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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CANDESARTAN BY DERIVATIVE SPECTROSCOPY (FIRST ORDER AND SECOND ORDER)

Gunjan Kalyani*, Vishal S. Deshmukh, Yogesh Vaishanv and Pranita Kashyap

Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

ABSTRACT

Keywords:

Candesartan,
UV method development,
UV derivative spectroscopy,
First order,
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Correspondence to Author:

Gunjan Kalyani

E - 82 Block 7, ACC Colony Jamul, Dist –
Durg- 490024, Chhattisgarh, India

Candesartan cilexetil, a prodrug is a racemic mixture containing one chiral center at the cyclohexyloxy-carbonyloxy- ethyl ester group. It is soluble in dimethyl formamide, acetone, methanol, 0.1 N sodium hydroxide solution and insoluble in water. Objective of the present study is to develop a simple, sensitive, accurate, precise and rapid derivative spectrophotometric method for the estimation of candesartan in pure form. For the estimation of candesartan, solvent system employed was absolute methanol and wavelength of detection (λ_{det}) was 268.8 nm for first order derivative spectroscopy. The linearity was obtained in the range 10 – 20 μ g/ml. The limit of detection is 0.5 μ g/ml and limit of quantification was found to be 1.63 μ g/ml. For the estimation of candesartan, solvent system employed was absolute methanol and wavelength of detection (λ_{det}) was 284.3 nm for second order derivative spectroscopy. The linearity was obtained in the range 40 – 160 μ g/ml. The limit of detection is 6.7 μ g/ml and limit of quantification was found to be 20.4 μ g/ml. Obtained results showed that there is minimum intra day and inter day variation. Both the developed methods were validated and recovery studies were also carried out. Sample recovery using the above methods was in good agreement with their respective labeled claims, thus suggesting the validity of the method and non-interference of formulation excipients in the estimation. First and second order derivative spectroscopy methods are simple, rapid and reproducible and further it can be used for the analysis.

INTRODUCTION: Candesartan cilexetil (prodrug is a racemic mixture containing one chiral center at the cyclohexyloxy-carbonyloxy- ethyl ester group. Angiotensin II receptor blockers (ARBs) are a new class of therapeutic agents for hypertension. The ARBs have a more direct mechanism of action than other drugs affecting the angiotensin converting enzyme inhibitors¹.

Candesartan is a potent, highly selective ARB that is devoid of agonist activity. It is administered orally as

Candesartan cilexetil, which is rapidly and completely hydrolyzed to Candesartan, the active moiety, during absorption from the gastrointestinal tract.

Candesartan cilexetil is white to off white powder. It is soluble in dimethyl formamide, acetone, methanol, 0.1 N sodium hydroxide solution and insoluble in water².

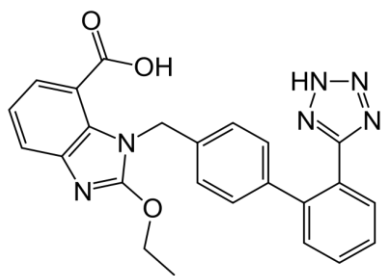


FIG. 1: STRUCTURE OF CANDESARTAN CILEXETIL

Mechanism of action¹: Candesartan cilexetil is a prodrug that has little pharmacological activity until it is hydrolyzed to candesartan during absorption. Candesartan competes with angiotensin II for binding at the AT1 receptor subtype. The drug blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland⁹. Candesartan, ARB, is used alone or with other antihypertensive agents to treat hypertension. The action is different from ACE inhibitors, which block the conversion of angiotensin I to angiotensin II, meaning that the production of angiotensin II is not completely inhibited, as the hormone can be formed via other enzymes. Also, unlike ACE inhibitors, candesartan and other ARBs do not interfere with response to bradykinins and substance P, which allows for the absence of adverse effects that are present in ACE inhibitors (e.g., dry cough).

Literature review suggested several analytical methods that have been reported for the estimation of Candesartan in bulk or pharmaceutical formulations include High Performance Liquid Chromatography, and UV-Visible Spectrophotometry. Literature review also suggested that there is no First Order Derivative Spectroscopic method. The objective of the work was to develop simple, accurate, precise and economic First order derivative Spectroscopic method to estimate the candesartan in bulk. The method should be simple, accurate, precise, reproducible and statistically valid.

UV spectrophotometry is generally preferred especially by small-scale industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis is based on measuring the absorption of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum

(190-380nm). The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to the photoelectric cell that transforms the radiant energy into electrical energy.

Thus, the objectives of project:

- I. To develop a simple, precise, accurate method, less time consuming & economical derivative spectroscopic method.
- II. Under derivative spectroscopy, the development of First and Second Order derivative Method.
- III. Validation of developed method using common parameters:
 - a) Linearity
 - b) Precision
 - c) Accuracy
 - d) Sensitivity
 - e) Limit of Detection (LOD)
 - f) Limit of Quantification (LOQ)

MATERIALS AND METHODS:

Year of experimentation: 2011

Site of experimentation: Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh.

Drug: The standard sample of CANDESARTAN was obtained as gift sample from Dr. Reddy's Laboratory Pvt. Ltd., Hyderabad, A.P., India. The candesartan tablets were procured from local market, brand name CANDESAR (8 mg), manufactured by RANBAXY Laboratories, India.

Instrument specifications: UV Spectrophotometer, Shimadzu, model 1800.

Chemicals and reagents used: Methanol obtained from local market, manufactured by Merck Pharmaceuticals.

For First order Derivative Spectroscopy

Preparation of stock solution: The stock solution of candesartan is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flask with continuous shaking. 1 ml of sample was withdrawn and diluted to 100 ml methanol to get 10 µg/ml of solution. The solution was then scanned in UV range between 200-400nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as methanol.

Wavelength scanning and determination of Absorption Maximum: From the stock solution of Candesartan, known concentration of 10µg/ml is prepared by suitable dilution with methanol. Wavelength scanned for the maximum absorption of drug solution using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against blank methanol. Convert the normal mode obtained spectra to first order derivative. The wavelength that shows the peak with a highest absorbance is considered as absorbance maximum of the drug. The result is presented in **fig. 2**.

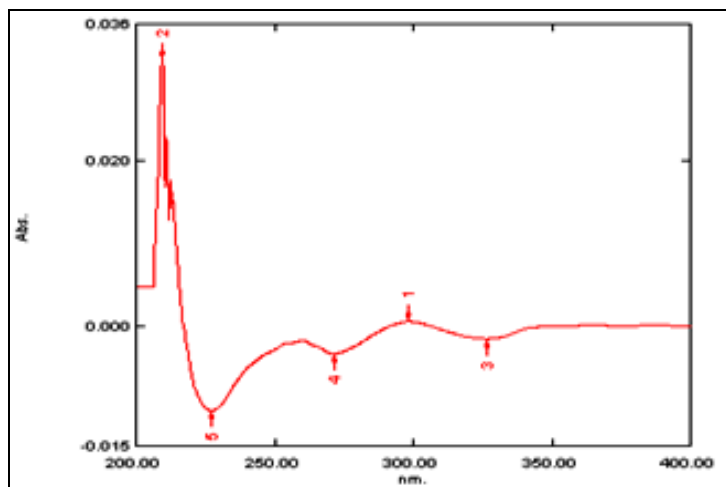


FIG. 2: WAVELENGTH SCANNING AND DETERMINATION OF ABSORPTION MAXIMUM

Linearity studies for Candesartan Analytical Method:

Stock solution was subsequently diluted with methanol to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, 10µg/ml, 12µg/ml, 14µg/ml, 16µg/ml, 18µg/ml, 20µg/ml, 22µg/ml, 24µg/ml, 26µg/ml, 28µg/ml, 30µg/ml. Convert the normal mode obtained spectra to first order derivative. The results are tabulated and the linearity curve was constructed by plotting concentration Vs. D^1 value. The result is presented in **table 1 and fig. 3**.

TABLE 1: LINEARITY OF CANDESARTAN FOR FIRST ORDER

CONCENTRATION (µg/ml)	D^1 VALUE AT DETECTION WAVELENGTH (268.8 nm)
2	0.003
4	0.004
6	0.006
8	0.007
10	0.008
12	0.010
14	0.012
16	0.014
18	0.016
20	0.018

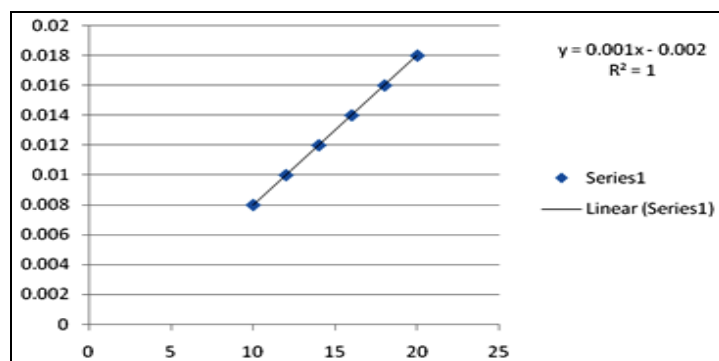


FIG. 3: STANDARD GRAPH OF CANDESARTAN

X – Axis: Concentration; Y- Axis: D^1 value

Precision: The precision of method was ascertained; the percent relative standard deviation were calculated and presented.

Inter day and intraday studies for Candesartan

Analytical Method: The prepared stock solution was subsequently diluted to get 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml, and 10µg/ml. The resulting solutions absorbance was measured at detection wavelength of 268.8 nm using double beam UV spectrophotometer against blank of methanol. The findings was made at different time intervals in a day times in a day and performed continuously for six days. Convert the normal mode obtained spectra to first order derivative. The results obtained were tabulated and studied for inter day and intraday variation (**table 2(a) and (b)**).

Accuracy studies: The accuracy/recovery studies were carried out by adding a known amount of drug from the pre-analyzed tablet powder and percentage recoveries were calculated. Convert the normal mode obtained spectra to first order derivative. The reproducibility of estimation was determined by performing the tablet drug content of different samples. The results of accuracy studies were expressed in %. The result is presented in **table 3**.

TABLE 2 (A) INTRADAY PRECISION FOR FIRST ORDER

S.NO	CONC. ($\mu\text{g/ml}$)	D ¹ VALUE AT DETECTION WAVELENGTH (268.8 nm)				
		TIME (MINS)	I	II	III	MEAN
1	18	1:30 PM	0.019	0.020	0.019	0.0196
2	18	1:45 PM	0.021	0.022	0.022	0.0213
3	18	2:00 PM	0.022	0.022	0.022	0.022
4	18	2:30 PM	0.021	0.021	0.021	0.021
5	18	3:30 PM	0.021	0.021	0.021	0.021
6	18	4:30 PM	0.021	0.021	0.021	0.021
MEAN =						0.021
SD =						0.000191
% RSD =						0.90

TABLE 2 (B) INTER DAY PRECISION FOR FIRST ORDER

S. NO	CONC. ($\mu\text{g/ml}$)	DAYS & DATE	D ¹ at 268.8 nm
1	18	28.11.2011	0.019
2	18	29.11.2011	0.021
3	18	30.11.2011	0.022
4	18	1.12.2011	0.021
5	18	2.12.2011	0.021
6	18	3.12.2011	0.021
MEAN =			0.021
SD =			0.00028
% RSD =			1.33

TABLE 3 ACCURACY STUDIES FOR FIRST ORDER

TEST ($\mu\text{g/ml}$)	STANDARD ($\mu\text{g/ml}$)	D ¹ VALUE AT 268.8 nm	CONC. ($\mu\text{g/ml}$)	AMOUNT OF TEST RECOVERED ($\mu\text{g/ml}$)	% RECOVERY
5	10	0.013	15	5	100
10	10	0.018	20	10	100
15	10	0.023	25	15	100

For Second order derivative spectroscopy

Preparation of Stock Solution: The stock solution of candesartan is prepared by dissolving 100 mg of drug in 100 ml methanol in volumetric flask with continuous shaking. 4 ml of sample was withdrawn and diluted to 100 ml methanol to get 40 $\mu\text{g/ml}$ of solution. The solution was then scanned in UV range between 200-400 nm UV-VIS Spectrophotometer, Shimadzu, Japan to determine the absorption maxima of the drug against blank as methanol.

Wavelength scanning and determination of Absorption Maximum: From the stock solution of Candesartan, known concentration of 40 $\mu\text{g/ml}$ is prepared by suitable dilution with methanol. Wavelength scanned for the maximum absorption of drug solution using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against blank methanol. Convert the normal mode obtained spectra to second order derivative. The wavelength that shows the peak with a highest absorbance is considered as absorbance maximum of the drug. The result is presented in **fig. 4**.

Linearity studies for Candesartan Analytical Method: Stock solution was subsequently diluted with methanol to get 20 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$, 60 $\mu\text{g/ml}$, 80 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 120 $\mu\text{g/ml}$, 140 $\mu\text{g/ml}$, 160 $\mu\text{g/ml}$, 180 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, 220 $\mu\text{g/ml}$, 240 $\mu\text{g/ml}$. Convert the normal mode obtained spectra to second order derivative. The results are tabulated and the linearity curve was

constructed by plotting concentration Vs. D² value. The result is presented in **table 4** and **fig. 5**.

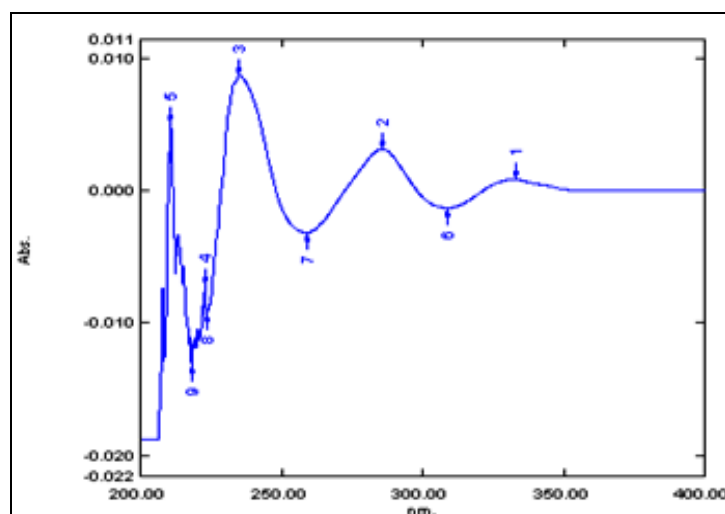


FIG. 4: WAVELENGTH SCANNING AND DETERMINATION OF ABSORPTION MAXIMUM

TABLE 4: LINEARITY OF CANDESARTAN FOR SECOND ORDER

CONCENTRATION ($\mu\text{g/ml}$)	D ² VALUE AT DETECTION WAVELENGTH (284.3 nm)
40	0.003
60	0.004
80	0.005
100	0.007
120	0.008
140	0.009
160	0.010

Precision: The precision of method was ascertained; the percent relative standard deviation were calculated and presented.

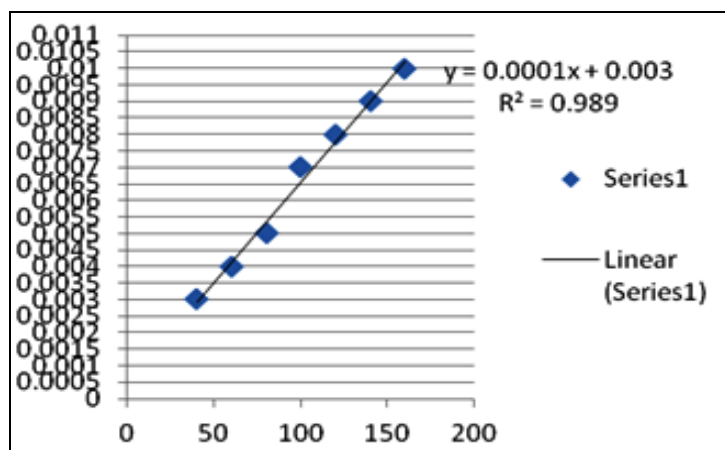


FIG. 5: STANDARD GRAPH OF CANDESARTAN

Regression equation; $y = 0.0001x + 0.003$; $R^2 = 0.989$; X - Axis: Concentration; Y- Axis: D^2 value

Inter day and intraday studies for Candesartan

Analytical Method: The prepared stock solution was subsequently diluted to get 120 µg/ml. The resulting solution absorbance was measured at detection wavelength of 284.3 nm using double beam UV spectrophotometer against blank of methanol. The findings was made at different time intervals in day times in a day and performed continuously for six days. Convert the normal mode obtained spectra to second order derivative. The results obtained were tabulated and studied for inter day and intraday variation. The results are tabulated in **table 5(a) and 5(b)**.

TABLE 5 (A) INTRADAY PRECISION FOR SECOND ORDER

S. NO	CONC.	D ² VALUE AT DETECTION WAVELENGTH
1	140	0.008
2	140	0.008
3	140	0.008
4	140	0.008
5	140	0.008
6	140	0.009
MEAN =		0.008
SD =		0.000135
% RSD =		1.68

TABLE 6: ACCURACY STUDIES FOR SECOND ORDER

TEST (µg/ml)	STANDARD (µg/ml)	D ² VALUE AT 284.3 nm	CONC. (µg/ml)	AMOUNT OF TEST RECOVERED (µg/ml)	% RECOVERY
5	60	0.004	65	5	100
10	60	0.005	70	10	100
15	60	0.006	75	15	100

RESULTS AND DISCUSSION: Candesartan Cilexetil is a novel, potent, highly selective non peptide angiotensin II type 1 (AT1) receptor blocker which is administered orally as Candesartan cilexetil, which is rapidly and completely hydrolyzed to Candesartan, the active moiety, during absorption from the gastrointestinal tract. The physico-chemical characteristic study of Candesartan like melting point is 165°C nearer to the literature value 168°C (www.drugbank.com. 2011). In previous literature Ganesh akula et.al developed the RP - HPLC method for determination of Candesartan in bulk sample and tablet dosage form. Subba Rao et al., developed and validated stability-indicating LC method for Candesartan and characterization of its degradation

(µg/ml)		(284.3 nm)				
		TIME (MINS)	I	II	III	MEAN
1	140	1:30 PM	0.008	0.008	0.008	0.008
2	140	1:45 PM	0.008	0.008	0.009	0.00833
3	140	2:00 PM	0.008	0.009	0.008	0.00833
4	140	2:30 PM	0.009	0.008	0.008	0.00833
5	140	3:30 PM	0.009	0.009	0.008	0.00866
6	140	4:30 PM	0.008	0.009	0.009	0.00866
MEAN =						0.008
SD =						0.00014
% RSD =						1.75

TABLE 5 (B) INTER DAY PRECISION FOR SECOND ORDER

S. NO	CONC. (µg/ml)	DAYS & DATE	D ² at 268.8 nm
1	140	8.2.2012	0.008
2	140	9.2.2012	0.008
3	140	10.2.2012	0.008
4	140	11.2.2012	0.008
5	140	12.2.2012	0.008
6	140	13.2.2012	0.009
MEAN =			0.008
SD =			0.000135
% RSD =			1.68

Accuracy studies: The accuracy/recovery studies were carried out by adding a known amount of drug from the pre-analyzed tablet powder and percentage recoveries were calculated. Convert the normal mode obtained spectra to second order derivative. The reproducibility of estimation was determined by performing the tablet drug content of different samples. The results of accuracy studies were expressed in %. The result is presented in **table 6**.

products. The literature survey ascertains that HPLC analytical method is developed for Candesartan, which is cost effective.

In our laboratory, we developed first order and second order derivative spectroscopic method for the analysis of Candesartan. The known concentration of Candesartan is prepared and scanned for absorption maximum. The detection wavelength according to spectra is 268.8 nm for first order and 284.3 nm for second order. Different measured D^1 values at detection wavelength 268.8 nm is plotted as the curve as D^1 value versus concentration. Candesartan obeys the beers law in the concentration range 10 to 20

µg/ml. Linearity study indicates the curve is linear in the range of 2 to 10 µg/ml. The linear regression equation is $Y = 0.001 * X - 0.002$ with correlation coefficient (R^2) = 1.

Different measured D^2 values at detection wavelength 284.3 nm is plotted as the curve as D^2 value vs. concentration. Candesartan obeys the beer's law in the concentration range 40 to 160 µg/ml. The linear regression equation is $y = 0.0001x + 0.003$ with correlation coefficient (R^2) = 0.989.

The developed method is validated for repeatability, reproducible and the accuracy and precision. In the inter day and intraday study of standard graph, the %SD is less than 2% indicating the developed method is reproducible. The different levels of standard concentration solutions are measured for D^1 and D^2 value and actual concentration is calculated. The results showed that the amount recovered is 100% indicating the first order derivative spectroscopic method is accurate and precise.

CONCLUSION: The developed analytical method for Candesartan by using first order derivative spectroscopy is found to simple, rapid and selective and the amount of drug recovered will be same as the label claimed and precise. It can be conveniently employed for the routine analysis and quantification of Candesartan.

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