

<https://doi.org/10.56770/jcp2025911>

HPLC METHOD DEVELOPMENT AND VALIDATION OF CANDESARTAN CILEXETIL FOR PHARMACOKINETICS PARAMETER ASSESSMENT

Israr Fatima 

Northwest Agriculture & Forestry University Yangling, China.

Submitted: October 13, 2024; Revised: April 25, 2025; Accepted: April 27, 2025; Published: June 30, 2025

ABSTRACT

In this study, healthy volunteers in Pakistan were asked to compare the single-dose pharmacokinetics of 16 mg of the angiotensin receptor blocker candesartan cilexetil following oral administration. Blood samples were taken over a 72-hour period while the medication was administered orally to 16 healthy individuals. Following a freeze-thaw cycle, the samples were subjected to reversed-phase HPLC analysis at a wavelength of 258 nm using an isocratic mobile phase consisting of potassium dihydrogen phosphate and methanol (25:75 v/v). Pharmacokinetic parameters such as C_{max} , T_{max} , $t_{1/2}$, K_a , V_d , and AUC were computed.

Keywords: Candesartan, RP-HPLC, Single-Dose Pharmacokinetics, Bioequivalence

*Corresponding Author. E-mail: israrfatima@nwafu.edu.cn

INTRODUCTION

Angiotensin Receptor Blockers (ARBs) and Angiotensin Converting Enzyme Inhibitors (ACEIs) are two classes of medications effective in treating cardiovascular diseases (CVDs) [1]. Candesartan is one of the ARBs approved in the United States for the treatment of heart failure [2]. It has a longer-lasting ability to bind to AT1 receptors compared to losartan [3]. Candesartan is marketed as an antihypertensive prodrug, known as Candesartan Cilexetil, which contains a chiral carbon atom in the cyclohexyloxycarbonyloxy ethyl ester group [4]. After oral administration, it is metabolized into its active form, candesartan, by intestinal wall esterases [5]. Candesartan has a partition coefficient greater than 1000 across a pH range of 1.1 to 8.9 [6]. Its pK_a value is 6.0, and it has a molecular mass of 61 kDa [7].

Candesartan is considered safe, even when a double dose is taken. Its antihypertensive effects and tolerability are comparable to those of amlodipine [8] and hydrochlorothiazide [9]. Major adverse events associated with candesartan include hypotension, renal dysfunction, and hyperkalemia. While no dose adjustment is necessary for patients with liver impairment, a lower starting dose is recommended for those with severe renal impairment [10].

Candesartan is characterized by low oral bioavailability (40%), high plasma protein binding (99%), and a volume of distribution of 0.13 L/kg. The primary site of clearance is the kidneys, with

an oral clearance rate ranging from 3.4 to 28.4 L/h. Currently, there are no reported drug-drug or drug-food interactions. Non-compartmental models are not suitable for explaining pharmacokinetic data, while two-compartment models account for the significantly longer half-life of 29 hours. Pharmacokinetic data remain unaffected by age, gender, or ethnicity. However, renal impairment does impact the pharmacokinetic profile, although elimination by hemodialysis is not observed; thus, a dose of 8 mg is deemed safe. Mild to moderate hepatic impairment does not alter pharmacokinetics, allowing for doses up to 12 mg/day without special precautions. Doses ranging from 8 to 32 mg/day are considered clinically effective [11].

The impact of gender on pharmacokinetics and pharmacodynamics has been well-documented in animal studies, yet there has been limited focus on its influence in humans. Several factors can affect the determination of pharmacokinetic parameters between genders, including differences in muscle and fat composition, physiological and hormonal variations, gastrointestinal blood flow, gastric acid secretion, gender-specific cytochrome P450 isozymes, plasma protein levels, and renal blood flow [12]. Studies have shown significant variations in the pharmacokinetics of antidepressants [13]. Additionally, opiates demonstrate a greater transfer from blood to brain in some volunteers [14].



Many studies have shown that the pharmacokinetics of candesartan are consistent between men and women. However, the pharmacokinetic parameters of a drug can vary significantly under different geographical conditions. In countries where the pharmaceutical landscape relies partially or completely on drug imports, it is crucial to re-evaluate drug prescriptions for the local population in their specific environment. This is due to "geonetical variations," which can lead to different responses to a particular drug among individuals in varied conditions [15].

For the first time, our study sought to ascertain the pharmacokinetic characteristics of candesartan tablets in healthy, fasting adult participants from Pakistan. Plasma samples were taken at different times from 0 to 72 hours after a single dosage of 16 mg candesartan cilexetil was given. A two-compartment model was used to derive the pharmacokinetic parameters, which included AUC, Vd, Cl, Cmax, tmax, t1/2, and Ka.

MATERIALS AND METHOD

Subject and Study design

This study was a single-dose pharmacokinetic evaluation of candesartan cilexetil aimed at assessing potential variations in pharmacokinetic parameters among healthy volunteers. The participants, aged between 19 and 22 years and weighing between 52 and 70 kg, provided written informed consent. Volunteers were included in the study only after undergoing complete physical examinations. Exclusion criteria for all participants included any type of disease, smoking, alcoholism, and abnormal liver and renal function test (LFT and RFT) results. Additionally, female participants were excluded if they were pregnant, menstruating, or met any of the other exclusion criteria. All volunteers received a single oral dose of 16 mg of candesartan cilexetil, marketed under the trade name Treatan® by PharmEvo Pvt. Ltd.

Blood Sampling

Blood samples of 5 ml were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, 24, 48, and 72 hours using an indwelling cannula. The samples were collected in heparinized vacutainers, then centrifuged to separate the plasma, which was preserved at -80 °C for further analysis.

Preparation of Drug Solution for Standard Curve

A stock solution of Candesartan cilexetil (1000 ng/ml) was prepared in methanol. Further dilutions (20, 40, 80, 120, 160, and 200 ng/ml) were made in blank plasma from this stock solution to construct a standard curve.

Validation of Analytical Methods

The analytical HPLC method was validated for specificity, linearity, limit of quantification, precision, and accuracy [16, 17].

Determination of Drug Concentration in Blood Samples by HPLC

Reverse phase HPLC was used to determine the levels of candesartan cilexetil in each blood sample. A Shimadzu 20-A (Japan) HPLC system with an auto-sampler and a photodiode array detector linked to a data gathering system was used. The RP-C18 (150 x 4.6 mm, 5 µm particle size) analytical column was utilized, and it was kept at 30 °C. The Quaternary Pump Model No. 20AT from Shimadzu, Japan, was the pump that was used. Candesartan cilexetil's detection wavelength was established at 258 nm. A potassium dihydrogen phosphate buffer (0.01 M) that had been pH 3.0 adjusted using orthophosphoric acid and methanol in a 25:75 (v/v) ratio made up the isocratic mobile phase. Each sample had an injection volume of 55 µL, and the flow rate was kept constant at 1 ml/min. An Eppendorf tube containing 500 µL of plasma and 500 µL of acetonitrile was vortexed for five minutes prior to being centrifuged for ten minutes at 10,000 rpm as part of the extraction procedure. A syringe was used to collect the supernatant, which was then filtered through a 0.2 µm filter and placed in an HPLC vial before being injected into the system.

Pharmacokinetic Parameters Calculation

Pharmacokinetic parameters, including AUC_{0-∞}, t1/2, ka, tmax, Cmax, Vd, Cl, and kel, were calculated from the plasma concentration-time curves, taking into account the bioavailability of Candesartan cilexetil following oral administration [18].

RESULTS AND DISCUSSION

Validation of Analytical Methods

Specificity

The chromatographic methods and conditions employed demonstrate no interference in control samples at the retention time of candesartan cilexetil.

Linearity

The standard calibration curve across each concentration showed strong linearity within the range of 20-200 ng/ml for candesartan cilexetil in human plasma. A linear relationship was observed between peak area and drug concentration, with an R² value of 0.998.

Limit of Quantification

The limit of quantification was determined based on a signal greater than five times the baseline noise, with sufficient precision (<20%) and accuracy (80-120%). Consequently, the limit of quantification for candesartan cilexetil was established at 18 ng/ml.

Precision and Accuracy

Intra-day and inter-day precision and accuracy were assessed using quality control samples in plasma, referencing a calibration curve on the same day and on different days. The mean intra-day

Table 1. Pharmacokinetic parameters of candesartan cilexetil in healthy volunteers.

Pharmacokinetic Parameters	Healthy Volunteers
	Mean $\pm$ SD
AUC (h.ng/L)	1450.82 $\pm$ 123.7
Elimination half life (hrs)	30.72 $\pm$ 8.25
Absorption rate constant (hr ⁻¹)	0.331 $\pm$ 0.059
Time to peak (hrs)	3.82 $\pm$ 0.7
Peak Conc. (ng/ml)	101.13 $\pm$ 6.64
Volume of distribution (L)	0.4865 $\pm$ 0.14
Clearance (L/h)	0.0146 $\pm$ 0.0129

accuracy as a percentage of the relative standard deviation (% RSD) at low, medium, and high concentrations was 94.4%, 99.1%, and 100.2%, respectively. The intra-day precision values were 0.03%, 0.02%, and 0.01%, while the inter-day precision values were 0.05%, 0.03%, and 0.01%.

Pharmacokinetic Parameters of Candesartan Cilexetil

Mean values and standard deviations for volunteers are presented in Table 1. The variance in pharmacokinetic parameters for groups was calculated using an unpaired t-test. The results indicated that there were no significant differences in all pharmacokinetic parameters (C_{max}, t_{max}, t_{1/2}, V_d, Cl, and K_a) except for AUC. Candesartan plasma concentrations were measured using a validated reversed-phase HPLC method, and pharmacokinetic parameters were calculated. The area under the curve (AUC) for Candesartan after oral administration in healthy volunteers was 1450.82 $\pm$ 123.7 ng·h/ml, which is significantly higher than previously reported values of 909 ng·h/ml [19] (P<0.005). This difference may be attributed to gender effects on drug absorption after oral administration, as well as varying environmental conditions.

The volume of distribution (V_d) calculated in healthy volunteers was 0.4865 $\pm$ 0.14 L/kg, compared to previously published data of 0.13 L/kg. This discrepancy may also be linked to

different environmental factors. The maximum concentration (C_{max}) of Candesartan was reached at 3.82 $\pm$ 0.7 hours, consistent with previously reported data of 4.3 hours [20]. The observed C_{max} for a 16 mg dose of Candesartan was 101.13 $\pm$ 6.64 ng/ml, aligning with earlier findings of 95.2 ng/ml.

The absorption rate constant (K_a) was determined to be 0.331 $\pm$ 0.059 per hour, and the half-life of Candesartan was found to be 30.72 $\pm$ 8.25 hours in healthy volunteers, which closely matches the previously reported value of 29 hours.

CONCLUSION

Candesartan plasma concentrations were measured using a simple, accurate, and reliable HPLC method after the drug was administered orally. The developed method effectively determines the pharmacokinetic parameters of Candesartan, including AUC, C_{max}, T_{max}, volume of distribution, absorption rate constant, and elimination half-life, in healthy volunteers.

Author(s) Contribution: Investigations and resources, I. Fatima.

Funding: Nill

Ethical Approval: Kind Edward Medical University (S.LP.SWE 4264 dated 03-06-2023).

Conflict of Interest: There is no conflict of interest.

Consent for Publication: All authors approved the manuscript for publication and agreed to be responsible for all aspects of this article.

REFERENCE

1. Azizi M and Wuerzner G. Rationale for combining blockers of the renin-angiotensin system. *Semin Nephrol.* (2007) 27(5): 544-54. Ripley TL, Chonlahan JS and Robin E. Candesartan in heart failure. *Interv Clin Agin.* (2006) 1(4): 357-366.
2. Lacourcière Y and Asmar R. A comparison of the efficacy and duration of action of candesartan cilexetil and losartan as assessed by clinic and ambulatory blood pressure after a missed dose, in truly hypertensive patient^s. A placebo-controlled, forced titration study. *Am J Hypertens.* (1999) 12(12): 1181-1187.
3. Amy R and Vasilios P. Candesartan cilexetil in cardiovascular disease. *Expert Rev. Cardiovasc Ther* (2004) 2 (6): 829-835.
4. Ferreir'os N, Dresen S, Alonso RM and Weinmann W. Hydrolysis and transesterification reactions of candesartan cilexetil observed during the solid phase extraction procedure. *J Chromatogr B.* (2007) 855: 134-138.
5. Omaria A, Omarib MA, Badwanb AA and Al-Sou'od AK. Effect of cyclodextrins on the solubility and stability of candesartan cilexetil in solution and solid state. *J Pharm Biomed Anal.* (2011) 54: 503-509.
6. Kamalakkannan V, Puratchikody A, Ramanathan L and Jayaprabha S. Development and validation of a dissolution test with reversed-phase high performance liquid chromatographic analysis for Candesartan cilexetil in tablet dosage forms. *Arabian Journal of Chemistry* (2011). Available from URL: <http://www.sciencedirect.com/science/article/pii/S1878535211002528>
7. Zuschke CA, Keys I, Munger MA, Car-r AA, Marinides GN, Flanagan TL, Cushing DJ, Hayes JL and Michelson EL. Candesartan Cilexetil: Comparison of Once-Daily Versus Twice-Daily Administration for Systemic Hypertension. *Clin Ther.* (1999) 21(3): 464-474.
8. Gamiz JLP, Pego M, Contreras EM, Anglada MP, Mart'nez JO, Esquerria EA and Gorostiza JDS. A Twelve-Week, Multicenter, Randomized, Double-Blind, Parallel-Group, Noninferiority Trial of the Antihypertensive Efficacy and Tolerability of Imidapril and Candesartan in Adult Patients with Mild to Moderate Essential

- Hypertension: The Iberian Multicenter Imidapril Study on Hypertension (IMISH). *Clin Ther.* (2006) 28(12): 2040-2051.
9. Bonner G, Landers B and Bramlage P. Candesartan cilexetil/hydrochlorothiazide combination treatment versus high-dose candesartan cilexetil monotherapy in patients with mild to moderate cardiovascular risk (CHILI Triple T). *Vasc Health Risk Manag.* (2011) 7: 81-95.
 10. Zeeuw D, Remuzzi G and Kirch W. Pharmacokinetics of candesartan cilexetil in patients with renal or hepatic impairment. *J Hum Hypertens.* (1997) 11: 37-42
 11. Gleiter CH and Morike KE. Clinical Pharmacokinetics of Candesartan. *Clin Pharmacokinetic.* (2002) 41(1): 7-17.
 12. Fletcher CV, Acosta E and Strykowski J. Gender differences in human pharmacokinetics and pharmacodynamics. *J Adolesc Health.* (1994) 15(8):619-629.
 13. Bigos KL, Pollock BG, Stankevich BA and Bies RR. Sex differences in the pharmacokinetics and pharmacodynamics of antidepressants: An updated review. *Gen Med.* (2009) 6(4): 522-543.
 14. Brenesel MD, Mimica-Dukic N, Pilija V and Tasic M. Gender related differences in the pharmacokinetics of opiates. *Forensic Sci Int.* (2010)194(1-3): 28-33.
 15. Muhammad F, Hussain F, Nawaz M, Javed I. Disposition kinetics of Kanamycin in mules. *Veterinarski Archiv.* (2003) 73(4): 221-226.
 16. Tayebbeh G, Farzad K and Seyed Alireza M. Development of a Simple RP-HPLC-UV Method for Determination of Azithromycin in Bulk and Pharmaceutical Dosage forms as an Alternative to the USP Method. *Iran. J. Pharma. Res.* (2013), 12: 55-61.
 17. Seyed Mohsen F, Afshin Z, Alireza S, Babak M, and Farshid A. Rapid High Performance Liquid Chromatographic Method for Determination of Clarithromycin in Human Plasma Using Amperometric Detection: Application in Pharmacokinetic and Bioequivalence Studies. *Iran. J. Pharma. Res.* (2013), 12: 63-67
 18. Peepliwal AK, Bonde CG and Mohanraj K. Bioanalytical method development and its validation for determination of candesartan cilexetil by high performance liquid chromatography with UV detection. *Acta Pharmaceutica Scientia.* (2010) 52: 247-253.
 19. Hoogkamer JFW, Kleinbloesem CH, Ouwerkerk M, Hoëgemann A, Nokhodian A, Kirch W and Weidekamm E. Pharmacokinetics and safety of candesartan cilexetil in subjects with normal and impaired liver function. *Eur J Clin Pharmacol.* (1998) 54: 341-345.
 20. Trachtman H, Hainer JW, Sugg J, Teng R, Sorof JM and Radcliffe J. Efficacy, safety, and pharmacokinetics of candesartan cilexetil in hypertensive children aged 6 to 17 years. *J Clin Hypertens.* (2008) 10(10): 743-50.