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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CANDESARTAN BY DERIVATIVE SPECTROSCOPY (FIRST 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 was to develop a simple, sensitive, accurate, precise and rapid derivative spectrophotometric bioanalytical 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 2 – 10 μg/ml with a regression coefficient of $R^2 = 1$. The limit of detection is 0.99 μg/ml and limit of quantification was found to be 3.1 μg/ml. Obtained results showed that there is minimum intraday 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. Thus, Bioanalytical method development by first order derivative spectroscopy method is 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 [1]. Angiotensin II receptor blockers (ARBs) are a new class of therapeutic agents for hypertension [2]. The ARBs have a more direct mechanism of action than other drugs affecting the angiotensin converting enzyme inhibitors [3].

Candesartan is a potent, highly selective ARB that is devoid of agonist activity. [4] 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 [5]. It is soluble in dimethyl formamide, acetone, methanol, 0.1 N sodium hydroxide solution and insoluble in water [6].

Literature review suggested several bioanalytical methods that have been reported for the estimation of Candesartan in bulk or pharmaceutical formulations include High Performance Liquid Chromatography. Literature review also suggested

that there is no bioanalytical derivative spectroscopic method. The objective of the work was to develop simple, accurate, precise and economic First order bioanalytical derivative spectroscopic method to estimate the candesartan in bulk. The method should be simple, accurate, precise, reproducible and statistically valid.

Candesartan SMEDDS - Formulation profile [2]

Each ml of Candesartan SMEDDS contains-

- Candesartan   –   24 mg

- Soyabean oil   –   0.28 ml

- Tween 80   –   0.24 ml

- Cremophor EL   –   0.24 ml

- L – 90   –   0.23 ml

Note:
SMEDDS has been used as the commercial preparation of candesartan for the method development by derivative spectroscopic technique. 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 bioanalytical derivative spectroscopic method.

II.   Under derivative spectroscopy, the development of First order bioanalytical 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 SMEDDS was used as commercial preparation for the estimation of candesartan.

Animal model:
Wistar Rats (200 – 250 g), for bioanalytical method development by first order derivative spectroscopy, were acquired from M/S Ghosh Enterprises, Kolkata.

Note: Permission has been obtained from IAEC for performing experiment on animal models, Form B, per rule 8(a).

Reg. no. 1188; Protocol No: SRIP/IAEC/2011-12/04 dated 13/02/2012.

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.

Preparation of plasma standard solutions:
A suitable amount of standard solution was spiked in 0.5 ml of blank plasma and then extracted with centrifugation method. The concentration of plasma standards at the respective standards at the respective points on the concentration graphs produced for plasma were 2, 4, 6, 8, 8, 10 μg/ml.

Extraction from plasma:

A suitable amount of standard solution of candesartan was added to 0.5 ml plasma sample. Methanol was used as the precipitating agent and was added to drug spiked plasma. The solutions were vortex mixed for 1minute and centrifuged at 5000 rpm for 15 mins using an Eltek, Model TC 650 D centrifugal device. The clear supernatant liquid was separated, the solutions were then filtered with 0.45 μ filter and the extracted candesartan was reconstituted with the solvent methanol. The appropriate dilutions were prepared in the above stated manner. And transfer it to quartz cell for analysis.

Wavelength scanning and determination of absorption maximum:

With the stock solution of Candesartan, known concentration of 10μg/ml plasma standard 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. 1**.
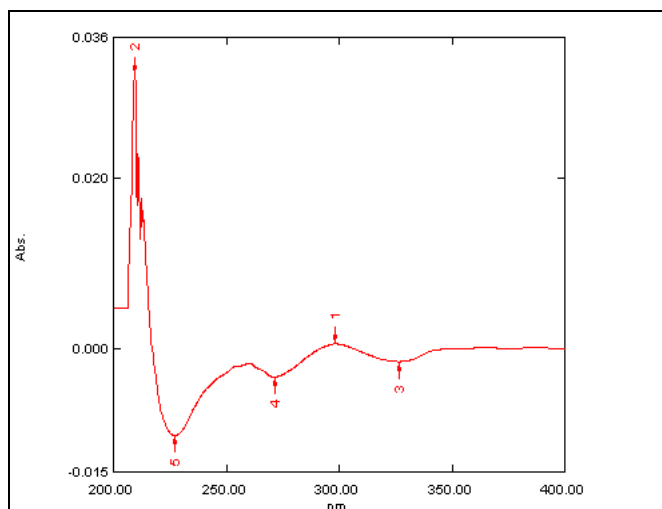

FIG. 1: WAVELENGTH SCANNING AND DETERMINATION OF ABSORPTION MAXIMUM

Method validation:

The validation of method was carried out by establishing linearity, limit of detection (LOD) and limit of quantification (LOQ) and within- and between- day precision and accuracy.

Linearity studies for Candesartan analytical method:

Plasma standard solutions were prepared of concentrations 2μg/ml, 4μg/ml, 6μg/ml, 8μg/ml, 10μg/ml, 12μg/ml 14μg/ml, 16μg/ml, 18μg/ml 20μg/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. 2** and **3** presents the linearity obtained from the derivative spectra.

TABLE 1: LINEARITY OF CANDESARTAN FOR FIRST ORDER

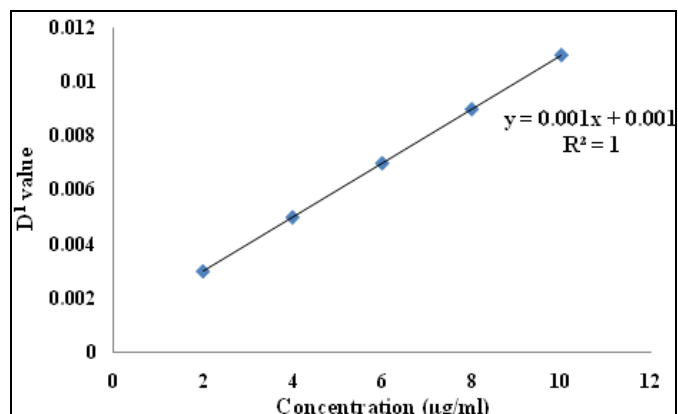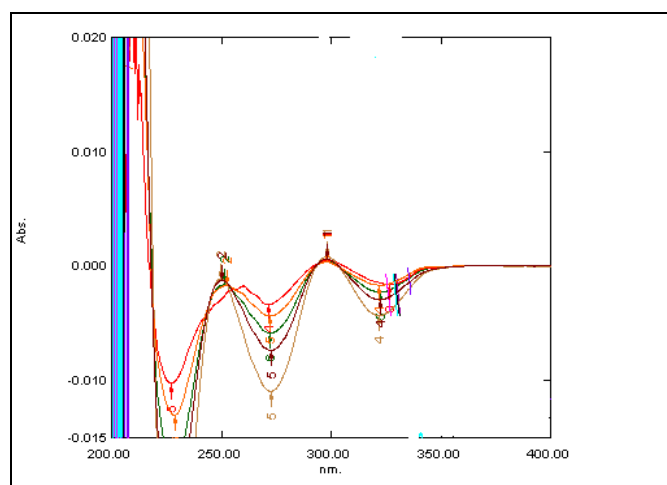| S.no.	Concentration (μg/ml)	D^1 value at detection wavelength (268.8 nm)
1	2	0.003
2	4	0.005
3	6	0.007
4	8	0.009
5	10	0.011

FIG. 2: STANDARD GRAPH OF CANDESARTAN

FIG. 3: DEPICTING THE LINEARITY FOR THE CONCENTRATION RANGE (2 – 10 μG/ML)

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. The results are presented in **Table 2 (a)** and **2 (b).**

TABLE 2 (a): INTRADAY PRECISION FOR FIRST ORDER

S. No.	Concentration (µg/ml)	Time	I	II	III	Mean
1	8	1:30 PM	0.009	0.009	0.009	0.009
2	8	1:45 PM	0.009	0.008	0.009	0.00866
3	8	2:00 PM	0.008	0.009	0.009	0.00866
4	8	2:30 PM	0.009	0.009	0.008	0.00866
5	8	3:30 PM	0.009	0.009	0.008	0.00866
6	8	4:30 PM	0.009	0.009	0.009	0.009
Mean =						0.009
SD =						0.000175
% RSD =						1.94

Header: D^1 value at 268.8 nm

TABLE 2 (b): INTER DAY PRECISION FOR FIRST ORDER

S.no	Concentration (µg/ml)	Days & Date	I	II	III	Mean
1	8	2.04.2012	0.008	0.008	0.008	0.008
2	8	3.04.2012	0.008	0.008	0.008	0.008
3	8	4.04.2012	0.009	0.009	0.009	0.009
4	8	5.04.2012	0.009	0.009	0.009	0.009
5	8	6.04.2012	0.009	0.009	0.009	0.009
6	8	7.04.2012	0.009	0.009	0.009	0.009
Mean =						0.009
SD =						0.000140
% RSD =						1.55

Header: D^1 value at 268.8 nm

Accuracy studies:
The accuracy/recovery studies were carried out with the commercial preparation of candesartan SMEDDS and percentage recoveries was calculated. Convert the normal mode obtained spectra to first order derivative. The reproducibility of estimation was determined by performing the drug content of different samples. The results of accuracy studies were expressed in %. The result is presented in **Table 3.**

TABLE 3: DEPICTING THE ACCURACY STUDIES FOR FIRST ORDER

S.no.	Test (µg/ml)	Standard (µg/ml)	D^1 value at 268.8 nm	Conc. (µg/ml)	Amount of test recovered (µg/ml)	% Recovery
1	5	4	0.008	9	5	100
2	10	4	0.014	14	10	100
3	15	4	0.018	19	15	100

Assay studies:
The assay studies were carried out with the help of candesartan SMEDDS. The percentage purity was calculated. Convert the normal mode obtained spectra to first 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 4**.

TABLE 4: DEPICTING THE ASSAY STUDY

S.no.	Conc. (µg/ml)	D^1 value at 268.8 nm	Conc. of drug in test solution (µg/ml)	% Purity (w/w)
1	6	0.006	5.90	98.2
2	6	0.007	6	100
3	6	0.006	5.90	98.2

Stability of Plasma samples:
To determine the stability of candesartan in plasma at ambient and refrigeration temperature, four sets of low (4 µg/ml), medium (7 µg/ml) and high (9 µg/ml) concentrations of the spiked calibration standards in plasma were divided in to 18 test tubes. One set of spiked samples was assayed immediately and was considered as standard (100 %). Two of the sets were stored at a temperature of -4° C in a refrigerator for 24 hours and one week. Then one of the sets was stored at -20° C in a deep freeze for one week. The remaining set was allowed to stand at ambient temperature for 24 hours. Stability measurements then carried out. The results were evaluated and comparing these measurements with those of standards and expressed as percentage deviation. The results are summarized in **Table 3**. The plasma samples were found to be stable for over one week at -20° C (deep freeze) and 24 hours at room temperature and at -4° C (refrigerator). The stability of analyte stock solution was tested for over 7 days in the same assay. The results indicated stability of stock solution for over 7 days at both room temperature and refrigerator temperature. The results are presented in **Table 5**.

TABLE 5: DEPICTING THE STABILITY STUDY

S. no.	Concentration (µg/ml)	Room temperature Stability (%) 24 h	Freeze thaw stability (%) 24 h	Freeze thaw stability (%) 1 week	Deep freeze stability (%) 1 week
1	4	100	100	100	100
2	7	100	100	90	88
3	9	100	100	90	90

CONCLUSION: 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 inhibitors. Candesartan is a potent, highly selective ARB that is devoid of agonist activity. Due to this important pharmacological response development of sensitive and accurate methods for determination of candesartan is desired. In the present study a simple, accurate, precise and economic first order bioanalytical derivative spectroscopic method was developed to estimate the candesartan in bulk and pharmaceutical dosage forms. The developed bioanalytical method for candesartan by using first order derivative spectroscopy is found to simple, rapid and selective and the amount of drug recovered were same as the label claim and precise. It can be conveniently employed for the routine analysis and quantification of Candesartan.

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