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“Stability Indicating Method Development and Validation of Candesartan in Bulk and Pharmaceutical Dosage Form by Derivative Spectrophotometric Method (First Order)”.

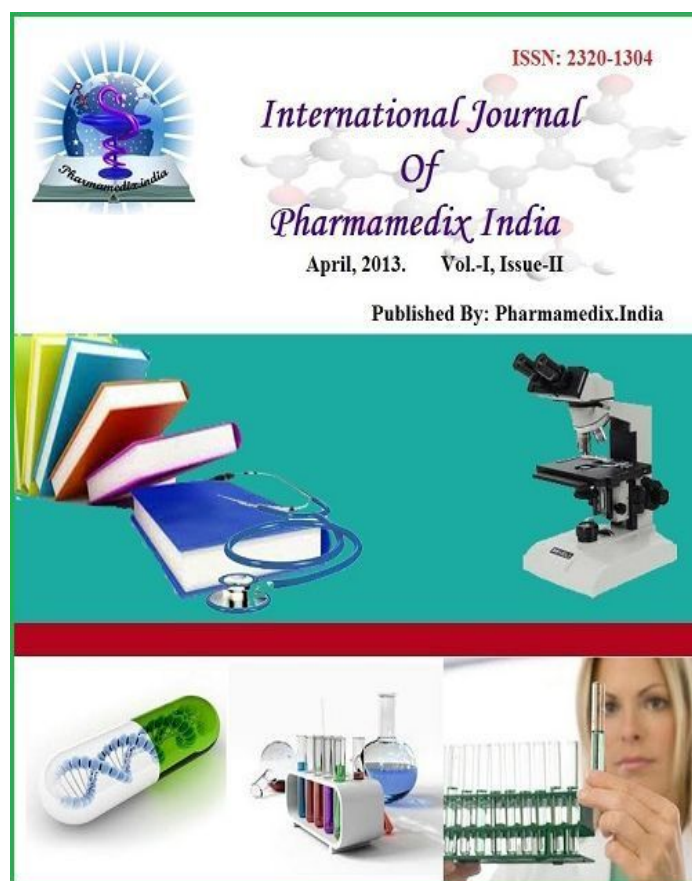
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Abstract:

A simple method for the estimation of candesartan in bulk and pharmaceutical dosage forms has been developed. Methanol was chosen as the solvent system. The λ_{det} was found to be 268.8 nm. The responses were linear in the range of 10 - 20 $\mu\text{g/ml}$. The regression equation of the calibration graph and correlation coefficient were found to be $y = 0.001x - 0.002$ and 1 respectively. The % RSD values for both intraday and interday precision were less than 2%. The recovery of the drug from the sample was found to be 100%. The proposed method was validated for precision, accuracy, intraday and interday assay. Commercial formulation used was candesartan SMEDDS, it was analyzed by the proposed method and the results were well within the claimed limits. Further stability studies of candesartan were carried out under acidic, alkaline, hydrolytic, oxidative and photolytic conditions as per SIAM (Stability Indicating Assay Methods) as described by International Conference on Harmonisation.

Keywords: Candesartan, UV method development, UV derivative spectroscopy, first order, Validation studies, Stability assay.

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 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 cilexetil 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].

Mechanism of action^[1]:

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).

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 and for the stability indicating assay by spectroscopic technique.

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 stability indicating assay method by Derivative Spectroscopy. The objective of the work was to develop SIAM by derivative spectroscopy 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.

Materials and method:

Chemicals & Reagents: Analytically pure candesartan was obtained as gift sample from Dr. Reddy's Laboratory Pvt. Ltd., Hyderabad, A.P., (India). A commercial formulation used was Candesartan SMEDDS. All chemicals and reagents used were of Analytical Grade, obtained from Merck.

Instruments: A SHIMADZU double beam UV/Visible recording spectrophotometer (Model: 1800) was employed for all spectrophotometric measurement using 10mm matched quartz cell and Borosil glass wares were used for the study. Calibrated electronic single pan balances Sartorius CP 225 D, Enertech Fast Clean Ultrasonicator were also used during the analysis.

Standard Stock Solution: The standard stock solution was prepared by dissolving 10 mg of drug in 10ml of methanol to get a concentration of 1000 $\mu\text{g/ml}$. It was appropriately diluted with methanol to get a concentration of 100 $\mu\text{g/ml}$ and was kept as the working stock solution.

Determination of detection wavelength: The standard solution of candesartan (10 $\mu\text{g/ml}$) was scanned in the wavelength region of 200-400 nm and the detection wavelength (λ_{det}) was found to be 268.8 nm. (Fig.2)

Preparation of calibration curve: The stock solution of candesartan was according diluted to obtain concentration in the range of 10 – 20 $\mu\text{g/ml}$. The D^1 were observed against methanol as blank and the calibration curve was plotted between concentration (x-axis) and D^1 value (y-axis).

Assay of tablet dosage form: SMEDDS has been used as the commercial preparation of candesartan for the method development and for the stability indicating assay by spectroscopic technique. The stock solution of the formulation Candesartan SMEDDS was prepared by following method (1mg/ml), each ml of SMEDDS contains candesartan, 24 mg. Therefore, 0.41 ml of candesartan SMEDDS was diluted with methanol to prepare a stock solution of formulation with a concentration (1 mg /ml). From this the working standard (100 $\mu\text{g/ml}$) was prepared. Sonicate for about 10 mins, filter and use.

Validation

1. Accuracy: The accuracy of the proposed method was tested by recovery studies by adding a known amount of pure drug to the pre-analyzed formulation of concentration.
2. Precision: The precision of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (18 $\mu\text{g/ml}$) within the Beer's range and

finding out the absorbance by the proposed method.

3. Intraday Assay: The intraday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (18 μ g/ml) within the Beer's range and finding out the absorbance by the proposed method at different time period of the same day.
4. Interday Assay: The interday assay of the proposed method was ascertained by actual determination of 6 replicates of a fixed concentration of the drug (18 μ g/ml) within the Beer's range and finding out the absorbance by the proposed method on different days.
5. Robustness: The robustness of the method was carried out by changing the solvent system from methanol to a mixture of methanol and water 90:10.
6. Ruggedness: In order to determine the ruggedness of the proposed method, the method was carried out by two analysts separately.

Stability studies

- **Acid degradation:** Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and few drops methanol were added to dissolve the drug. The volume was made up with freshly prepared 0.1N HCl. Then this solution was kept inside a water

bath maintained at a temperature of 70°C. Samples were withdrawn in regular interval of 1 hour and the absorbance were measured at the determined λ_{det} & recorded.

- **Alkali Degradation:** Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and minimum amount of methanol were added to dissolve the drug. The volume was made up freshly prepared 0.1N NaOH. This solution was kept inside a water bath maintained at a temperature of 70°C. The samples were withdrawn in regular interval of 1 hour and the absorbance were measured at the determined λ_{det} and recorded.
- **Oxidation Degradation:** Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and few drops of methanol were added to dissolve the drug and the volume was made up with freshly prepared 3% H₂O₂. The samples were withdrawn in regular interval of 6 hour for 36 hours and absorbance were measured at the determined λ_{det} and recorded.
- **Neutral conditions:** The stress medium selected was distilled water. Accurately weighed 10 mg of the drug was taken in a 10 ml volumetric flask and few drops of methanol were added to dissolve the drug and the volume

was made up with distilled water. This stock solution was used for the forced degradation studies, reflux it at 80⁰ C for 8 hrs, and cool it.

- **Photolytic conditions:** The stress medium selected was subjecting the drug to direct sunlight. Photolytic studies were done by exposing candesartan SMEDDS directly to sunlight for 48 h. Control samples were kept in dark for the same period.
- **Thermal conditions:** The stress medium selected was subjecting candesartan SMEDDS to 80⁰ C in a hot air oven. Thermal studies were done by subjecting the SMEDDS to 80⁰ C in a hot air oven.

Results and discussion:

The absorbance for the different concentrations of candesartan i.e. 10 – 20 µg/ml was recorded at 268.8 nm. The regression equation of the calibration curve was found to be $y = 0.001x - 0.002$. The calibration curve is shown in figure 3 and represented in table 1. The assay result of the commercial formulation of candesartan (SMEDDS) is shown in table 2. The method was found to be accurate and precise which was evident from its low % RSD values (Table 3). Similarly the % RSD for Intraday and Interday Assay were found to be 0.90 and 1.33 respectively (Table 4 & 5). The % RSD

for Robustness was found to be 1.20 and 1.39 for the proposed method by taking Methanol and Methanol and water (90:10) respectively while the % RSD for Ruggedness was found to be 1.86 and 1.29 respectively when performed by two analysts separately (Table 6). The results obtained in various stability studies are represented in table 7 and figure 4.

Conclusion:

The proposed method was found to be simple, precise, economic and rapid for the determination of candesartan from pure and its dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims as 100%. The % RSD for precision was found to be less than 2%. Thus the proposed method can be used as an alternative method to the reported ones for the routine analysis of the drug in bulk and pharmaceutical dosage forms and can also be used for dissolution or similar studies.

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Tables and Figures:

S.No.	Concentration (µg/ml)	Detection wavelength (268.8 nm)
1	10	0.008
2	12	0.010
3	14	0.012
4	16	0.014
5	18	0.016
6	20	0.018

Table 1: D¹ values for the calibration curve of candesartan.

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	10	0.013	15	5	100
2	10	10	0.018	20	10	100
3	15	10	0.023	25	15	100

Table 2: Assay result of the marketed formulation (Candesartan SMEDDS) by the proposed method.

S.No.	Concentration (µg/ml)	I	II	III	Mean
1	18	0.014	0.014	0.014	0.014
2	18	0.015	0.015	0.015	0.015
3	18	0.015	0.015	0.016	0.0156
4	18	0.015	0.015	0.016	0.0153
5	18	0.015	0.016	0.016	0.0156
6	18	0.015	0.016	0.016	0.0156
Mean =					0.01542
SD =					0.000268
% RSD =					1.73

Table 3: Precision of the proposed method.

S.No	Concentration (µg/ml)	Time	I	II	III	Mean
1	18	1:30 PM	0.019	0.020	0.019	0.01966
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 4: Results Showing Intraday Studies of the Proposed Method.

S.No	Concentration (µg/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 5: Results Showing Interday Studies of the Proposed Method.

S.NO	Concentration (µg/ml)	Methanol	Methanol: Water (90:10)
1	18	0.017	0.016
2	18	0.017	0.016
3	18	0.017	0.016
4	18	0.015	0.016
5	18	0.017	0.017
6	18	0.017	0.016
Mean =		0.016	0.017
SD =		0.000193	0.000237
% RSD =		1.20	1.39

Table 6: Results Showing Robustness of the Proposed Method.

S.No	Concentration (µg/ml)	Analyst X (Gunjan)	Analyst Y (Yogesh)
1	18	0.018	0.016
2	18	0.016	0.016
3	18	0.015	0.014
4	18	0.015	0.016
5	18	0.018	0.016
6	18	0.018	0.014
Mean =		0.018	0.015
SD =		0.000335	0.000194
% RSD =		1.86	1.29

Table 6: Results Showing Ruggedness of the Proposed Method.

S.NO.	Parameters	Obtained result	Acceptance of results
1	Acidic stress (0.1 N HCl)	10 % degradation	✓
2	Basic stress (0.1 N NaOH)	30 % degradation	✓
3	Oxidative stress (3 % H ₂ O ₂)	20 % degradation	✓

4	Thermal stress (Hot air oven; 80° C)	No degradation observed	✓
5	Aqueous hydrolysis (distilled water)	20 % degradation	✓
6	Photolytic stress (Sunlight)	No degradation observed	✓

Table 7: Stability Study Results of candesartan.

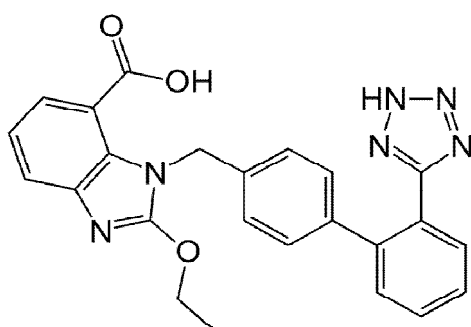


Fig. 1: Structure of Candesartan.

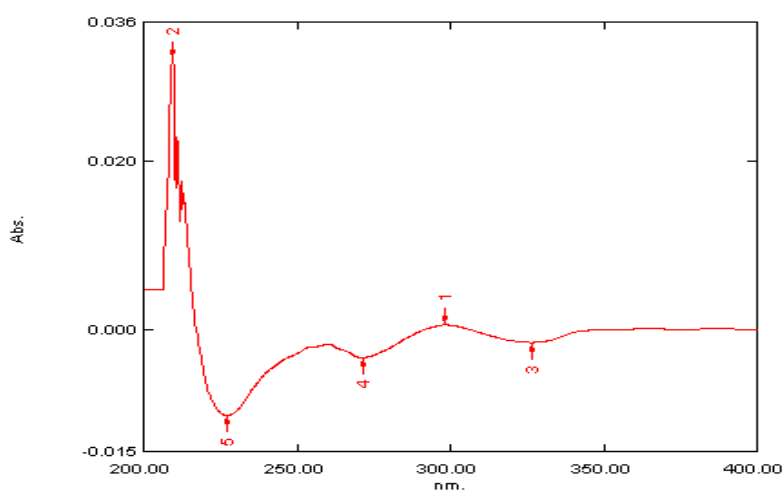


Fig. 2: A sample UV derivative spectrum of candesartan showing λ_{det} at 268.8 nm

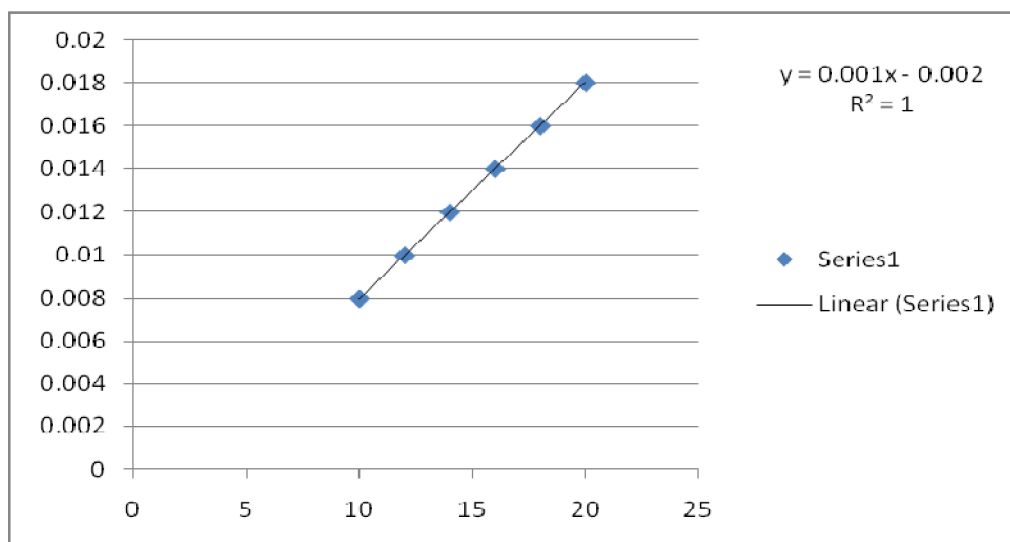


Fig 3: Calibration curve of Candesartan.

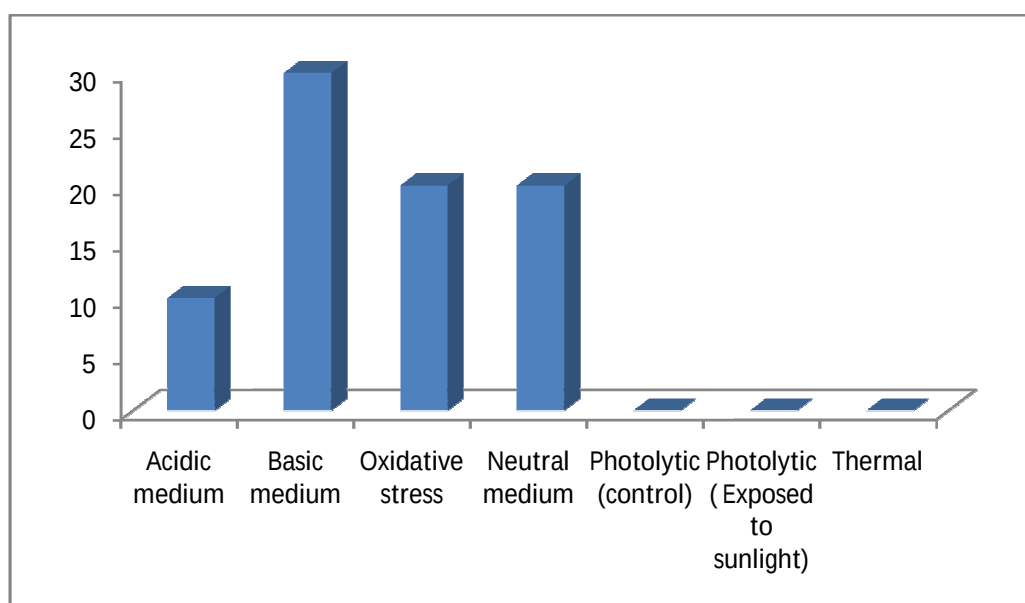


Fig. 4 depicting the comparative results of degradation studies

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