flow rate of 1.5 ml/min (Gradient). The mobile phase consists of acetonitrile:0.05 M KH_2PO_4 buffer in the ratio of 65:35 are degassed in a sonicator for about 10 minutes the injection volume is 20mL and the ultra violet detection was at 256 nm. Acetonitrile HPLC grade was obtained from Ranbaxy Fine Chemicals Ltd.

Reagents and Solutions:

Preparation of mobile phase: 3.4022 grams of potassium dihydrogen phosphate was dissolved in 500 ml of triple distilled water to get 0.05 M solution and sonicate for 10min.The prepared buffer and Acetonitrile were properly mixed in the ratio of 35:65.

Preparation of standard drug and internal standard solutions: Stock solution of the drug (pure) was prepared by dissolving 25mg of CDN in 20 ml of Acetonitrile in 25 ml volumetric flask and the final volume was made upto 25 ml using Acetonitrile. Then the stock solution of internal standard (Bromhexine) was prepared by dissolving 25mg of Bromhexine in 25 ml volumetric flasks containing 20 ml of mobile phase and then the final volume was made upto 25 ml with the mobile phase.

Preparation of sample drug solution for pharmaceutical formulations: Twenty tablets containing CDN of each marketed formulation were taken and powdered. The powder equivalent to 8mg of CDN was dissolved in 8 ml of acetonitrile to get a stock solution of 1 mg/ml and then sonicated for 15 min. This solution was filtered through a Whattman filter paper. The solution was further diluted stepwise with mobile phase and spiked with required amount of internal standard and diluted with mobile phase to get concentrations with in the linearity range.

RESULTS & DISCUSSION:

Linearity: The linear fit of the system was illustrated graphically. Least square regression analysis was carried out for the slope, intercept and correlation coefficient. The linearity range was found to be in between 0.5-400µg/ml. The linearity range and linearity graphs were shown in **Table 2** and **Fig. 2**.

TABLE 2: LINEARITY

Concentration (µg/ml)	Ratio (drug/IS)
0.5	0.0067
1.0	0.0131
2.5	0.0335
5.0	0.0671
10	0.1341
20	0.5262
30	0.6705
40	1.3421
50	2.6850
100	4.0260
200	5.3640
300	0.5262
400	0.6705

Slope (a): 0.0134; Intercept (b): -0.0009; Correlation coefficient: 0.9999

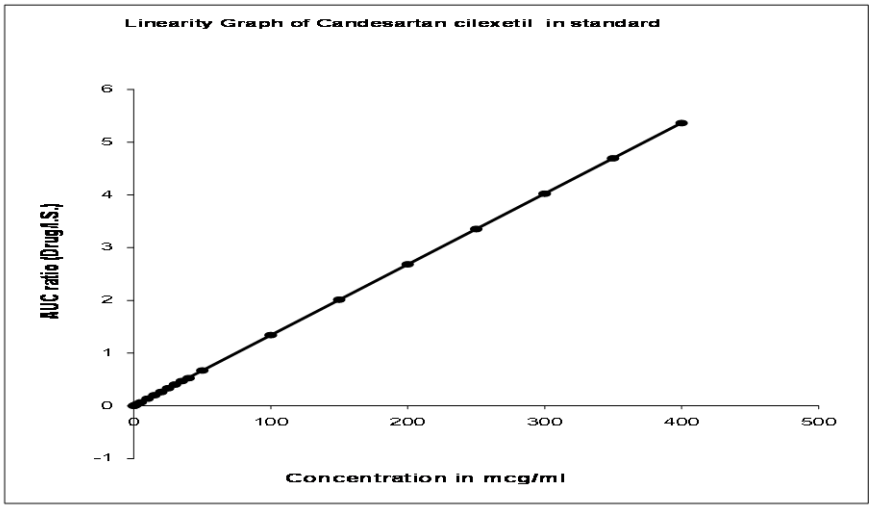

FIG. 2: LINEARITY GRAPH OF CANDESARTAN CILEXETIL

Precision: The precision of each method was ascertained separately from the peak area ratios obtained by actual determination of eight replicates of a fixed amount of drug and internal standard. The percent relative standard deviation and percentage range of errors (at 0.05 and 0.01 confidence limits) were calculated for CDN and presented in the **table 3**.

TABLE 3: PRECISION

Concentration (μg/ml)	Ratio (drug/IS)
0.5	0.526
1	0.524
2.5	0.522
5	0.524
10	0.526
15	0.523
20	0.526
25	0.523

Mean: 0.5242; SD: 0.0016; %RSD: 0.3058

The precision of the assay was also determined in terms of intra-and inter-day variation in the peak areas for a set of drug solutions on three different days. The intra-and inter-day variation in the peak area ratio of the drug solution to that of internal standard was calculated in terms of % RSD and the results are presented in table 3.

Accuracy: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of bulk samples of Candesartan Cilexetil along with internal standard (I.S) within the linearity range were taken and added to the pre-analyzed formulation of concentration 20 μg/ml. From that percentage recovery values were calculated. The results were shown in **table 4**.

TABLE 4: ACCURACY

Sample ID	Concentration (μg/ml)		%Recovery of pure drug	Statistical Analysis	
	Pure drug	Formulation			
S_1 : 80 %	16	20	100.18	**Mean**	100.04%
S_2 : 80 %	16	20	99.14	**SD**	0.2671
S_3 : 80 %	16	20	100	**%RSD**	0.2673
S_4 : 100%	20	20	99.98	**Mean**	99.98%
S_5 : 100%	20	20	100.01	**SD**	0.4283
S_6 : 100%	20	20	99.95	**%RSD**	0.4284
S_7 : 120%	24	20	100.41	**Mean**	99.64%
S_8 : 120 %	24	20	99.54	**SD**	0.607
S_9 : 120 %	24	20	98.97	**%RSD**	0.6092

System suitability parameters: System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed or The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Resolution (R), Tailing factor (T), LOD (μg/ml) and LOQ (μg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Candesartan Cilexetil in pharmaceutical formulations was validated or not. System suitability parameters were shown in **table 5**.

TABLE 5: SYSTEM SUITABILITY PARAMETERS

Parameters	Obtained Values
Theoretical plates (N)	2253
Resolution (R) between drug and I.S.	3.65
Tailing factor (T)	1.333
LOD (μg/ml)	0.358
LOQ (μg/ml)	1.196

Analysis of formulations: The amount of drug present in each pharmaceutical formulation was calculated through peak area ratio of drug to that of internal standard by using the standard calibration curve (concentration in µg/ml was taken on x-axis and peak area ratio on y-axis). The results were shown in **Table 6**. A typical chromatogram of Candesartan cilexetil with I.S and win formulation was shown in **Fig. 3** & **4** respectively.

TABLE 6: AMOUNT OF CANDESARTAN CILEXETIL PRESENT IN TABLETS

Formulations (mg)	Labeled amount	Amount obtained (mg) proposed Method*	%RSD
CANTAR	8	7.99	± 0.059
CANDEZ* 8	8	7.95	± 0.174

* Each value is average of five determinations ± standard deviation

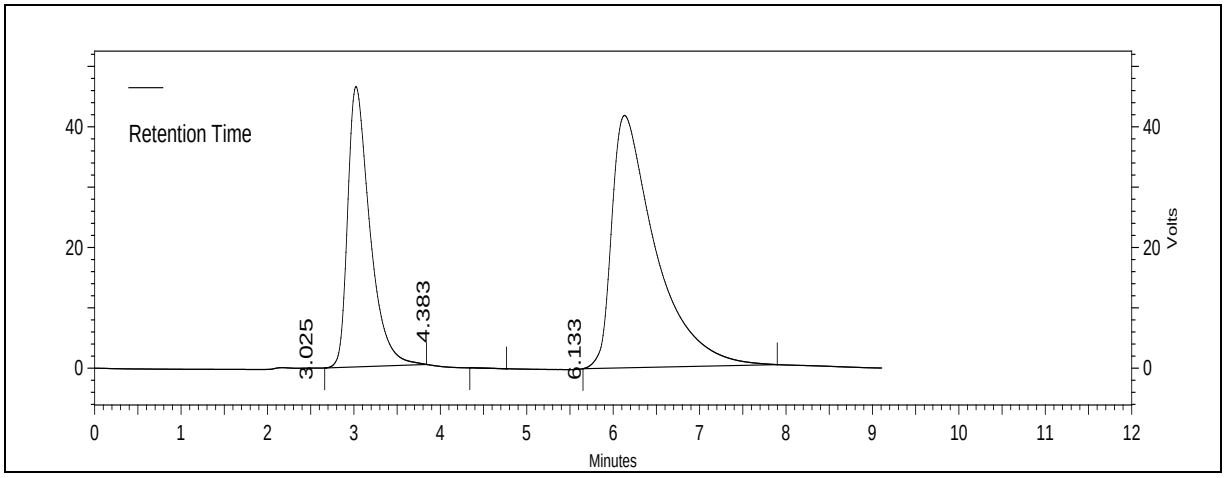

FIG. 3: TYPICAL CHROMATOGRAM OF CANDESARTAN CILEXETIL (STANDARD DRUG)

Name of the Peaks	Retention time
Candesartan cilexetil	3.025
Bromhexine	6.133

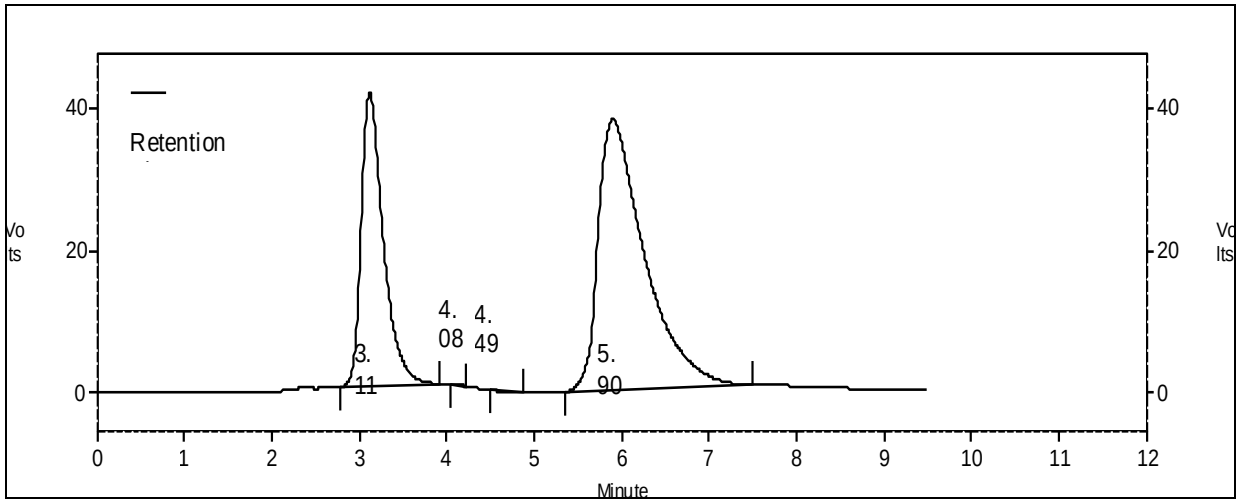

FIG. 4: TYPICAL CHROMATOGRAM OF CANDESARTAN CILEXETIL (FORMULATION)

Name of the Peaks	Retention time
Candesartan cilexetil	3.117
Bromhexine	5.900

CONCLUSION: The proposed method was found to be simple, precise, accurate and rapid for determination of Candesartan cilexetil from pure and its dosage forms. A simple reverse phase HPLC method was developed for the determination of Candesartan cilexetil (CDN) present in pharmaceutical dosage forms. An ODS C_{18} (250×4.6 mm, 5µ) column from Shimadzu in gradient mode, with mobile phase acetonitrile: KH_2PO_4 buffer [0.05M] (65:35) was used. The flow rate was 1.5 ml/ min and effluent was monitored at 256 nm. Bromhexine was used as the internal standard. The retention times were 3.0 min and 6.1 min for Candesartan cilexetil and Bromhexine respectively. The linearity range was found to be 0.5-400 µg/ml. The proposed method was also validated.

The proposed method can be used as alternative method to the reported one for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

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