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Der Pharmacia Lettre, 2015, 7 (6):237-243
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

UV spectrophotometric estimation of candesartan cilexetil by area under curve and second order derivative methods in bulk and pharmaceutical dosage form

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ABSTRACT

Simple and precise UV spectrophotometric methods by area under curve [AUC] and second order derivative methods have been developed and validated for the estimation of candesartan cilexetil in bulk and its tablet formulation. The standard and sample solutions of candesartan cilexetil were prepared in methanol. Candesartan cilexetil was estimated in area under curve (AUC) method (A), the zero order spectrum was measured in between 250 nm to 260 nm and at 232.7 nm for the second order derivative UV-spectrophotometric method (B). In AUC method, Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 0.9999. Similarly Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 0.9997 for second order derivative method. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation were of 0.6182 % and 2.5548 % for the above two methods respectively. The proposed methods were successfully applied for the determination of Candesartan cilexetil in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

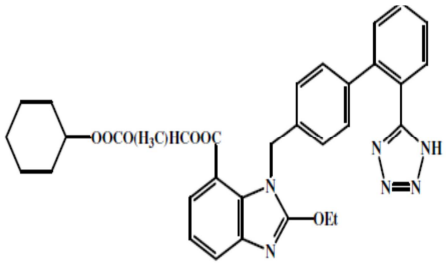
Keywords: Candesartan cilexetil, UV spectroscopy, Derivative spectroscopy, Area under curve method.

INTRODUCTION

Candesartan is an antihypertensive drug commercially available as cilexetil (cyclohexyl 1- hydroxy ethyl carbonate) ester form. It is a pro-drug and is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT1 subtype angiotensin II receptor antagonist. It is a non-peptide, chemically described as (±)-1-Hydroxyethyl 2-ethoxy-1- [*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester) Candesartan cilexetil is white to off-white crystalline powder with a molecular weight of 610.67.
It is practically insoluble in water and soluble in methanol. Candesartan cilexetil is a racemic mixture containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group.

Following oral administration, Candesartan cilexetil undergoes hydrolysis at the ester link to form the active drug, Candesartan. Literature survey reveals the Spectrophotometric [1-4], HPLC [5-11], UPLC [12] methods for the estimation of candesartan cilexetil. Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of candesartan cilexetil. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.

Structure of candesartan cilexetil



Material and Methods
Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.
A Shimadzu analytical balance with 0.01 mg was used.

CHEMICAL AND REAGENTS
Reference standard of candesartan cilexetil was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

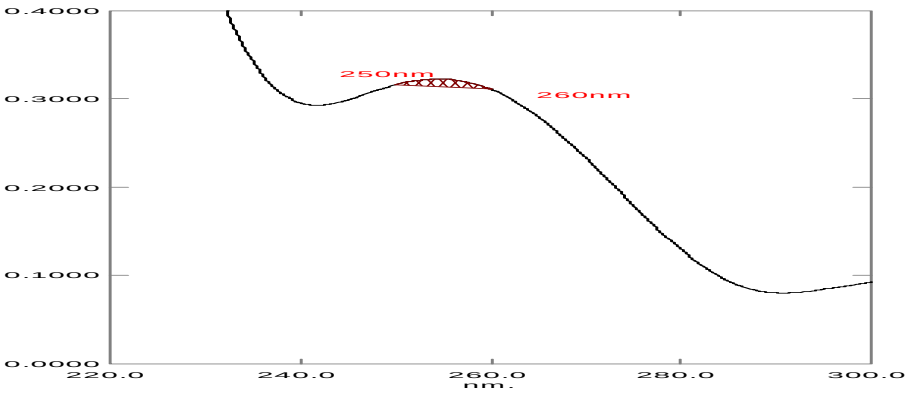
PREPARATION OF STANDARD SOLUTION
About 10 mg of standard candesartan cilexetil was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of methanol was added and sonicated for 15 minutes. The volume was adjusted up to the mark with absolute alcohol to give concentration as 100 µg /ml.

Estimation from tablets
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 8 mg of candesartan cilexetil was weighed and transferred in 100 ml of volumetric flask. A 30 ml of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 80 µg /ml. Such solution was used for analysis.

MATERIALS AND METHODS

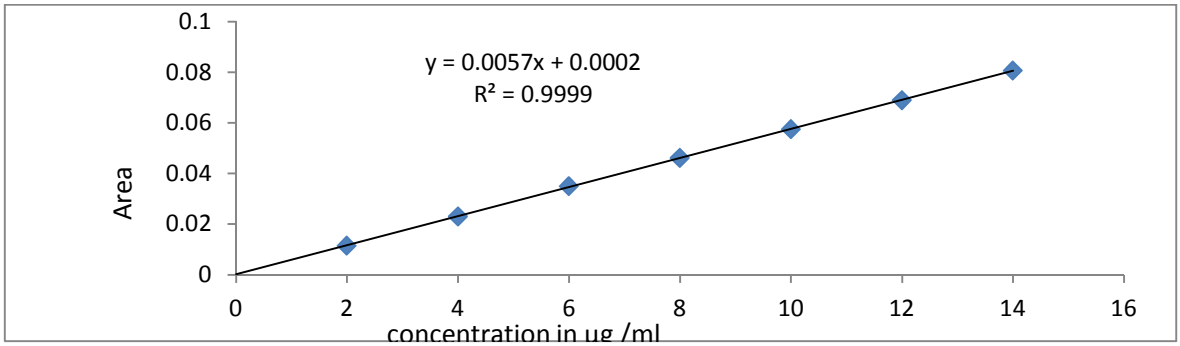
Method A: Area under curve (AUC) method
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of candesartan cilexetil was scanned in the spectrum mode from 350 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 250 nm to 260 nm (Fig. 1).

Fig. 1. Area under curve spectrum of candesartan cilexetil (10 µg/ml) showing area from 250 nm to 260 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of candesartan cilexetil was pipette out and volume was adjusted with methanol. Solutions were scanned between 350 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for candesartan cilexetil was plotted in the concentration range of 1 to 14 µg/ml (Fig. 2).

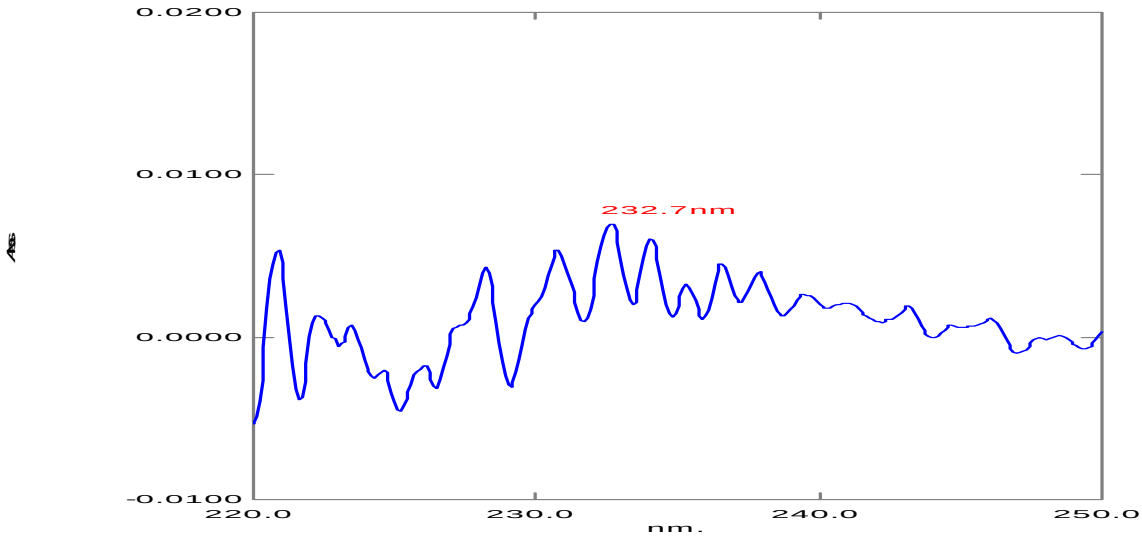
Fig. 2. Calibration curve for candesartan cilexetil by area under curve spectroscopy



Method B: second order derivative method

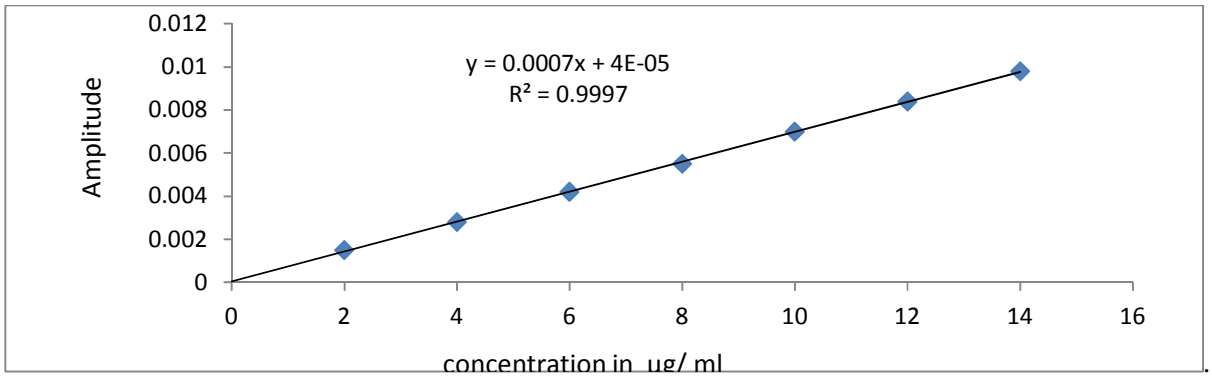
For the selection of analytical wavelength, 10 µg /ml solution of candesartan cilexetil was scanned in the spectrum mode from 350 nm to 200 nm by using methanol as blank. The second order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 232.7 nm (Fig. 3).

Fig. 3. Second order derivative spectrum of candesartan cilexetil (10 µg/ml) showing absorbance at 232.7 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of candesartan cilexetil was pipette out and volume was adjusted with methanol as solvent. Solutions were scanned between 350 nm to 200 nm in spectrum mode. The second order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at 232.7 nm by using methanol as blank. The calibration curve was prepared in the concentration range of 1 to 14 µg/ml. (Fig. 4)

Fig. 4. Calibration curve for candesartan cilexetil at 232.7 nm by second order derivative Spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	Second order derivative method	Area under curve (AUC) method
Detection Wavelength (nm)	232.7	250-260
Beer Law Limits (µg/ml)	1-14	1-14
Correlation coefficient(r^2)	0.9997	0.9999
Regression equation (y=b+ac)		
Slope (a)	0.0007	0.0057
Intercept (b)	0.00004	0.0002

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of candesartan cilexetil for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.9855	99.2753	0.02311	1.164
2	2	4.002899	100.072	0.02559	0.6395
2	4	6.084058	101.400	0.02995	0.4923
2	6	8.023188	100.289	0.0142	0.1769
				Mean =0.02331	Mean =0.6182

Table 3: Results of recovery of candesartan cilexetil for second order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery (%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.000	100.00	0.09035	5.125
2	2	3.9285	98.2142	0.07824	3.364
2	4	5.9523	99.2063	0.11166	1.694
2	6	8.0238	100.2976	0.1404	1.613
				Mean =0.1064	Mean =2.5548

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard

deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	Weight of candesartan cilexetil taken in mg	Weight of candesartan cilexetil found in mg	Weight of candesartan cilexetil found in mg
		Area under curve method	second order derivative method
1	8	8.034	8.142
2	8	8.017	7.857
3	8	8.034	8.000
4	8	8.000	8.142
5	8	8.034	7.857
6	8	8.017	8.142
		Mean= 8.022	Mean =8.023

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 8 mg of candesartan cilexetil was transferred to 100 ml of volumetric flask. A 30 ml of methanol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with methanol to give concentration as 80 µg /ml. Such solution was used for analysis.

For area under curve method

Solution was scanned between 350 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 250 nm to 260 nm by using methanol as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 250 nm to 260 nm (method A). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of candesartan cilexetil was estimated by comparison with standard at 250 nm to 260 nm, table 5.

For second order derivative method

Solution was scanned between 350 nm to 200 nm in spectrum mode. The second order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 232.7 nm by using methanol as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell 232.7 nm for second order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of candesartan cilexetil was estimated by comparison with standard at 232.7 nm for second order derivative, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	Area under curve (AUC) method	Second order derivative method
(A)	Intra-day precision (n=3) Amount found ±% RSD	100.28% 0.1769	99.206 % 1.694
(B)	Inter-day precision (n=3) Amount found ±% RSD	99.275% 0.2765	98.227% 1.613
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.1823	1.815

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	Area under curve (AUC) method	Second order derivative method
Limit of Detection (µg/ml)	0.0764	0.2981
Limit of Quantification (µg/ml)	0.231	0.9034

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of candesartan cilexetil sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of candesartan cilexetil was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The area under curve and second order derivative UV-spectroscopic methods are useful for routine analysis of candesartan cilexetil in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. For candesartan cilexetil, in the AUC spectrum method areas were measured between 250 nm to 260 nm (method A) and for second order derivative method absorbance maxima at 232.7 nm (method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 14 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of candesartan cilexetil in pharmaceutical formulation.

Acknowledgment

Authors express sincere thanks to the Principal, Dr. Tushar M. Desai of D. G. Ruparel college.

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