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Development and Validation of Simplified RP-HPLC Method for Quantification of Candesartan Cilexetil in Commercial Formulations

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ABSTRACT

In order to produce antihypertensive effects, Candesartan Cilexetil (CC), a candesartan inactive prodrug, was quickly converted into active candesartan after absorption in the Gastrointestinal (GI) tract. This research study describes the development and validation of an accurate, precise, repeatable, easy and speedy Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) approach for measuring CC in a formulation using reverse-phase HPLC. In this study, liquid chromatography was performed on a Zorbax SB C-18 analytical column with dimensions of 2504.6 mm, 5 m, using a mobile phase consisting of Acetonitrile (ACN) and 0.1 percent orthophosphoric acid (pH 2.5) in a ratio of 35:65 vol/vol as the mobile phase. Results were presented as mean Standard Deviation (SD). This experiment used an injection volume of 20 microliters to measure the sample at 258 nano liters per minute while using a flow rate of 1.5 microliters per minute. 4.2 minutes was determined to be the retention time for both the reference and sample drugs. There was no evidence of nonlinearity in the calibration curve for CC when the concentration ranged from 50 ppm to 160 ppm and the Regression coefficient (R^2) was determined to be 0.9996. When the percentage recovery of CC was achieved, it was in the range of 98.10 percent to 98.70 percent, indicating that the present approach was very accurate. Recovery trials using Percent Relative Standard Deviation (percent RSD) with intra and inter-day accuracy were found to be less than 2 percent, demonstrating that the established procedure is repeatable. The technique was verified in accordance with the International Conference on Harmonization (ICH) requirements and may be highly recommended for regular analysis of CC due to the fact that it is the most reliable and quick methodology currently available. Despite the fact that a great deal of research has been done on calculating CC in dosage forms, this study was determined to be under green chemistry conditions, as well as being quick and inexpensive. As a result, the statistical validation of the data revealed that the suggested approach may be used to estimate the CC in commercial formulations, which is encouraging.

Keywords: Candesartan cilexetil; Method validation; RP-HPLC; ICH

INTRODUCTION

Because of its widespread incidence and the resultant cardiovascular illness and chronic renal disease, hypertension is a significant worldwide health concern [1-2]. Premature mortality and disability are associated with high blood pressure, which is a modifiable risk factor for both [3]. Since at least the 1970's, the mean blood pressure and the pre valence of high blood pressure have both decreased significantly in high-income countries of the world. Blood pressure, on the other hand, has been increasing in East, South and Southeast Asia, Oceania and sub-Saharan Africa [4]. As a result of these developments, the prevalence of hypertension is currently greater in low and middle income nations than it is in high income ones. Prior research showed that 26.4 percent of the worldwide adult population or 972 million individuals had hypertension in 2000 and based on data from the world health organization. The incidence of hypertension has increased in low and middle income nations since 2000, but it has remained stable or decreased in high income countries [5-6], according to national reports published since 2000. Since 1990, the number of adults (aged 30-79 years) living with hypertension has more than doubled, rising from an estimated 331 million women and 317 million men in 1990 to 626 million women and 652 million men in 2019. The vast majority of this increase has occurred in low and middle income countries, with the majority of the increase occurring in developing countries (LMICs). The multinational research, which was published in the lancet, looked at blood pressure readings gathered by more than 100 million individuals in 184 countries over three decades and published in the lancet [7-8].

Candesartan cilexetil is a racemic combination that has one chiral centre at the cyclohexyloxycarbonyloxy ethyl ester group of the cyclohexyloxycarbonyloxy ethyl ester group. During absorption from the gastrointestinal system, Candesartan cilexetil, a prodrug, is hydrolyzed to become candesartan, which is then administered. Candesartan is an angiotensin II receptor antagonist that is selective for the AT1 subtype. Candesartan cilexetil is a non-peptide with the chemical formula is (±)-l-hydroxyethyl 2-ethoxy-l-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). Its empirical formula is $C_{33}H_{34}N_6O_6$. It is hydrophobic drug which belongs to BCS class II and its half-life is 5.1 h with 15%-40% bioavailability. It is essentially insoluble in water, sparingly soluble in methanol and somewhat soluble in acetonitrile [9-10], with the exception of acetonitrile. In addition, it is a hydrophobic medication that belongs to BCS class II. Its half-life is 5.1 hours, and it has a bioavailability of 15-40 percent. CC is promptly and totally biaoactivated by ester hydrolysis at the ester link to generate the active achiral candesartan following absorption from the Gastrointestinal (GI) tract, which is due to the fast hydrolysis of double esters in the blood or tissues. In order to produce antihypertensive effects, candesartan, a very powerful and selective Angiotensin II type 1 (AT1) receptor antagonist, must first inhibit the vasoconstrictor and aldosterone secreting activities of angiotensin II [11-12].

In the past, numerous techniques for the simultaneous or single measurement of CC have been developed, which have made use of a variety of equipment, such as the UV spectrophotometer, the high-performance liquid chromatography, the HPLC-MS, and the LC/MS. In contrast, the current approach, which was developed *via* RP-HPLC for the determination of CC in dosage forms, was found to be simple, exact, quick and cost-effective to perform. Although multiple RP-HPLC approaches for measuring CC were discovered in various works of literature, they were found to be complex and time consuming. To promote green chemistry, the present research sought to create a new RP-HPLC technique for the measurement of CC in dosage form that was accurate, sensitive, cost-effective, and stability indicating, while employing the fewest amount of hazardous chemicals possible. According to ICH Q2 guidelines, the validation of the newly designed CC RP-HPLC technique was completed (R1). The accuracy, precision, linearity, specificity, Limit of Detection (LOD), and Limit of Quantification (LOQ) of the technique for DRV, as well as the quantification of DRV in indicated tablet dosage form, were evaluated throughout the development of the method for DRV [13-16].

MATERIALS AND METHODS

Chemicals and reagents

Dr. Reddy's laboratories provided a gift sample of CC (99.5 percent pure) (Hyderabad, India). The HPLC-grade solvents used in this study were obtained from Merck Ltd. in Bangalore, India, including acetonitrile, methanol, orthophosphoric acid and water. All of the chemicals used were of the highest quality for HPLC.

Instruments and chromatography condition

The HPLC was carried out on a waters 2695 Alliance system equipped with a 2996 Photodiode Array Detector (Waters) (PDA). The standards were resolved on a reverse phase Zorbax SB C18 column with a diameter of 250 4.6 mm and a thickness of 5 microns (Agilent, Mumbai, India). When the mobile phase was ready, it was made by diluting it with Acetonitrile (ACN) and 0.1 percent orthophosphoric acid (pH 2.5) in a ratio of 35:65 vol/vol, followed by centrifugation. Before use, the mobile phase was degassed and filtered through a 0.45 m filter to remove any remaining gas. It was necessary to inject the sample at a volume of 20 mL. With the temperature kept constant at 25 degrees Celsius, the flow rate was reduced to 1.5 ml/min at a wavelength of 258 nm. A software system called Water Empower 3 was used to collect the chromatographic data for this study.

Selection of lambda max

Lambda max is selected using a UV spectrophotometer, and this decision is critical for the sensitivity of the RP-HPLC technique. By using an optimum wavelength, it is possible to detect an exact absorbance for any medication. In the current investigation, pure CC concentrations of 100 ppm were determined by scanning in the range of 200 nm-400 nm using a high performance liquid chromatography system (Waters 2695 Alliance system).

Preparation of standard stock solutions

In order to create stock A, 10 mg of pure CC was weighted and put into a 10 ml volumetric flask containing 5 ml of diluent (HPLC grade methanol), which was then sonicated for 5 minutes and allowed to cool before being made up to volume with diluent to prepare stock B. (1000 ppm). In order to create multiple concentration standard working solutions ranging from 50 ppm to 160 ppm, this solution was further dilute. 10 mL of the aforementioned stock A solution was drawn and transferred to a volumetric flask with a capacity of 100 mL, which was then allowed to cool and make up the volume with diluent (100 ppm). The resultant solution should be used as the standard solution.

Preparation of sample solutions

Weighing and finely grinding the sample composition resulted in a powder of uniform size. An equal amount of 50 mg CC was extracted from this powdered tablet and placed in a volumetric flask with a capacity of 500 ml, where it was diluted with methanol and then subjected to 30 minutes of sonication. The basic RP-HPLC technique was used to evaluate a sample solution with a final concentration of 100 ppm.

Method of analysis

The chromatographic conditions were maintained as previously mentioned, and the baseline stabilization procedure was carried out for 30 minutes total. Following stabilization, the repeatability of the blank and the prepared concentration solution of the standard drug was tested in the respective peak regions of the blank and the prepared concentration solution of the standard drug. For the purpose of quantification, the solution of the sample was injected. We estimated the response factor based on the standard peak ratio and the sample peak ratio. The same technique was done six times to ensure that the created method was adhering to the established standard of repeatability [17-20].

Validation of RP-HPLC method

Accuracy: Accuracy is defined as the degree of agreement between the measured value and the genuine value. The three separate CC standard and sample solutions were obtained from concentrations ranging from 80 mg/ml to 120 mg/ml in the concentration range. These solutions were injected into the test subjects in order to determine the correctness of our devised procedure. A sample solution was generated for three duplicate concentrations, and the results were quantified in the meanwhile. The percentage of recovery was calculated using the methodology shown below. The recovery rate must be in the range of 98-102 percent in order to be considered satisfactory.

Precision: Precision may be defined as a measurement of real value between different outcomes of the same amount or quantity range. Analyzing six duplicate concentration solutions from 100 ppm on the same day and three separate days allowed researchers to examine intra-day and inter-day fluctuations in the concentrations. Calculation of the percent Relative Standard Deviation (RSD) was accomplished by the use of the following equation: The normal acceptance restriction for percent RSD acceptance is less than 2 percent of the total.

Linearity: For the purpose of evaluating the linearity of CC solution, several concentration solutions ranging from 50 to 160 mg/ml (54, 65, 83, 108, 130, 139 and 160 ppm) were injected into the test tube. Each concentration solution was examined six times in the column under the identical conditions by injecting it into the column six times. The linearity calibration curve was developed based on the area peak obtained from the chromatogram and each concentration of CC solution used in the experiment. It was possible to calculate the coefficient of correlation (R^2) using regression analysis calculations since the slope and intercept values were known. It is recommended that the standard limit of regression analysis be greater than 0.999.

System suitability: The appropriateness of the system was determined in accordance with the United States Pharmacopeia (USP). To determine the metrics such as column efficiency, resolution, peak symmetry factor, percentage coefficient in peak area or height [28], it was necessary to employ the prepared concentration solution of CC for six duplicate injections. A percentage RSD, theoretical plate value, tailing factor and system accuracy were all calculated using the observed value as a starting point.

Specificity and selectivity: It was necessary to test the specificity and selectivity of the newly devised approach in order to identify the excipients that were interfering with the estimate of the CC. The blank solution, which did not include the CC, was produced and injected. The chromatogram produced from the blank was compared to the chromatograms obtained from the standard and sample and the difference was examined to determine if excipients interfered with the drug quantification.

Robustness and ruggedness: The robustness and ruggedness of the created CC RP-HPLC technique were proved by modest modifications in the chromatographic conditions that were used in the development. In order to determine if any impacts were induced by the changes in parameters such as column temperature, flow rate and the usage of various percentage ratios of mobile phase, the parameters were varied. The difference in chromatographic conditions was taken into consideration in the current investigation. Flow rates of 1.3 ml and 1.7 ml were maintained throughout the injection process. The differing column temperatures (20 degrees Celsius and 30 degrees Celsius) were maintained. The various wavelengths (253 and 263) were retained [21-24].

RESULTS AND DISCUSSION

Development of RP-HPLC method

The development of an RP-HPLC technique for the measurement of CC in dosage form was completed. An aqueous mobile phase containing 0.1 percent orthophosphoric acid as an organic phase and 0.1 percent ACN as an organic phase was used. A variety of chromatographic conditions, including flow rate, column temperature and the components ratio in the mobile phase used, were tested in order to generate a crisp and symmetric peak with an appropriate retention period. In order to get a superior peak, the column Zorbax SB C-18 was employed. Because of the mobile phase employed in the procedure, the characteristics of the chromatographic settings such as retention duration, theoretical plate number (N), retention factor and selectivity could be tailored to meet the needs of the researchers. The determination of wavelength was carried out using a Waters 2695 Alliance system equipped with a 2996 photodiode array detector in order to ensure adequate sensitivity of CC using the RP-HPLC technique (PDA). In order to discover the wavelength maxima, the standard solution of CC was scanned throughout a range of 200 nm-400 nm, with a better peak being identified at 258 nm (Figure 1). In light of the aforementioned considerations, the present investigation produced an acceptable result using Acetonitrile (ACN) and 0.1 percent orthophosphoric acid (pH 2.5) in the ratio of 35:65 v/v with a flow rate of 1.5 ml/min for the estimate of CC in the dosage form [25-27].

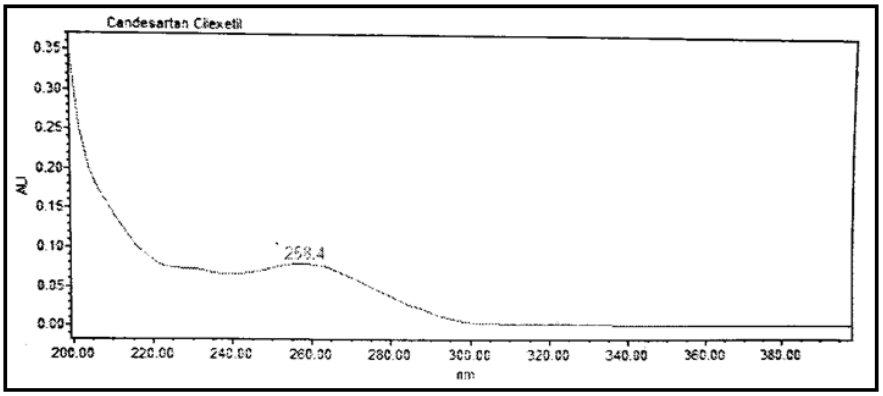

Figure 1: HPLC spectra of CC.

Accuracy

A specified level of concentration ranging from 50 Parts Per Million (ppm) to 160 Parts Per Million (ppm) was used to test the percentage recovery and accuracy of the proposed CC RP-HPLC technique process. As demonstrated in Table 1, the information received was deemed to be accurate. According to the findings, the percentage recovery of standard and sample CC was 98.52 percent, 98.12 percent and 98.13 percent, respectively. It

was determined that the acquired result is within the range of typical recovery values (98.0 percent to 102.0 percent).

Table 1: Accuracy studies of developed method.

Analyte	Recovery level	% Recovery	Average % Recovery
Candesartan cilexetil	80%-1	97.91	98.52
	80%-2	98.73	
	80%-3	98.93	
	100%-1	97.8	98.12
	100%-2	98.51	
	100%-3	98.03	
	120%-1	98.21	98.13
	120%-2	97.89	
	120%-3	98.3	

Precision: In this research, precision was examined in terms of system precision, technique accuracy and intermediate accuracy. Using six duplicate injections of the same standard from the same vial, the accuracy of the system was measured and quantified in terms of percent Relative Standard Deviation (percent RSD), tailing, plate count and resolution. The sample was subjected to the above mentioned method a total of six times. The percent assay for each analyte was represented as a percentage of the Relative Standard Deviation (percent RSD). Intermediate precision was performed on two different systems, one using a waters e2695 alliance system with a 2996 PDA and the other using a 2489 Ultraviolet (UV) detector, by different analysts by analysing six different samples of extract, and the results were expressed as a percent relative standard deviation. The results of this investigation demonstrated a more exact and accurate approach for detecting CC in the dose form than previous methods (Table 2).

Table 2: Method precision and intermediate precision results.

Sample	Analyst-1	Analyst-2
	Method precision	Intermediate precision
1	100.3	99.5
2	100.5	99.3
3	101.1	98.9
4	99.8	99.9
5	100	99.4
6	100.4	100
Mean	100.3	99.3
SD	0.501	0.438
%RSD	0.5	0.44
Overall mean	99.8	
Overall RSD	0.741	
Overall % RSD	0.74	

Linearity and range: The linearity calibration curves were found to have an R^2 value of 0.9996, which was determined to be 0.9996. The calibration curve that was drawn in the concentration range of 50 ppm-160 ppm was found to be linear in nature (Table 3). Y=5095.4x-7505.5 was determined to be the equation, and the Regression coefficient (R^2) was found to be 0.9996. The equation and Regression coefficient (R^2) are presented in Figure 2. According to the results, the relative standard deviation was in the range of 1.0 to 1. A higher correlation value was discovered between the observation derived from peak value and the concentration of the drug solution than had previously been seen.

Table 3: Linearity of Candesartan cilexetil.

% Level	Conc. of CC (ppm)	Average peak area of CC
50	54	263099
60	64.8	328186
80	82.9	418169
100	108	537461
120	129.6	653765
140	138.2	695671
150	159.8	808601
(Correlation coefficient) R^2		0.9996
Slope of regression line		5095.4

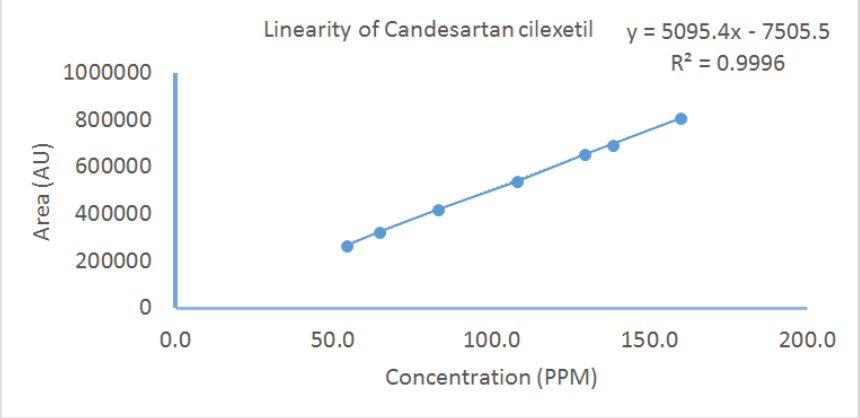

Figure 2: Linearity graph for Candesartan cilexetil

System suitability: For the system suitability investigations, a standard CC solution with a concentration of 100 ppm was used, and the results were evaluated. The time required for separation under chromatographic conditions was determined to be 4.2 minutes. A variety of frameworks, including the tailing factor, retention duration, theoretical plate number (N), and system accuracy, were determined to be within acceptable limits of 2 percent. The results obtained were within the acceptability requirements specified in the United States Pharmacopeia at the time of testing. Detailed data were displayed in Table 4, which may be seen here.

Table 4: System suitability parameters.

Sr. No.	Solution name	Retention time	USP tailing	USP plate count
1	Standard	4.229	1.3	53014
2	Sample	4,256	1.2	53213

Specificity and selectivity: When comparing the results of the current study to those of past research studies on the same medication, it was discovered that the retention period with readily accessible mobile phase was superior. The approach was quantified accurately and with high resolution. It was not possible to draw any conclusions from the blank sample. A cost effective mobile phase consisting of acetonitrile and 0.1 percent orthophosphoric acid (pH 2.5) in a volumetric ratio of 35:65 v/v is utilized in this procedure. Figure 3 shows the retention time for standard and sample CC concentrations. The retention time for standard and sample CC concentrations was 4.2 minutes. When compared to other approaches that have been developed, the retention time attained was found to be shorter.

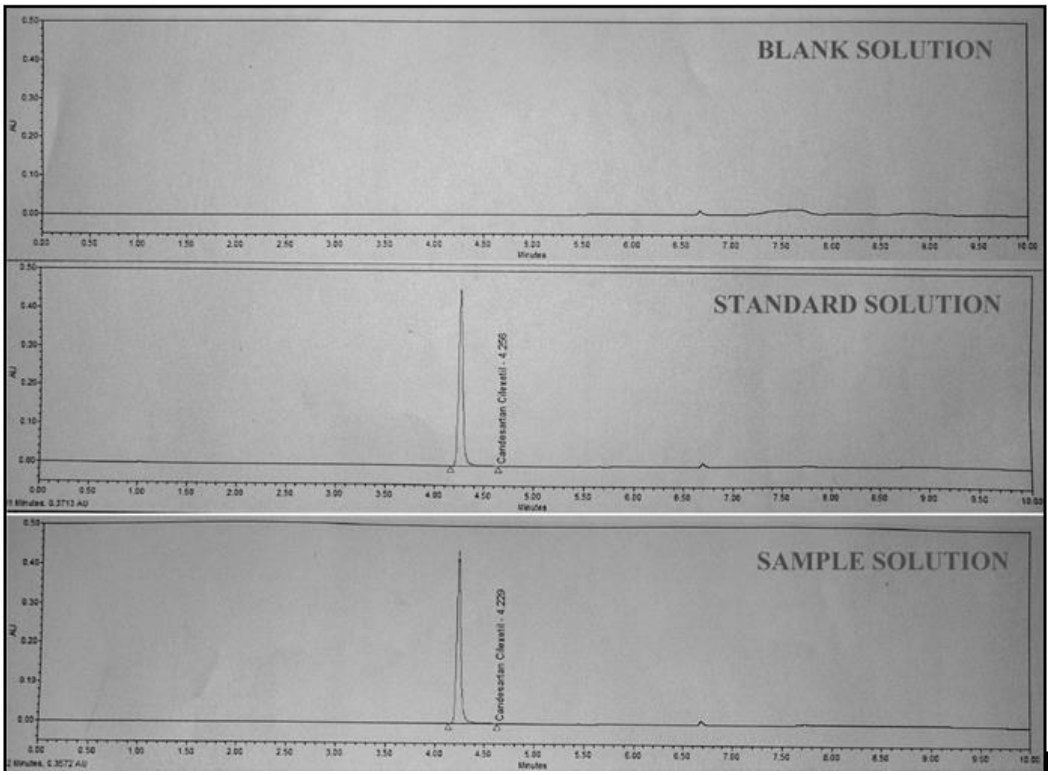

Figure 3: Chromatograms showing retention time for standard and sample CC concentration.

Robustness and ruggedness:
In order to determine the robustness and ruggedness of the currently developed CC RP-HPLC method, minor variations in chromatographic parameters, such as the rate of flow of mobile phase (1.3 ml/min and 1.7 ml/min), different wavelengths (253 and 263) and temperature of the column (20 degrees Celsius and 30 degrees Celsius), were applied. The collected results did not reveal any statistically significant differences in peak area or retention duration. The percentage recovery of CC for the standard solution was almost identical to 99.0 percent, whereas the percentage recovery of CC for the sample solution was nearly identical to 99.2 percent. According to the findings, the percent RSD was less than 2.0 percent under various situations, indicating that the current approach is robust and tough [28-30] .

CONCLUSION

The RP-HPLC technique was used to produce an accurate, precise, robust, reliable and repeatable approach for the quantitative measurement of CC in dosage form. The solvent that was utilized as the mobile phase has shown a very high resolution rate while requiring less retention time. The procedure was carried out in accordance with ICH and FDA rules and the report received fulfilled all of the required standards. The accuracy, precision and linearity of the procedure were all evaluated in order to ascertain the quality of the drug content. The RP-HPLC technique presented here enables for the measurement of CC in a repeatable manner. According to the statistical data, the proposed approach may be effectively used to our regular determination method with success. The specificity report demonstrated that the excipient had no effect on the results. Kinetics investigation using plasma and biological fluids may be included to this research as a further extension. The fact that this innovative approach demonstrated a greater cost effectiveness ratio when compared to the previously reported studies was a significant finding.

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