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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD AND RP-HPLC METHOD FOR ESTIMATION OF CANDESARTAN CILEXITIL IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

The main objective of the present work is to develop and validate a simple, novel, specific, accurate, and reliable method for the estimation of candesartan in bulk and pharmaceutical dosage forms using UV-visible spectroscopy and sensitive Reverse Phase High Performance Liquid Chromatographic method. The uv-visible spectrophotometric determination was performed with Elico double beam SL 210 UV-visible spectrophotometer having deuterium lamp at λmax 238 nm using water as a medium. Linearity was noted over a concentration range of 2-10µg/ml with a correlation coefficient of 0.99. HPLC analysis was performed with Agilent 1260 infinity DAD detector using Eclipse XDB C18 column with 5 µm particle size having dimensions 4.6 X 250 mm column, 1260 infinity quaternary pump using Ezchrome software at a flow rate of 1 ml/min and a run time pressure of 2140 psi. The mobile phase used was 0.01m mono basic potassium phosphate buffer: Acetonitrile (40:60) and the effluents were analyzed at 238 nm at a flow rate of 0.7 ml per minute. As per International Conference on Harmonization (ICH) guidelines, both the proposed methods were validated for various parameters like linearity, precision, accuracy, robustness, ruggedness, selectivity, detection, quantification limits and formulation analysis. Linearity for UV and HPLC method was noted over a concentration range of 25-200 µg/ml with a correlation coefficient of 0.99. The retention time was considered to be 6.7 min. The % RSD for interday and intraday precision studies and recovery analysis of both UV and HPLC methods was found to be less than 1% which is less than the official RSD limit (2%). Recovery analysis performed using marketed formulation Candelong was considered to be greater than 99% for both the methods. Validation of both the methods was performed according to the ICH guidelines. Hence it was evident that the proposed methods were novel, sensitive, precise and reliable for estimation of Candesartan in bulk and were successfully applied for estimation of pharmaceutical dosage forms.

INTRODUCTION:

Candesartan is an angiotensin- II receptor blocker (ARB), used to treat hypertension. It competes with angiotensin II binding at the AT1 receptor subtype by blocking the vasoconstrictor aldosterone-secreting effects[1]. Chemically, Candesartan cilexetil is 2-ethoxy-3-[21- (1H-tetrazol-5-yl)-4-yl methyl]-3H- benzoimidazole-4-carboxylic acid 1-cyclohexyloxy carbonyl oxy ethyl ester with a molecular formula of

C33 H34 N6 O6 and a molecular weight of 610[(2)].The chemical structure of the drug was shown in the **figure -1**.

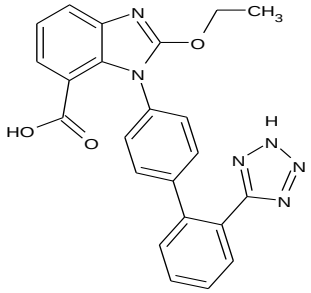

Fig 1: Chemical Structure of Candesartan Cilexetil.

Candesartan cilexetil gets metabolized completely by esterases to the active candesartan moiety in the intestinal wall during absorption**.** Based on the detailed review of the literature, there are several reported analytical methods for the estimation of candesartan in biological fluids or pharmaceutical formulations such as stability indicating LC method [(3)], HPLC method for simultaneous analysis of candesartan cilexetil and hydrochlorthiazide [(4,5)]. HPTLC densitometric method and Q-absorbance ratio method for analysis of candesartan cilexetil and hydrochlorothiazide were developed [(6,7)]. First derivative UV spectroscopic method for determination of candesartan cilexetil and dissolution testing were also prescribed [(8)]. The literature survey is revealed about its pharmacological action [(9,10)]. The main objective of the present work was to develop a simple, accurate, precise and economic UV and RP-HPLC methods to estimate the candesartan cilexitil in bulk and pharmaceutical dosage forms.

2. MATERIALS AND METHODS

Chemicals and reagents

The reference sample Candesartan cilexitil was secured from Natco pharma Ltd. Hyderabad. analytical grade. Acetonitrile (HPLC grade), Monobasic potassium phosphate and acetic acid Ethanol(HPLC grade) were acquired from Merck specialty's private ltd., Mumbai, India. All the reagents used were of analytical grade. Commercial tablet Candelong was procured from the local market.

Instrument specifications

The UV analysis was performed using Elico double beam SL 210 UV visible spectrophotometer having deuterium lamp associated with spectra treats software. The HPLC analysis was performed using Agilent 1260 infinity system (Ezchrome elite software) consisting of DAD VL detector adjusted to a wavelength of 304 nm. The instrument also consisted of Inertsil ODS-3V C-18 (250 x 4.6 mm, 5µm) and a 1260 infinity VL quaternary pump.

Spectrophotometric and chromatographic conditions

Spectrophotometric analysis was performed using triple distilled water as mobile phase. The detection was carried out at an absorption maximum (λmax) of 238 nm. Chromatographic separation was achieved using mobile phase 0.01m mono basic potassium phosphate buffer: AcetonItrile (40:60). A flow rate of 0.7 ml/min was maintained throughout the separation process with a Run time pressure of 600 bars. All the contents of the mobile phase were filtered through a 0.45 mm membrane filter and degassing was performed using ROHS sonicator to remove dissolved gasses if any. For each trial, 20 µl samples were injected manually, and a total run time of 10 min was maintained. The eluent was detected at 238 nm. Various systems suitability parameters were assessed as mentioned in **table 1.**

Preparation of stock solutions and sample solutions

a. UV-Visible method

Preparation of standard solution:

Candesartan cilexetil (100mg) was accurately weighed into a 100ml volumetric flask and dissolved in a small quantity of ethanol. The volume was made up with ethanol to get a concentration of 1000µg/ml. From this 10 ml was withdrawn and diluted to 100ml in pH 6.8 phosphate buffer to get a concentration of 100µg/ml.

Preparation of working solutions:

From the standard stock solution aliquots 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipetted out into 10ml volumetric flask. The volume was made up with phosphate buffer pH6.8 to get a final concentration of 2 µg/ml, 4 µg/ml,6 µg/ml,8 µg/ml and 10µg/ml respectively. The absorbance of each concentration was measured at 238nm.The UV-visible spectral scan was shown in **figure 2.**

b. HPLC METHOD

Standard preparation (200ppm)

Accurately weighed and transferred 20 mg of Candesartan into 100 mL volumetric flask to this add 70 mL of diluents and sonicated for 15 mins. Then made up to the volume with diluents.

Preparation of stock solution (2000ppm)

Accurately weighed and transferred 100 mg of Candesartan into 50 mL volumetric flask to this add 30 mL of diluents and sonicated for 15 mins. Then made up to the volume with diluents and used as a stock solution.

c. Validation of developed methods [11,12]

Linearity and range

Linearity is defined as the ability to obtain test results, which were directly proportional to the concentration of an analyte in the sample within a given range. Linearity data for the spectrophotometric method was obtained at an absorption maximum of 238 nm as shown in **figure 3** by using five concentrations in the range of 2–10µg/ml. A calibration curve was obtained by plotting absorbance against concentration by considering five observations as shown in **figure 4**. Linearity data for the chromatographic method was obtained by using five concentrations within the range of 25–200 µg/ml. A calibration curve was obtained plotting peak area against concentration by considering five observations as shown in **figure. 4**. Both the methods were studied using six replicates of each sample concentrations.

Precision

The degree of closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition was determined. The intra-day precision was performed by analyzing six replicate standard solutions on the same day, and inter-day precision was performed by analyzing a series of standard solutions for 3 consecutive days using the proposed U V and HPLC methods. The data obtained was presented in **table 5.**

Robustness

Robustness is defined as the measure of its capacity to remain unaffected by small but deliberate variation in method parameters, and it provides an indication of its reliability during normal range. Robustness of both the methods was studied using six replicates of the sample at a concentration level of 100µg/ml(for HPLC) and 10 µg/ml (for UV).

Ruggedness

Ruggedness was calculated by considering the same sample at different labs by different analysts.

Detection and quantification limits

Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected. Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively determined. The above parameters are calculated based on the standard deviation of the response and the slope. The standard deviation was calculated based upon the calibration curve. LOD = 3.3σ/SLOQ = 10σ/S

Selectivity and specificity

The ability to measure accurately and specifically the analyte of interest in the presence of other components like excipients in the tablet formulation were analyzed. The blank, standard, placebo, placebo along with analyte and test preparations were analyzed as per the method to identify interference of blank and placebo with candesartan peaks.

Table 1: System suitability parameters for HPLC

HPLC system	Azilent 1260 Infinity
Column	Inertsil ODS-3V C-18 (250 x 4.6 mm, 5 µm)
Mobile phase	0.01M mono basic potassium phosphate buffer: Acetonotrile(40:60) pH 6.0 adjusted with 10% Acetic Acid
Flow rate	0.7 mL/min
Injection volume	20µL
Detection	238 nm
Temperature	Ambient
Retention time	6.7 min
Run time	10min

Table 2: Summary of validation parameters obtained for proposed UV and HPLC methods

Validation parameters	UV	HPLC
Beer's law limit	2-10	25-200
Correlation coefficient (r2)	0.998	0.999
Regression equation	Y=0.0986x-0.0129	Y=307.72x+0.00
slope	0.0986	307.72
intercept	-0.0129	0
LOD	1.361119µg/ml	15.34251µg/ml
LOQ	4.124604µg/ml	46.49247µg/ml

Table 3: Linearity data table for proposed HPLC and UV methods (where n=6)

HPLC Linearity data		UV Linearity data	
Concentration(mcg/ml)	**Peak area±RSD**	**Concentration(mcg/ml)**	**Absorbance**
25	7562±2785.32	2	0.191± 0.005
50	15608±639.22	4	0.363±0.011
100	32165±2074.65	6	0.560±0.006
150	46324±3019.35	8	0.791±0.002
200	61321±1820.09	10	0.982±0.003
Correlation coefficient	0.999	Correlation coefficient	0.998
slope	307.72	Slope	0.0986
Intercept	0	Intercept	-0.0129

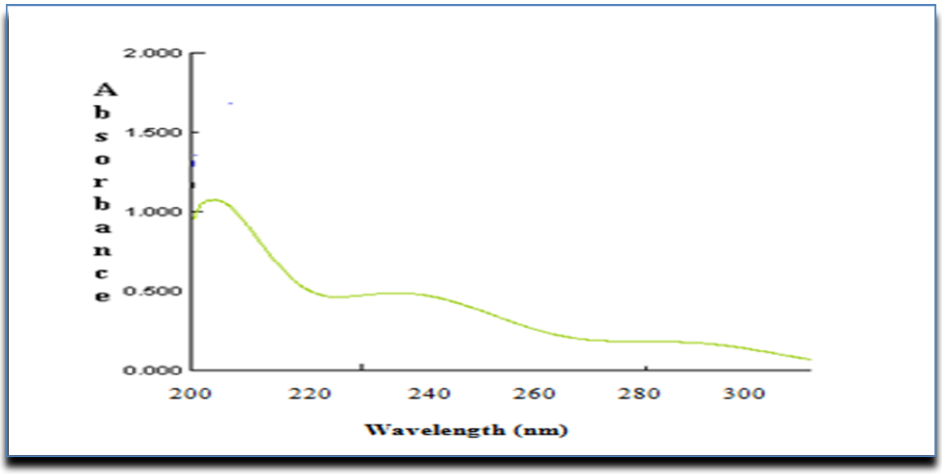

Fig. 2: UV-visible spectrum scans of Candesartan

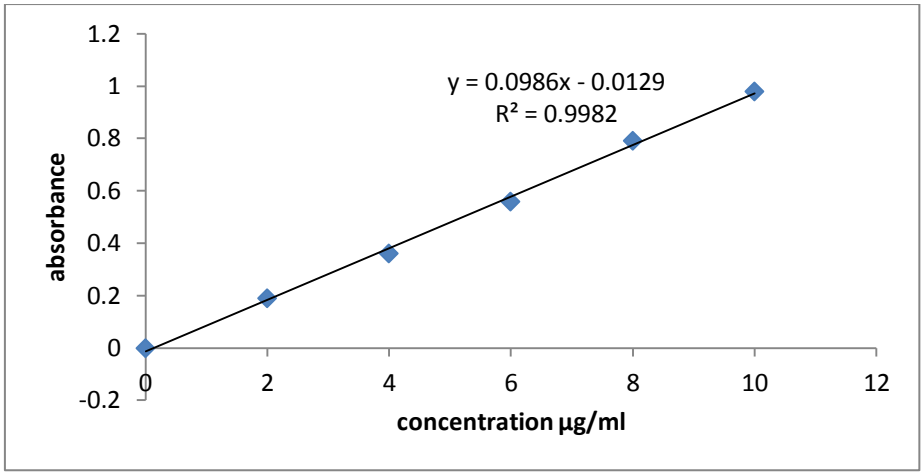

Fig. 3: Linearity curve of candesartan cilexitil in 6.8 pH phosphate buffer by UV – Visible Spectrophotometry

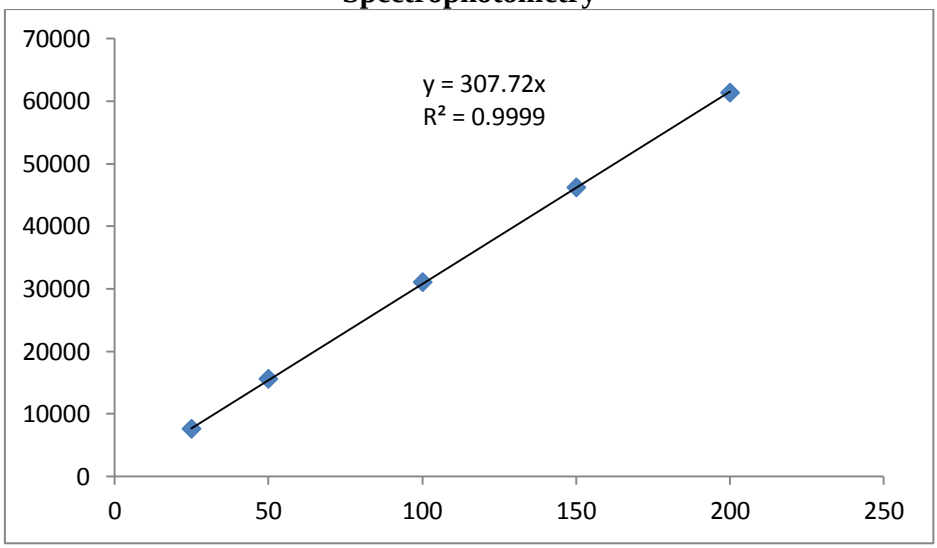

Fig 4: Linearity curve of candesartan cilexitil using HPLC method

Table 4: Precision analysis data of Candesartan for UV and HPLC

Parameter	UV	HPLC
Interday(%RSD)	0.021	0.39
Intraday(%RSD)	0.72	0.91

Table 5: Recovery analysis for Candesartan by the proposed UV and HPLC methods

Method	Std. solution	Conc. level	Amount added **(µg/ml)**	Total amount **(µg/ml)**	Amount founded **(µg/ml)**	Amount recovered **(µg/ml)**	% Recovery	% RSD
UV	10 µg / ml	50%	5	15	14.97	4.97	99.4	0.054
		100%	10	20	19.73	9.81	98.1	0.063
		150%	15	25	24.82	14.78	98.53	0.059
HPLC	100 µg / ml	50%	50	150	149.92	49.92	99.84	0.113
		100%	100	200	198.65	98.65	98.65	0.247
		150%	150	250	249.74	149.74	99.83	0.123

Table 6: Single factor ANOVA for recovery studies performed using UV method

Source of variation	ss	df	MS	F cal	p-value	F tab
Between groups	0.0384	1	0.0384	0.001559	0.970397	7.708647
Within groups	98.5294	4	24.63235			

Table 7: Single factor ANOVA for recovery studies performed using HPLC method

Source of variation	ss	df	MS	F cal	p-value	F tab
Between groups	0.476017	1	0.476017	0.000191	0.989642	7.708647
Within groups	9982.944	4	2495.736			

Table 8: Results obtained for robustness study of HPLC method (n=6)

S.no	Parameter	Condition	Area ± RSD	% of change
1	Standard solution (100 mcg/ml)	0.01m mono basic potassium phosphate buffer: Acetonotrile (40:60)	32165	-------
2	Mobile phase change	0.01m mono basic potassium phosphate buffer: Acetonotrile (37:73) 0.01m mono basic potassium phosphate buffer: Acetonotrile (43:67)	31291±1820.09 34718±639.22	0.027 0.062
3	Flow change	0.8 ml/min 0.6 ml/min	33027±1023.34 30864±912.63	0.027 0.04
4	Wavelength change	240 nm 236 nm	32027±1023.34 31864±912.63	0.004 0.009

Table 9: Results obtained for robustness study of UV-Visible spectrophotometric method (n=6)

S.No	Parameter	Condition	Absorbance	% of change
1	Standard solution (10 µg/ml)	phosphate buffer pH6.8	0.982	
2	Mobile phase change	phosphate buffer pH6.8: methanol (98:2) phosphate buffer pH6.8: water (98:2)	0.943±0.011 0.906±0.023	0.04 0.07
3	Wavelength change	240 nm 236 nm	0.979±0.013 0.973±0.009	0.003 0.009

Table 10: Detection and quantification limits of proposed UV and HPLC methods

Detection and Quantification limits	UV Method	HPLC Method
LOD	1.361119µg/ml	15.34251 µg/ml
LOQ	4.124604µg/ml	46.49247 µg/ml

Table 11: Selectivity and specificity of Candesartan samples using proposed UV and HPLC methods

Method	Mobilephase/ Dilution liquid	Placebo	Candesartan sample Peak area/absorbance
UV METHOD	No absorbance	No absorbance	0.560 ± 0.006
HPLC METHOD	No peak	No peak	32165 ± 2074.65

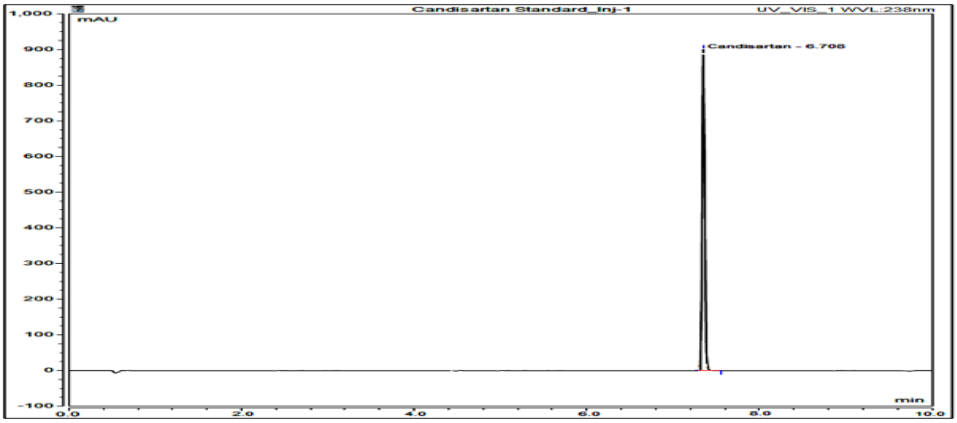

Fig. 4: Typical chromatogram of candesartan

Table 12: Formulation analysis results

S.No	Tablet name	Dose	Sample concentration	Sample estimated	% of drug estimated in tablet
1	(HPLC)	4 mg	1 mg/ml	0.957± 0.0012	95.7
2	(UV)	4 mg	3 mg/ml	2.985 ± 0.0016	99.5

Estimation of an active ingredient in bulk and in tablet dosage form (Formulation analysis):

Twenty tablets (Candelong 4 mg) were weighed accurately and crushed into powder form. Accurately weighed the quantity of powder taken and a standard solution of 1000 µg/ml was prepared using the mobile phase and the diluting fluid. Serial dilutions were taken to ensure the standard solution prepared, and the solutions were analyzed spectrophotometrically and chromatographically using the proposed methods.

3. RESULTS AND DISCUSSION

The summary of validation parameters obtained for proposed UV and HPLC methods were given in **table 2**

Linearity and range

The linearity of candesartan employing UV method was constructed by considering concentration (µg/ml) on X–axis and Absorbance on Y– axis. The regression coefficient was considered to be 0.998 over a concentration range of 2–10 µg/ml. The representative linearity equation was found to be y = 0.0256x+0.0002 as shown in **figure 3**and data were shown in **table 3.** The linearity of proposed Candesartan employing HPLC method was constructed by considering concentration (µg/ml) on X–axis and peak area on Y-axis. The regression coefficient was considered to be 0.999 over a concentration range of 25–200 µg/ml. The representative linearity equation was found to be Y=307.72 xs+0.00 as shown in **figure 4** and the corresponding data were shown in **table 3**. For both the methods the % RSD was found to be within the acceptable theoretical limits of ≤ 2%.

Precision

The % RSD for intra-day precision (six independent series in the same day) and inter-day precision (3 consecutive days) analysis performed for six different individual samples of drug solution using the proposed UV and HPLC methods was found to be 0.021%, 0.72%and 0.39%, 0.91%respectively. Since the values

obtained as shown in **table 4** were within the proposed theoretical limits<2% RSD according to IP, the method was demonstrated to be precise.

Recovery studies

The accuracy of the proposed UV-visible spectroscopic method and HPLC method was established by recovery experiments. The recovery analysis studies were carried out at three different concentration ranges (50, 100 and 150%). All studies were carried in triplicate, and the results obtained were presented in **table 5.** The analyzed samples yielded high recovery values from the proposed methods. % RSD values were found to be less than 0.2% for both UV and HPLC analysis, indicating that the proposed methods were accurate. All the RSD values obtained were less than the theoretical limit of<2% RSD according to IP. F-test results for both the UV and HPLC methods revealed that the F cal value is less than the tabulated value as shown in **table 6 & 7**, proving that null hypothesis is accepted. Hence it was proved that there is no significant difference between the actual amount added, and the amount recovered.

Robustness

The robustness of the proposed HPLC method was checked in terms of variation in mobile phase, flow rate change and wavelength change. Experimental findings proved that the change of mobile phase is the most influential factor on repeatability of the proposed HPLC method. Suitable measures have been adopted to maintain similarity in various instrumental aspects like injection and capillary conditioning. Since % RSD values for all the parameters were found to be less than **0.1%** (less than the acceptable theoretical limit of<2% RSD) the proposed HPLC method was found to be robust. The results obtained were presented in **table 8.** The robustness of the proposed UV method was checked in terms of variation in the mobile phase and change in wavelength. Experimental findings proved that change in the mobile phase has a higher influence on repeatability of the proposed UV method compared to change in wavelength. % RSD values for all the parameters were found to be less than **0.02%** (less than the acceptable theoretical limit of<2% RSD) which proved that the proposed UV method was found to be robust. The results obtained were presented in **table 9.**

Ruggedness

Standard solutions of candesartan were analyzed using both the proposed methods for ruggedness, the difference between labs, analysts or between instruments. Thus both the methods are proven to have ruggedness.

Detection and quantification limits

The LOD and LOQ for candesartan utilizing the proposed UV method were determined to be 1.36 µg/ml and 4.12µg/ml respectively. The LOD and LOQ for Candesartan using the proposed HPLC method were found to be15.34 µg/ml and 46.49µg/ml respectively. The results obtained were presented in **table 10**. Both the methods indicate the accuracy and precision to detect a very low quantity of analyte which is a favorable sign for extending the method to plasma drug analysis.

Specificity

The selectivity and specificity of the proposed methods were tested by studying the effect of various excipients and other additives usually present in the formulations of candesartan. The chromatograms didn't yield any peaks for mobile phase and placebo when analyzed with the proposed HPLC method. No absorbance was found for blank/dilution fluid when analyzed spectrophotometrically using the proposed UV method. The results obtained were presented in **table 11**. The well-shaped peaks and the linearity of the results indicate that the proposed methods are selective and specific. A model chromatogram was illustrated in **figure. 4**.

Determination of an active ingredient in bulk and in tablet dosage form (Formulation analysis)

Twenty solutions of candesartan were prepared using bulk drug and tablet dosage form (candelong). The samples were analyzed with both the proposed methods using the same experimental conditions and the drug content was found to be within the

limits specified by I. P. The results obtained were presented in **table 12**. F-test results for UV and HPLC method revealed that the Fcal value<F tab value proving that null hypothesis is accepted. Hence it was proved that in both the methods, there is no significant difference between sample concentration and the sample estimated. The results also assured that both the proposed methods are selective for estimation of formulations.

4. CONCLUSION

A novel, precise, economical, accessible, reliable and reproducible method for estimation of candesartan in bulk and tablet dosage form using UV and HPLC methods were developed and were validated as per ICH guidelines. The wide range of linearity establishes a further scope of promoting the proposed methods for estimation of candesartan. The RSD values for all the validation parameters were found to be less than 1, indicating that the proposed UV and HPLC methods were trusts worthy. Both the methods have ample scope and application in industry for estimation of Candesartan.

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