



DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD FOR CANDESARTAN CILEXETIL IN PURE AND FORMULATION PRODUCTS

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ABSTRACT

A simple and sensitive determination of candesartan cilexetil in pure and formulation products was done by high performance liquid chromatographic method. The present method consists of protein precipitation, extraction of analytes from samples into acetonitrile and separation using reversed-phase C₁₈ column. Losartan potassium was used as an internal standard and the eluent was monitored by UV detector at 252 nm. The mobile phase used was 62:38 ratio of 20 mM phosphate buffer pH adjusted to 4.0 with OPA and acetonitrile at a flow rate of 1 mL min⁻¹. The retention times for drug and internal standard were found to be 3.8 and 7.0 minutes respectively. The intraday and inter-day coefficient of variation and percent error values of assay method were less than 5 % and mean recovery was more than 98% for each analyte in both pure and formulation products and the method was found to be precise, accurate and specific during study. The method was successfully developed and validated in pure and formulation products, also applied for pharmacokinetic studies.

KEY WORDS

candesartan cilexetil, high performance liquid chromatography, UV detector, assay, precision.

INTRODUCTION:

Candesartan cilexetil (CC), is an ester prodrug of candesartan, a non-peptide angiotensin II type 1 (AT₁) receptor antagonist used in the treatment of hypertension and heart failure. Candesartan cilexetil is rapidly and completely bioactivated by ester hydrolysis during absorption from gastro intestinal tract to candesartan (Sever, 1997). The major drawback in the therapeutic application and efficacy of candesartan cilexetil as an oral dosage form is its very low aqueous solubility and first-pass metabolism. Low solubility of CC across the physiological pH range is reported to result in incomplete absorption from the gastrointestinal (GI) tract. Based on its solubility across physiologically relevant pH conditions and absorption characteristics,

candesartan cilexetil is classified in the Biopharmaceutics Classification System (BCS) as a class II drug. Hence there is a need to develop a validated method for the analysis of S(-)-Candesartan cilexetil from API and marketed formulations.

Single analytical approaches are available for the related substances of candesartan cilexetil in tablet formulations and drug substances.[1] A method for the isolation of degradation products is also available.[2] A number of assay methods for determination of candesartan cilexetil in pharmaceutical formulations and human plasma are also available.[3-7] A number of assay methods for determination of candesartan cilexetil in combination pharmaceutical formulations are also available.[8,9] However; all the above-

mentioned methods are orientated to the determination of the active pharmaceutical compound. Nowadays, the pharmaceutical industry is forced to assess a strict control of impurities when manufacturing drug substances and drug products. Determination of impurities during the development of separation methods is one of the main and difficult tasks for pharmaceutical analysts, especially if increasingly impurities of closely related structure require determination. Methods are available for the estimation of candesartan cilexetil with spectrofluorimetry.[10]. To the best of our knowledge, this is the first report to develop and validate a simple and efficient HPLC method using Phenomenex Lux Cellulose-4 column as chiral stationary phase and UV detection for the analysis of Candesartan cilexetil from API and marketed formulations.

MATERIALS:

2.1 Materials

Candesartan cilexetil and Losartan potassium pure samples were donated respectively by Aurabindo pharmaceuticals, Hyderabad, India. Acetonitrile, methanol (HPLC) and potassium dihydrogen orthophosphate, ortho phosphoric acid (GR) and sodium hydroxide were purchased from Merck, Mumbai, India. Double distilled water was used during the entire HPLC procedure.

Chromatographic conditions

Analysis of samples was performed using HPLC. The HPLC system (Shimadzu, Kyoto, Japan) consisted of two LC-20AD Prominence liquid chromatography pumps, LC-20AD UV detector, CTO-20AC Prominence column oven with Lab solutions (LC solutions) software. The analytical column used was Onyx monolithic C₁₈ column (Phenomenex, 100mm² 4.6mm i.d, particle size 5μ) at 25°C temperature. The mobile phase used was 62:38 ratio of 20 mM phosphate buffer pH adjusted to 4.0 with OPA and acetonitrile at a flow rate of 1 mL min⁻¹ and detection was carried out at 254 nm. The retention times for drug and internal standard were found to be 3.8 and 7.0 minutes respectively. The injection volume was 20 μL and detector sensitivity was set to 0.0001 AUFS.

Standard solution preparation:

Stock solutions of Candesartan cilexetil at a concentration 0.1 mg/mL were prepared by dissolving accurately weighed respective standard drugs in HPLC grade methanol.

Sample preparation:

Twenty tablets of Candesartan cilexetil (ATACAND®) were weighed and then finely powdered. An accurately weighed portion of the powder, equivalent to about 8mg of Candesartan cilexetil, was transferred to a 100 ml volumetric flask and 40 ml of Methanol was added. Then the volumetric flask was shaken mechanically for 5 minutes, sonicated for 10 min, and diluted to volume with Methanol. 1 ml of this solution was transferred to a 100-ml volumetric flask and diluted to volume with Methanol. A portion of this solution was filtered through syringe-driven PVDF hydrophilic membrane filter of pore size 0.22 μm and which was used as sample.

Method validation:

According to the ICH guidelines, the developed method was validated to assure the reliability of the results of the analysis for different parameters like system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness [12].

System suitability

The system suitability was determined by calculating retention factor, separation factor and tailing factor of drug and IS peaks resulted for the HPLC method analysis of Candesartan cilexetil. The retention factor (*k*) was calculated as $k = (t_R - t_M)/t_M$ where, *t_R* refer to the retention time of the analyte and *t_M* refer to the elution time of the non-retained components. The separation factor (*α*) was calculated as the ratio of retention factors, $\alpha = k_2/k_1$. The resolution factor (*R_s*) was calculated as $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ where, *t₁*, *t₂* refer to the retention time of the first and second enantiomers; *w₁* and *w₂* are the peak widths for the first and second eluting enantiomers, respectively. Tailing factor (*T*) was calculated as $T = W_{0.05}/2f$ where, *W_{0.05}* is the width of peak at 5% height from base line and *f* is the distance from peak maximum to the leading edge of the peak.

Linearity

Linearity was assessed by plotting calibration curve of C. For this, five different concentration solutions of CC like 0.1, 0.25, 0.5, 1, 2.5 and 5μg/mL were prepared from the stock solution using Methanol as diluent. The calibration curve was obtained by plotting peak area versus concentration, using the least squares method. Linearity was checked for three consecutive days in the same concentration range from the same stock

solution. The percentage relative standard deviation (RSD) of the regression coefficient was calculated.

LOD

LOD was calculated using the following formula;

$$LOD = 3.3\sigma/S$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

LOQ

LOQ was calculated using the following formula;

$$LOQ = 10\sigma/S$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Accuracy

Accuracy was determined by the standard addition method. Previously analyzed marketed samples of CC (1 μ g/mL) were added up with 50, 100, and 150% of standard CC separately. Each sample was prepared in triplicate (n=3) and all the samples were analyzed by the proposed method. Recovery (%), RSD (%), and standard error (SE) were calculated.

Precision

Precise method should have the reproducibility and was determined by measuring repeatability (intra-day) and intermediate precision (inter-day precision) of peak areas of the drug. In order, to determine the repeatability of the method, three different concentration solutions of CC like 15, 2450 and 4500 ng/mL were prepared in triplicate (n=3) and were analyzed by the proposed method. The intermediate precision was determined by analyzing six samples (n=6) of 50 ng/ml CC solution on three consecutive days.

Robustness

To determine robustness of the method, experimental conditions were purposely altered, and after analysis changes in retention time of the CC was observed. The effect of change in flow rate on retention time of CC was studied by changing the mobile phase flow rate to 0.9 mL/min as well as 1.1 mL/min instead of 1mL/min, while the other chromatographic conditions were held constant. The effect of temperature on retention time of the enantiomer was studied at 38°C and 42°C instead of 40°C while the other mobile phase components were held constant, as stated in chromatographic condition section.

Results and Discussion:

Various solvent mixtures were tried for the analysis of Candesartan cilexetil with good resolution. However, 62:38 ratio of 20 mM phosphate buffer pH adjusted to 4.0 with OPA and acetonitrile was given well-resolved sharp peaks with very good symmetry. The flow rate was set depending on the resolution factor, peak shape, tailing factor and time required for the analysis. Candesartan cilexetil (API) and Candesartan cilexetil tablet formulation (ATACAND[®]) were analyzed by this method and their chromatograms were shown in Figure 1, 2 and 3, respectively. The retention time of standard CC and IS were found as 3.8 and 7.0 minutes respectively. As the retention time of standard enantiomers were comparable to that of the Candesartan cilexetil API and ATACAND[®], the present method can be used for the analysis of Candesartan cilexetil from various marketed formulations.

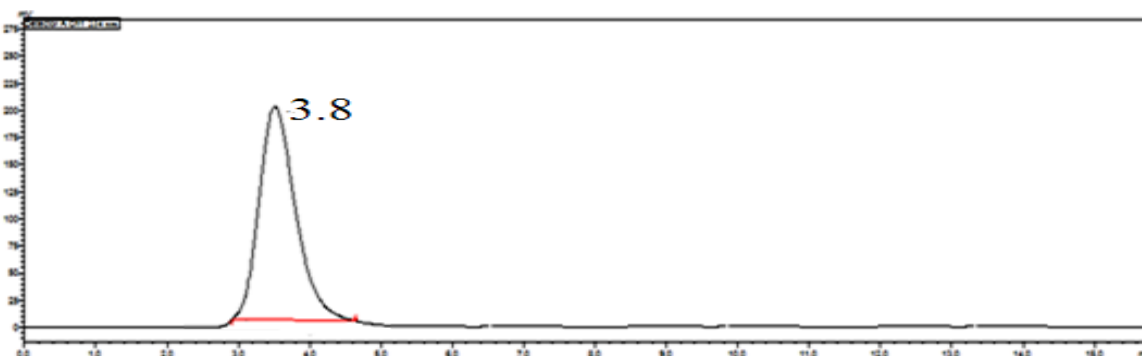


Figure 1. Chromatogram of Candesartan cilexetil (1000 ng/mL)

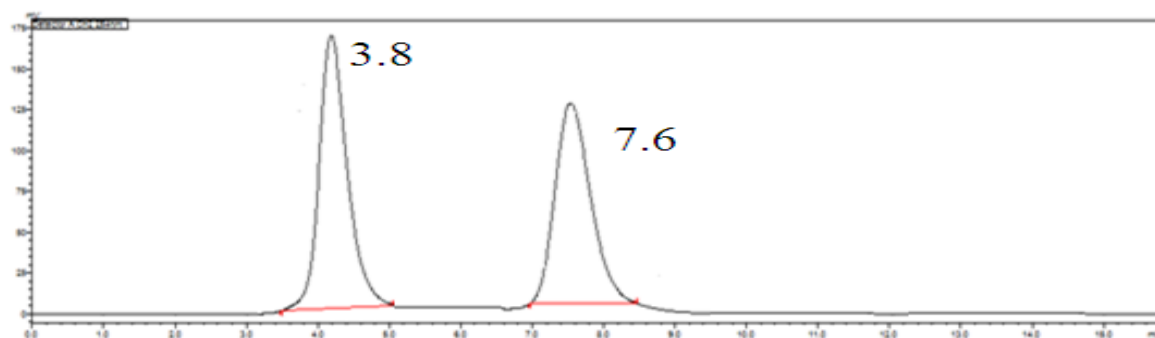


Figure 2. Chromatogram of drug and IS (1000 ng/mL)

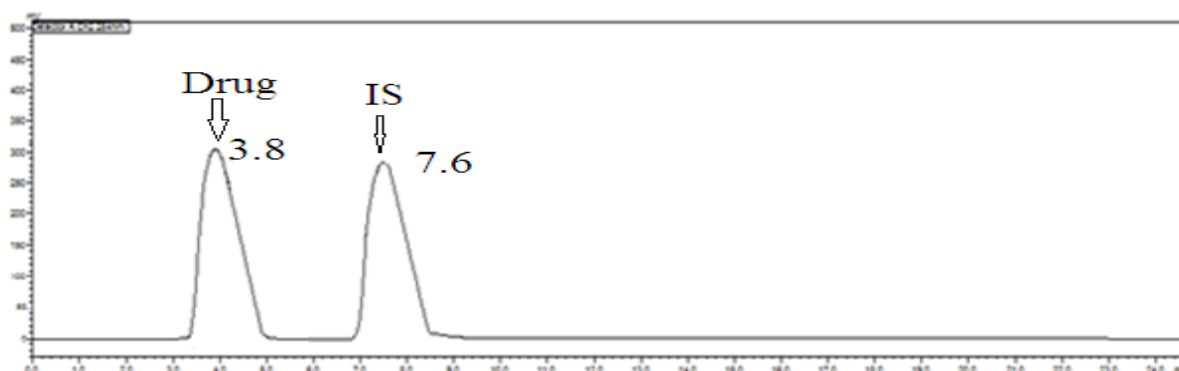


Figure 3. Chromatogram of ATACAND and IS (1000 ng/mL)

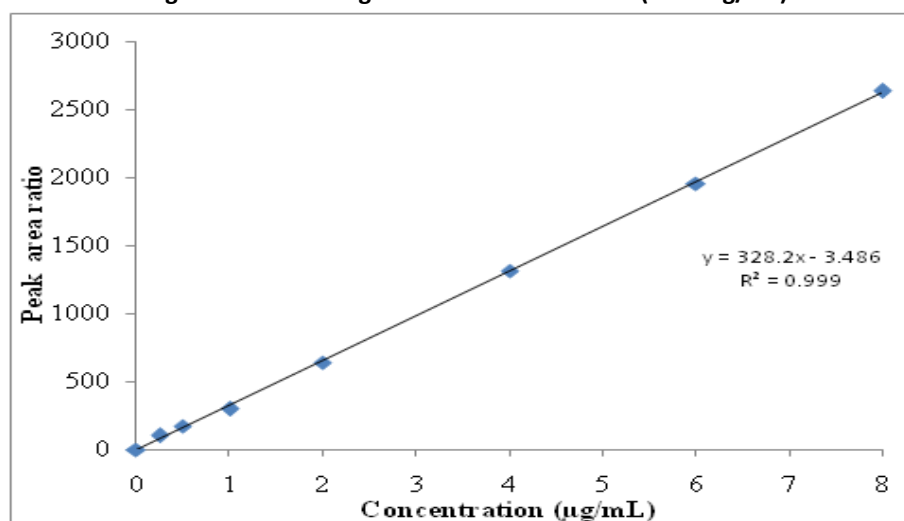


Figure 4: Standard graph of Candesartan cilexetil

Validation of the method

System suitability parameters were evaluated. The separation factor and resolution factor of enantiomers of the Candesartan cilexetil and IS were found as 1.55 and 1.82, respectively. These values were greater than the minimum values mentioned in ICH guidelines and hence the method proved to provide good resolution of enantiomers of c Candesartan cilexetil. As per ICH guidelines, the tailing factor should be less than 2 and

the present method has shown tailing factors of drug and IS as 0.33 and 0.42, respectively. Hence the method offers well resolved sharp peaks for the Candesartan cilexetil with good symmetry. The retention factor is often used to describe the migration rate of an analyte on a column and as per ICH guidelines it should be between 1 and 10. The method has given retention factors of drug and IS as 1.9 and 2.22, respectively and was found to be in the limit of ICH guidelines. Hence the

both drugs will take less time for elution and the analysis will complete in short duration of time. All the parameters were proved that the chromatographic system used was suitable for the analysis of Candesartan cilexetil from API and formulations.

Linearity

The calibration curve of CC was plotted using peak area ratio vs. concentration and was shown in Figure 4. The experiment was repeated for three times (n=3) and the relative standard deviation (RSD) was calculated for regression coefficient. The method has shown good linearity for CC ($r^2=0.9997$) in the concentration range of 0.25–8 µg/mL. As the %RSD of regression coefficient was found to be very less (<2%), which is within the limits of ICH guidelines and the methods linearity was proved to be reproducible.

LOD and LOQ

The LOD and LOQ values of CC found by this method. The low values of LOD and LOQ indicates that the method can be used for detection and quantification of CC over a very wide range of concentrations.

Accuracy

Accuracy of the method was ascertained by recovery method. For this purpose previously analyzed ATACAND test solutions of concentration 1µg/mL were added up with 50, 100, 150% of standard drug, separately. The samples were prepared in triplicate (n=3) and analysis was carried out by the proposed method. The % recoveries along with relative standard deviation (RSD) and standard error (SE) values were shown in Table 1. The overall average % recovery of CC was found as 99.05 % with very less standard deviation and negligible standard error, which proves the developed method as accurate.

Precision

To determine intra-day precision, three different concentrations of CC (15, 2450 and 4500 ng/mL) were prepared in triplicate (n=3) and analyzed at specific time intervals in the same day. The Coefficient of Variance (%CV) was calculated for all the samples and the values along with RSD and SE were given in Table 2. All the three different concentration samples of CC were shown insignificant RSD and SE in their Coefficient of Variance, which indicates that the method has good repeatability.

For inter-day precision analysis, six samples of CC, 15 ng/mL concentration solution (n=6), were prepared and analyzed for three consecutive days. Average % Coefficient of Variance of CC on each day was calculated and given along with their RSD and SE in Table 1. The RSD and SE of % CV of CC for all the three consecutive days was found to be less than 2%, which is the limit as per ICH guidelines and hence the method proved to be precise.

Robustness

Chromatographic conditions of the method were altered briefly and analysis of CC was performed for three times (n=3). Changes in retention time were recorded and the %RSD of retention time of CC respective to change in each parameter was shown in Table 3. As the %RSD of retention time of CC for each change in parameters was less than 2 %, which is the limit as per ICH guidelines and hence the method proved to be robust. Hence slight changes in temperature and mobile phase flow rate will not affect the method in analysis of Candesartan cilexetil from API and formulations.

Table 1: Intra-day and inter-day precision and accuracy data for assay of CC (n=6)

Added conc (ng mL ⁻¹)	Calculated conc (ng mL ⁻¹)		%CV		% Relative error	
	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
15	14.88	14.90	14.83	14.88	0.19	-1.5
2450	2448.8	2443.2	5.2	4.3	-2.3	-1.0
4500	4502.6	4497.7	4.4	2.9	0.9	-1.3

Table 2: Recovery and accuracy of the proposed method

Conc (ng mL ⁻¹)	Absolute recovery				Accuracy (%)		
	Conc (ng mL ⁻¹) (Mean ± SD)	Mean (%) ± S.D (n=5)	Range (Min–Max)	% CV	Mean ± SD (n=5)	Range (Min–Max)	% CV
15	14.99 ± 0.05	98.5 ± 1.7	95.1–99.2	2.2	96.9 ± 1.9	93.6 – 99.7	3.6
2450	2448.4 ± 36.8	99.1 ± 2.6	93.6–99.6	2.6	95.1 ± 3.0	93.9 – 97.9	2.03
4500	4495.7 ± 121.4	98.9 ± 2.3	93.2–99.8	2.82	94.6 ± 1.9	91.8 – 99.7	2.4

Table 3: Stability study results of the proposed method

Stability	Spiked conc (ng mL ⁻¹)	Calculated comparison sample concentration (ng mL ⁻¹) ^d		Calculated stability sample concentration (ng mL ⁻¹) ^d		Avg %
		Mean ± SD	% CV	Mean ± SD	% CV	
Bench top ^a	15	14.89 ± 0.09	3.8	14.86 ± 0.15	8.5	98
	2450	2448.5 ± 22.8	2.5	2447.3 ± 32.5	3.2	98.5
	4500	4492.6 ± 72.6	2.1	4495.8 ± 92.	1.3	100
Freeze and thaw ^b	15	14.94 ± 0.1	5.8	14.82 ± 0.2	8.4	96.4
	2450	2448.6 ± 75.2	2.7	2449.8 ± 46.2	3.1	98.9
	4500	4503.7 ± 104.4	1.9	4498.2 ± 121.3	1.5	100
Long term ^c	15	14.88 ± 0.1	4.2	14.79 ± 0.14	5.2	97
	2450	2448.5 ± 72.6	2.4	2445.3 ± 55.6	3.0	99
	4500	4451.6 ± 113.4	2.6	4448.2 ± 88.2	1.7	99.5

CONCLUSION:

The present work was intended for the analysis of Candesartan cilexetil from API and formulations, both qualitatively and quantitatively. For this purpose, a simple and rapid HPLC method was developed and validated. Statistical analysis results of the method validation revealed that the method has high accuracy with a good precision. Hence the method is reliable and convenient for routine quality control and stability assays of CC in both API and marketed tablet formulations.

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