Research Article

Analytical Method Development and Validation for the Estimation of Candesartan by Derivative Spectroscopy (Fourth Order).

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ABSTRACT

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 304.3 nm for fourth order derivative spectroscopy. The linearity was obtained in the range 320 – 440 µg/ml. The limit of detection is 6.7 µg/ml and limit of quantification was fund to be 20.4 µg/ml. Obtained results showed that there is minimum intra day and inter day variation. The developed method was validated and recovery studies were also carried out. Sample recovery using the above method 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. Third order derivative spectroscopy method is simple, rapid and reproducible and further it can be used for the analysis.

Keywords: Candesartan, UV method development, UV derivative spectroscopy, Fourth order, Validation studies.

INTRODUCTION

Candesartan cilexetil (prodrug is a racemic mixture containing one chiral center at the cyclohexyloxy-carbonyloxy-ethyl ester group (Fig. 1). 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 inhibitors[1]. 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 cilexitil is white to off white powder. It is soluble in dimethyl formamide, acetone, methanol, 0.1 N sodium hydroxide solution and insoluble in water[1].

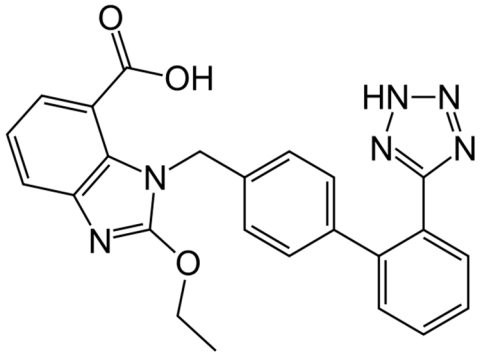

Figure 1: Structure of candesartan cilexetil

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 fourth order Derivative Spectroscopic method. The objective of the work was to develop simple, accurate, precise and economic fourth 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 Fourth 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

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.

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. 3.2 ml of sample was withdrawn and diluted to 100 ml methanol to get 320 µg/ml of solution. The solution was than 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 320µg/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 fourth 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 table 1.

Linearity studies for Candesartan analytical method

Stock solution was subsequently diluted with methanol to get 320 µg/ml, 340 µg/ml, 360 µg/ml, 380 µg/ml, 400 µg/ml, 420 µg/ml, 440 µg/ml, Convert the normal mode obtained spectra to fourth order derivative. The results are tabulated and the linearity curve was constructed by plotting concentration Vs. D^4 value. The result is presented in table 1 and fig. 2.

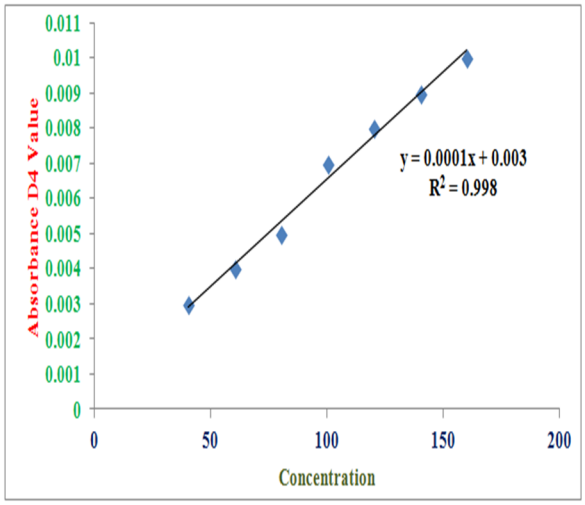

Figure 2: Standard graph of candesartan

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 400 µg/ml. The resulting solution absorbance was measured at detection wavelength of 304.3 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 fourth order derivative. The results obtained were tabulated and studied for inter day and intraday variation. The results are tabulated in table 2 and 3.

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 fourth 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 4.

Assay studies

The assay studies were carried out with the help of candesartan marketed formulation (Candesar, 8 mg). The percentage purity was calculated. Convert the normal mode obtained spectra to fourth order derivative. The reproducibility of estimation was determined by performing the drug content of different samples. The results of assay studies were expressed in %. The result is presented in table 5.

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. Different measured D^4 values at detection wavelength 304.3 nm is plotted as the curve as D^4 value vs. concentration. Candesartan obeys the beer's law in the concentration range 320 to 440 µg/ml. The linear regression equation is y = 0.0001x + 0.003 with correlation coefficient (R^2) = 0.999. The developed method is validated for repeatability, reproducible and the accuracy and precision. In the inter day and intraday study of standard graph, the % RSD is less than 2% indicating the developed method is reproducible. The different levels of standard concentration solutions are measured for D^4 value and actual concentration is calculated. The results showed that the amount recovered is 100% indicating the third order derivative spectroscopic method is accurate and precise.

Table 1: Linearity of Candesartan for fourth order

S. No.	Concentration (µg/ml)	D^4 value at detection wavelength (304.3 nm)
1	320	0.003
2	340	0.004
3	360	0.005
4	380	0.007
5	400	0.008
6	420	0.009
7	440	0.010

Table 2: Intraday precision for fourth order

S. No	Concentration (µg/ml)	D^4 value at detection wavelength (304.3 nm)				
		Time	I	II	III	MEAN
1	400	1:30 PM	0.008	0.008	0.008	0.008
2	400	1:45 PM	0.008	0.008	0.009	0.00833
3	400	2:00 PM	0.008	0.009	0.008	0.00833
4	400	2:30 PM	0.009	0.008	0.008	0.00833
5	400	3:30 PM	0.009	0.009	0.008	0.00866
6	400	4:30 PM	0.008	0.009	0.009	0.00866
Mean =						**0.008**
SD =						**0.00014**
% RSD =						**1.75**

Table 3: Inter day precision for fourth order

S. No.	Concentration (µg/ml)	Days & Date	D^4 at 304.3 nm
1	400	18.6.2012	0.008
2	400	19.6.2012	0.008
3	400	20.6.2012	0.008
4	400	21.6.2012	0.008
5	400	22.6.2012	0.008
6	400	23.6.2012	0.009
Mean =			**0.008**
SD =			**0.000135**
% RSD =			**1.68**

Table 4: Accuracy studies for fourth order

S. No.	Test (µg/ml)	Standard (µg/ml)	D^4 value at 304.3 nm	Conc. (µg/ml)	Amount of test recovered (µg/ml)	% Recovery
1	5	340	0.004	345	5	100
2	10	340	0.005	350	10	100
3	15	340	0.006	355	15	100

Table 5: Depicting the assay study

S. No.	Conc. (µg/ml)	D^4 value at 304.3 nm	% Purity (w/w)
1	380	0.007	100
2	380	0.007	100
3	380	0.007	100

CONCLUSION

The developed analytical method for Candesartan by using fourth 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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