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Validated UV spectrophotometric method for quantitative determination of candesartan cilexetil in bulk and pharmaceutical dosage form

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ABSTRACT

In the present work simple, precise, specific, accurate and cost effective UV spectrophotometric method has been developed for the estimation of Candesartan Cilexetil in pharmaceutical formulation. The wavelength of the Candesartan Cilexetil detection was 258 nm in methanol and obeys Beer's law in the concentration range of 10 - 50 µg /mL with a correlation coefficient of 0.9999. The results of analysis were validated by recovery studies. The percentage recovery of the drug after standard addition was found to be 99.45 - 99.85 %. The relative standard deviation was found to be < 2.0 % in all cases. The result of analysis was found to be 99.83±0.226 %. The Proposed spectrophotometric method was validated as per the ICH Q2 (R1) guidelines. The proposed method was found to be suitable for the quantitative determination of Candesartan Cilexetil in bulk form and pharmaceutical formulations.

Key words: Candesartan Cilexetil, UV spectrophotometry, Validation, ICH Q2 (R1).

INTRODUCTION

The chemical name for Candesartan Cilexetil is (+)-1-[[(cyclohexyloxy)carbonyl]oxy]ethyl-2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'biphenyl]-4-yl]methyl]-1H-benzimidazole-7-carboxylate. Candesartan Cilexetil is used for treating high blood pressure (hypertension) in adult patients. Treating adult heart failure patients with reduced heart muscle function, in addition to ACE (Angiotension Converting Enzyme) inhibitors or when ACE inhibitors cannot be used. Candesartan is marketed as cyclohexyl 1-hydroxyethyl carbonate (Cilexetil) ester, known as Candesartan Cilexetil. Candesartan Cilexetil is metabolized completely by esterases in the intestinal wall during absorption to the active Candesartan Cilexetil moiety.

Literature survey revealed that very few analytical methods have been reported hitherto for estimation of Candesartan Cilexetil. Majority of methods for determination of Candesartan Cilexetil in biological fluids includes RP-HPLC [1-6], UV spectrophotometric method [7], liquid chromatography and fluorimetric detection [8], spectrofluorimetry [9], LC-MS/MS [10,11]. The present investigation the authors have developed a new, simple, accurate, precise, fast and reproducible UV spectrophotometric method for the determination of Candesartan Cilexetil in bulk and its tablet dosage form. This work describes the validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1). Figure 1 shows chemical structure of Candesartan Cilexetil.

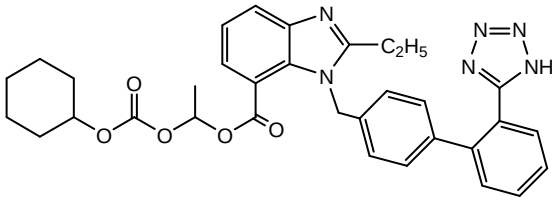

Figure 1: Chemical structure of Candesartan Cilexetil

MATERIALS AND METHODS

Selection of solvent

Numerous trails were performed to find out the suitable solvent system for dissolving the drug. The solvents like acetonitrile, double distilled water and methanol were tried based on the solubility of the drug. The drug showed maximum absorption at 258 nm in methanol. So methanol was chosen as optimized solvent in the present spectrophotometric method.

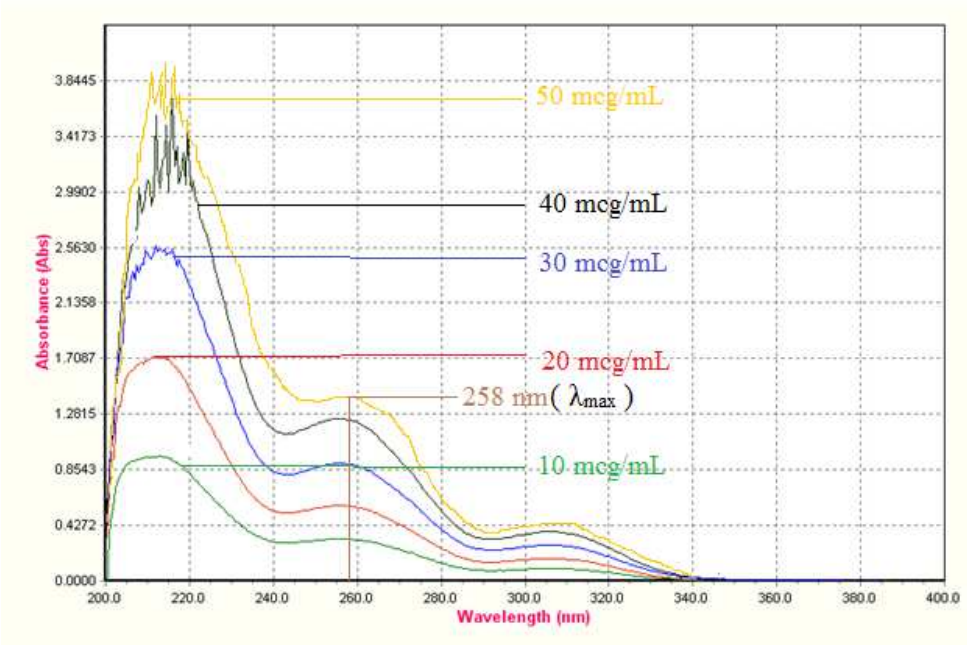

Figure 2: UV Spectrum of Candesartan Cilexetil

Instruments used

ELICO Double beam SL 210 UV-VIS spectrophotometer was used to record the absorption spectra. Spectrophotometer with 1 cm matched quartz cells were used for the estimation of Candesartan Cilexetil. Essae vibra AJ (0.001g) analytical balance was used for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai was used for sonication.

Reagents and Materials

Candesartan Cilexetil pure drug was supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. The marketed formulation Candesar tablets containing 8 mg of Candesartan Cilexetil tablets were obtained from local market. Analytical grade methanol was procured from E. Merck specialties private Ltd., Mumbai, India.

Selection of detection wavelength

Suitable dilutions of Candesartan Cilexetil were prepared from the standard stock solution. By using ELICO Double beam SL 210 UV VIS spectrophotometer, the dilutions of Candesartan Cilexetil were scanned in UV range of 200 -

400 nm using methanol as a blank. It was observed that the drug showed maximum absorbance at 258 nm which was chosen as the detection wavelength for the estimation of Candesartan Cilexetil. The spectrum of Candesartan Cilexetil is shown in Figure 2.

Preparation of standard drug solutions:
An accurately weighed 10 mg of Candesartan Cilexetil pure drug was dissolved and transferred to 10 mL volumetric flask. Then the volume was made up to the mark with methanol to obtain the stock (primary) solution of Candesartan Cilexetil having concentration l000 µg/mL. From this stock solution prepared the concentration of 100 µg/mL. The above stock solution was further diluted with the methanol to get a working standard solution of 10 to 50 µg/mL.

Preparation of Calibration curve:
Linearity range solutions containing 10, 20, 30, 40, 50 µg/mL of Candesartan Cilexetil were prepared. After setting the instrument for its spectral properties the solutions were scanned in the wavelength ranging from 200 nm - 400 nm. The wavelength of maximum absorption for Candesartan Cilexetil was found at 258 nm. Calibration data is presented in Table 1. Calibration curve was prepared by plotting concentration of Candesartan Cilexetil on X-axis and their respective absorbance's on Y-axis. The calibration curve is shown in Figure 3. The optical characteristics are presented in Table 2.

Table 1: Linearity data for Candesartan Cilexetil

Concentration(µg/mL)	Absorbance
0	0
10	0.32
20	0.62
30	0.93
40	1.24
50	1.56

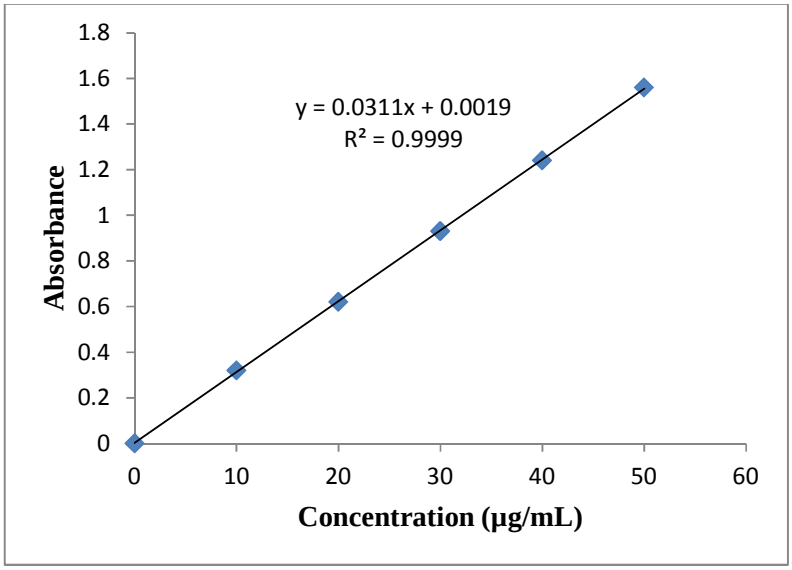

Figure 3: Calibration curve of Candesartan Cilexetil by UV method

Validation of the developed method: [12,13]
The developed UV method of analysis was validated according to the International Conference Harmonization ICH Q2 (R1) guidelines for the different parameters such as precision, accuracy, specificity, linearity, ruggedness, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Table 2: Optical characteristics, regression data of the proposed method

Parameter	Result
λmax (nm)	258
Beer's law limits (µg / mL)	10-50
Molar absorptivity (L.mole^{-1} cm $^{-1}$)	13653.95
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.03225
Regression equation (Y= a+bc) ; Slope is	0.03105
Standard deviation of slope (S_b)	0.00013
Intercept (a)	0.00190
Standard deviation of intercept (S_a)	0.00408
Standard error of estimation (S_e)	0.00564
Correlation coefficient (r^2)	0.9999

Precision:

The precision of an analytical procedure states the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for three times of the sample solution. The percent relative standard deviation (% RSD) was calculated. The results obtained are given in Table 3.

Table 3: Results of precision study

Parameter	Intra-day	Inter-day		
		Day -1	Day -2	Day -3
Mean % recovery	0.929	0.932	0.9287	0.933
SD	0.0025	0.0027	0.0024	0.0024
% RSD*	0.258	0.285	0.236	0.256

**average of 3 determinations*

Accuracy (Recovery studies):

The accuracy of analytical method was determined by closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different percentage determinations (50 %, 100 % and 150 %) by standard addition method. For each percentage level the analysis was repeated for three times (n = 3) for Candesartan Cilexetil. The recovery studies were carried out by adding known amount of pure Candesartan Cilexetil at 50 %, 100 % and 150 % of preanalyzed formulation. From the amount of Candesartan Cilexetil found, % recovery was estimated. The results are presented in Table 4.

Table 4: Results of accuracy study

Recovery study %	Label claim mg/tab	Amount of substance added	Amount recovered	Mean Percent recovery ±RSD*
50 %	8	4	11.98	99.83±0.213
100 %	8	8	15.98	99.85± 0.205
150 %	8	12	19.89	99.45±0.173

***** = Average of 3 determinations, RSD: relative standard deviation***

Ruggedness

Ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. The results obtained are shown in Table 5.

Table 5: Ruggedness results

Parameter	Instrument-1 (Systronics model 2203)	Instrument-2 (Elico SL 159)	Analyst -1	Analyst -2
Mean	0.928	0.948	0.928	0.952
SD*	0.0024	0.0026	0.0024	0.0025
% RSD$^{\#}$	0.258	0.276	0.258	0.266

** = Standard deviation; % RSD: % Relative standard deviation # = average of 3 determinations.*

Robustness

According to ICH the robustness is defined as a measure of its capacity to remain unaffected by small deliberate changes in method parameters. The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be 0. within the method's robustness range. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6.

Table 6: Results for Robustness study

Parameter	λ_{max} 1	λ_{max} 2
Mean	0.929	0.948
SD*	0.0024	0.0026
% R.S.D# (n=6)	0.256	0.276

** = Standard deviation; % RSD# = % Relative standard deviation*

LOD and LOQ:

The limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3(SD) / S and LOQ = 10 (SD) / S, where SD = standard deviation of response (absorbance) and S = slope of the calibration. The results of LOD and LOQ are shown in Table 7.

Table 7: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Parameter	Results
Limit of Detection (LOD)	0.2554 µg/mL
Limit of Quantitation (LOQ)	0.7743 µg/mL

Procedure for assay of pharmaceutical formulations:

Twenty Candesartan Cilexetil (Candesar) marketed tablets were accurately weighed, finely powdered in glass mortar and average weight of each tablet was determined. A quantity of tablet powder equivalent to 8 mg of Candesartan Cilexetil was transferred to 100 mL volumetric flask and to this 25 mL of methanol was added. The solution was sonicated for 20 minutes and filtered through Whatman filter paper no. 42 to remove insoluble materials and the volume was diluted with the a diluent to make 80 µg/mL. which was further diluted to obtained concentration 8 µg/mL and was used in analysis. Assay results are presented in Table 8.

Table 8: Result of analysis for Candesartan Cilexetil in tablet formulation

S.NO.	Formulation	Labeled amount	Amount found *(mg) (mean ± SD)	% Assay	% RSD*
1	Candesar	8 mg	7.987 ± 0.483	99.83	0.226

** Average of five determinations. RSD: relative standard deviation*

RESULTS AND DISCUSSION

For the selection analytical wavelength, Candesartan Cilexetil solution were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 - 400 nm by ELICO Double beam SL 210 UV- VIS spectrophotometer. The λ_{max} of 258 nm was chosen for the determination of Candesartan Cilexetil and the absorption maxima curve was shown in Figure 2. The calibration curve for Candesartan Cilexetil was prepared in the concentration range of 10-50 µg/mL. The proposed method obeyed Beer's law in the concentration range of 10-50 µg/mL with a good correlation coefficient of r^2 = 0.9999. Calibration data is presented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of Candesartan Cilexetil is shown in Figure 3**.** The optical characteristics and the data concerning to the proposed analytical method is represented in Table 2. Accuracy studies were carried out by recovery study using standard addition method at three different concentration levels (50, 100 and 150 %). The known amount of standard drug solution of Candesartan Cilexetil was added to pre-

analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery study results were found to be in the range of 99.45 to 99.85 percentages with percentage RSD less than 2 (Table 4). The same solutions of recovery study was further determined on same day at three different times for intra-day and on three different days for inter-day precision study. The precision of the method was found to be good with % RSD less than 2 which indicates that the method was precise and the results are presented in Table 3**.** Ruggedness was performed by changing two different analysts and two instruments and the results are tabulated in Table 5. It reveals that the proposed method was found to be rugged. For the determination of a method's robustness, parameters such as detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated for six times. The results of robustness are presented in Table 6. The results are within the specified limits which states that this method is robust. The LOD and LOQ were found to be 0.2554 µg/mL and 0.7743 µg/mL respectively which shows that this method was very sensitive as they were within the permitted levels. The LOD and LOQ results are shown in Table 7. The developed method was eventually utilized in analysis of tablet formulation and was found to be within the proposed limits and also the mean % assay value was found to be 99.83 ± 0.226 %. The assay results are shown in Table 8. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

CONCLUSION

The developed and validated UV spectrophotometric method was found to be economical due to the use of methanol as a solvent all the way through the experiment. None of the usual excipients employed in the formulation of Candesartan Cilexetil dosage forms interfered in the analysis of Candesartan Cilexetil by the developed method. The system suitability parameters and system precision are determined and found within the limits. The plot is drawn between the concentration and absorbance which is found to be linear in the concentration range of 10-50 µg/mL with good correlation coefficient greater than r^2 = 0.9999. Low % relative standard deviation and high percent of recovery indicates that the method is highly precise and accurate. Thus, the developed method for Candesartan Cilexetil was found to be simple, precise, accurate and cost effective and in actual fact feasible for routine sample analysis of Candesartan Cilexetil in pharmaceutical dosage forms.
Conflict of interest: We declare that we have no conflict of interest.

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