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Method development and validation of candesartan cilexetil in bulk drug by RP-HPLC

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ABSTRACT

Candesartan Cilexetil (Candesartan) is a medication utilized for the treatment of Hypertension. The main objective of the present work is to determine a simple, precise, accurate RP-HPLC method. In RP-HPLC, the method was developed (Waters Alliance 2690 with PDA detector) at 250nm. The mobile phase used was Methanol: Water (75:25) and Inertsil C18 ODS (4.6×150mm, 5µ) column was used for separation with a flow rate of 1.0 ml/min. The method was validated for System suitability, Specificity, linearity, accuracy, precision, limit of detection, limit of quantification, Robustness. The linearity was observed in a concentration range of 20-70ppm. The %RSD was found to be <2.0% in all cases and all the validation parameters were found to be within the limits. The proposed method was suitable for the quantitative determination of Candesartan Cilexetil in bulk drug.

Keywords: RP-HPLC, CDC, Method Development, Validation.

INTRODUCTION

High Performance Liquid Chromatography

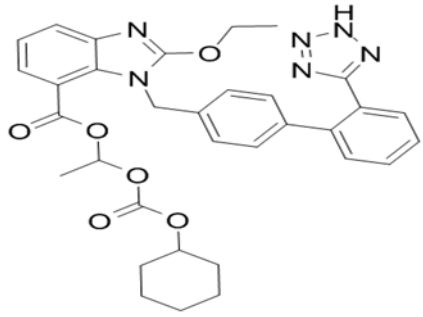
HPLC is a chromatographic technique which is used in the partition of a mixture of individual compounds. This technique is most extensively used in biochemistry and in analytical chemistry to identify, quantify and purify the substances [1, 2]. In the field of analytical chemistry, HPLC offers the resulting favorable circumstances:

1. Improved resolution of separated substances
2. Column packed with minute (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Continuous flow detectors is proficient of managing little flow rates.
6. Easy recovery, handling and maintenance.

Candesartan Cilexetil [8]

Candesartan cilexetil (candesartan) is a medication utilized for treating hypertension [4]. It is an angiotensive receptor blocker (ARB) prodrug

which swiftly converts to candesartan (active metabolite), through absorption from the gastrointestinal tract. CDC, chemically, 1-cyclohexyloxycarbonyloxyethyl2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] methyl] benzimidazole-4-carboxylate [6]. It is also used to treat Congestive Heart Failure, First Line Agent to Delay Progression of Diabetic Nephropathy [**7].** It is fine white powder and freely Soluble in Methanol, Acetonitrile and Acetone. Several methods have been developed for determination of candesartan cilexetil in pharmaceutical preparation by UV Spectrophotometry, HPTLC and HPLC [9, 10]. The proposed methods were validated in accordance with USP and as per ICH guidelines.



MATERIALS AND METHOD [3]

Materials

Candesartan cilexetil was procured as a gift sample from Mylan Laboratories, Hyderabad, India. HPLC grade Methanol (Qualigens) and Milli-Q water was used.

Instruments

HPLC (Waters alliance 2690 separation module Software: Empower 2, PDA detector), Digital pH meter and Digital weighing balance (MettlerToledo), Volumetric flask, Pipettes and burettes, Beakers (Borosil), Digital ultra sonicator (Fast clean).

Method

Preparation of mobile phase

Accurately measured 750ml of methanol (75%), 250ml of water (25%) were taken and mixed. The solution was degassed in ultrasonicator for 20 mins and then filtered through 0.45µ filter under vacuum filtration.

Diluent preparation: The mobile phase is used as diluent.

Preparation of standard solution

Accurately weigh and transfer 10 mg of candesartan working standard into a 10ml clean, dry volumetric flask and add about 5ml of diluent. Sonicate to dissolve it completely and make up the volume up to the mark with the same solvent (stock solution)

Preparation of sample solution

Weigh and transfer 10mg of sample into a 10 ml volumetric flask. Dissolve in 5ml of diluent and make up the volume.

Optimized Chromatographic Conditions

- Instrument used: Waters HPLC with auto sampler and PDA detector
- Temperature: Ambient
- Column: Inertsil C18 (4.6 ×250mm) 5µ
- Mobile phase: Methanol: Water (75:25v/v)
- Flow rate: 1.0 ml/min
- Wavelength: 250nm
- Injection volume: 20µl
- Run time: 6min.

VALIDATION PARAMETERS [5]

System Suitability

From the standard stock solution, pipette out 1.0 ml solution into a 10ml volumetric flask and dilute up to the mark with the diluents. The standard solution was injected for five times and the area was measured for all the injections in HPLC. The %RSD for areas of five replicate

injections was found to be within the specified limits.

Specificity of the Drug

From the already prepared stock solution pipette out 1 ml and dilute to 10ml with the diluent and later from the sample solution pipette out 2ml and dilute to 10 ml in the volumetric flask with the diluent. Now inject standard and sample solutions and calculate the assay by using the formula.

$$\% \text{ Assay} = \frac{\text{Area of Sample} \times \text{Wt. of STD} \times \text{Potency of STD} \times \text{Dilution Factor}}{\text{Peak Area of STD} \times \text{Wt. of Sample} \times 100 \times \text{Avg. Wt}} \times 100$$

Linearity

From the standard stock solution prepare aliquots of 20, 30, 40, 50, 60, 70 µg/ml and inject each level into chromatographic system and determine the peak area.

Plot a graph of peak area v/s concentration (X axis- concentration and Y axis- peak area) and calculate the correlation coefficient.

Precision

Repeatability

From the standard stock solution, pipette out 1.0 ml into a 10 ml volumetric flask and dilute it with the diluent.

The standard solution is injected for 5 times and the area is measured for all the five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the limits.

Intermediate Precision

The prepared stock solution is subsequently diluted to 200 µg/ml and was measured at a wavelength of 250nm. The findings were made on different days. The results obtained were tabulated and studies for inter-day and intra-day variation. The %RSD for all the injections was found to be within the specified limit.

Accuracy

Accuracy of the method is ascertained by standard addition method at 3 levels. Inject three replicate injections of individual concentration (50%, 100%, and 150%) under the optimized conditions. Record the chromatograms and measure the peak responses. Calculate the amount found and amount added for the candesartan and calculate the individual recovery and mean recovery values.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guideline.

$LOD = 3.3 \times (\sigma/S)$

$LOQ = 10 \times (\sigma/S)$

Where σ = the standard deviation of the response and S = the standard deviation of y-intercept of regression lines.

Robustness

Robustness was tested at the flow rate deviation from 0.9ml/min to 1.1 ml/min and mobile phase ratio difference from more organic phase to less organic phase ratio. The method is robust only in less flow conditions and even by change of ±5% in the mobile phase. The standard samples were injected by changing the conditions of chromatography.

RESULTS AND DISCUSSION

HPLC

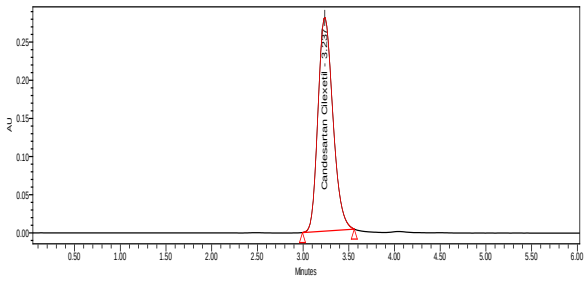


Figure-1: Optimized chromatogram for standard

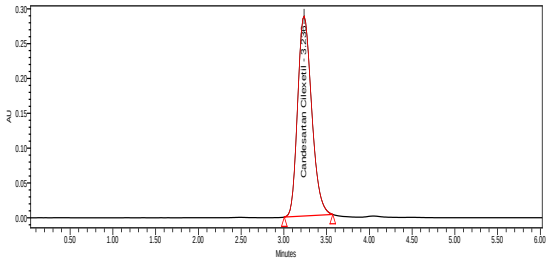


Figure-2: Optimized chromatogram of sample

Table-1: peak results for optimized chromatogram (standard & sample)

Peak name	R_t	Area	Height
STD	3.237	3058561	65411
SAM	3.236	5685421	57465

VALIDATION

System Suitability

The ICH document determines that system suitability test is an integral part of the analytical procedures and is used to ensure its capability for a particular analysis.

Table-2: Data of System Suitability.

Injection	R_t	Peak Area	USP Plate count
1	3.236	3058296.64	10087
2	3.235	3058388.08	10095
3	3.237	3058422.84	10080
4	3.232	3058359.19	10060
5	3.235	3058555.05	10070
Mean	3.235	3058404.36	10078
SD	0.001871	96.1222	-------
% RSD	0.055641	0.00314	-------

Specificity

Analytical method was tested for specificity to determine accurately the quantity of candesartan cilexetil in the given drug sample.

Table-3: Peak results for specificity (Standard & Sample)

Peak name	R_t	Area	% Assay
Standard	3.235	3056846	100.24
Sample	3.232	3056985	

The % Assay of Candesartan Celixitel was found to be 100.24%

Linearity

Table-4: Chromatographic data for linearity study

S.No	Concentration (ppm)	Average Area
1.	0	0
2.	20	1529106.51
3.	30	2293659.45
4.	40	3058213.02
5.	50	3822765.15
6.	60	4587318.61
7.	70	5305123.45

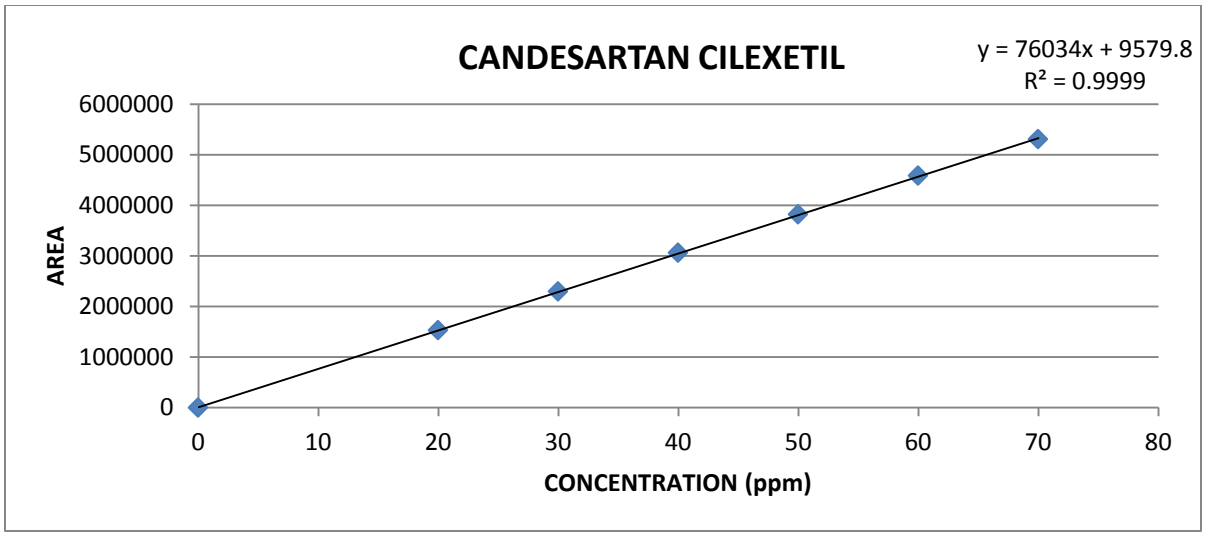


Figure-3: Linearity Curve for Candesartan Cilexetil

Linearity plot

The plot of concentration(X) versus the average peak area(Y) data of candesartan cilexetil is a straight line.

Y= m x + c

Slope (m) = 76034

Intercept (Y) = 9579

Correlation coefficient (r) = 0.999

Precision

Repeatability

Table-5: Data of Repeatability

No. of Injections	R_t	Peak Areas
1	3.234	3058546.85
2	3.237	3058624.18
3	3.236	3058471.28
4	3.235	3058770.08
5	3.237	3058581.59
Mean	3.235	3058598.79
SD	-------	110.9474
% RSD	-------	0.00362

Intermediate precision

Table-6: Data of Intermediate Precision

No of Injection	Day-1 Peak Areas	Day-2 Peak Areas
1	3058687.12	3058349.65
2	3058458.26	3058446.95
3	3058588.92	3058601.09
4	3058622.48	3058594.07
5	3058782.28	3058650.31
Mean	3058632.20	3058537.12
SD	107.9303	114.2974
% RSD	0.00352	0.00373

Limit of Detection

The LOD is found to be 0.0041µg/ml.

Limit of Quantification

The limit of quantification was found to be 0.0126µg/ml.

Accuracy

Table-7: Data of Accuracy for concentrations 50%, 100%, 150%

Conc % of spiked level	Area	Amt added (ppm)	Amt found (ppm)	% Recovery	Statistical Analysis of % Recovery
	1529202	20	19.98	99.93	**MEAN**-99.93
50	1529306	20	19.99	99.95	**%RSD**- 0.011
	1529256	20	19.98	99.93	
	3058222	40	40.09	100.22	**MEAN**-100.23
100	3058341	40	40.09	100.22	**%RSD**-0.016

	3058485	40	40.10	100.25	
	4587368	60	60.19	100.31	**MEAN**-100.33
150	4587497	60	60.20	100.34	**%RSD**-0.017
	4587402	60	60.20	100.34	

Robustness

Table-8: Results for Robustness

Parameter used for sample analysis	Peak area	Retention time	Theoretical plates
Actual flow rate of 1.0ml/min	3066548	3.237	10081
Less flow rate of 0.9 ml/min	3065541	3.544	15235
More flow rate of 1.1ml/min	3023542	2.985	9954
Less organic phase	3055641	3.548	15648
More organic phase	3023531	2.976	9800

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for Candesartan Cilexetil in bulk drug. This strategy is basic as the diluted samples are straightforwardly utilized with no primer substance derivatization or cleansing advances. The solvent systems used in these methods were economical. The %RSD values were within 2.0% and the methods were found to be precise.

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