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Analytical Method Development and Validation for the Simultaneous Estimation of Levocetirizine and Candesartan cilexetil by RP-HPLC Method in Bulk and Tablet Dosage Form

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A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Levocetirizine and Candesartan cilexetil by using Inertsil-ODS C_{18} (250 x 4.6 mm, 5 µ), flow rate was 1 ml/min, mobile phase ratio was Methanol: Acetonitrile (90:10 v/v), detection wavelength was 230 nm. The Spectroscopic method was done in solvent using mobile phase and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2690, PDA detector, Empower-software version 2. The retention times were found to be 2.789min for Levocetirizine and 3.480 for Candesartan cilexetil. The % purity of Levocetirizine and Candesartan cilexetil was found to be 99.71% and 99.56% respectively. The system suitability parameters for Levocetirizine and Candesartan cilexetil such as theoretical plates and tailing factor were found to be 7983, 1.13 and 9827, 1.07. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Levocetirizine and Candesartan cilexetil was found in the concentration range of 20 ppm-80 ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be within the range of 98% and 101% respectively. %RSD for repeatability and precision was found to be <2. LOD values were 0.33 and 0.32 and LOQ values were 1.01 and 0.98 respectively for Levocetirizine and Candesartan cilexetil.

Keywords: Levocetirizine, Candesartan cilexetil, RP-HPLC.

A R T I C L E I N F O

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1. Introduction

Levocetirizine is a potent second-generation histamine H1 antagonist that is effective in the treatment of allergic rhinitis, chronic urticaria, and pollen-induced asthma. Unlike many traditional antihistamines, it does not cause drowsiness or anti-cholinergic side effects. Cetirizine competes with histamine for binding at H_1-receptor sites on the effector cell surface, resulting in suppression of histaminic edema, flare, and pruritus. The low incidence of sedation can be attributed reduced penetration of cetirizine into the CNS as a result of the less lipophilic carboxyl group on the ethylamine side chain.

Candesartan is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension. It is administered orally as the prodrug, Candesartan cilexetil, which is rapidly converted to its active metabolite, Candesartan, during absorption in the gastrointestinal tract. Candesartan lowers blood pressure by antagonizing the rennin-angiotensin-aldosterone system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the blood pressure increasing effects of angiotensin II. Unlike angiotensin-converting enzyme (ACE) inhibitors, ARBs do not have the adverse effect of dry cough. Candesartan may be used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction and coronary artery disease. Candesartan selectively blocks the binding of angiotensin II to AT1 in many tissues including vascular smooth muscle and the adrenal glands.

This inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in an overall decrease in blood pressure. Candesartan is greater than 10,000 times more selective for AT1 than AT2. Inhibition of aldosterone secretion may increase sodium and water excretion while decreasing potassium excretion.

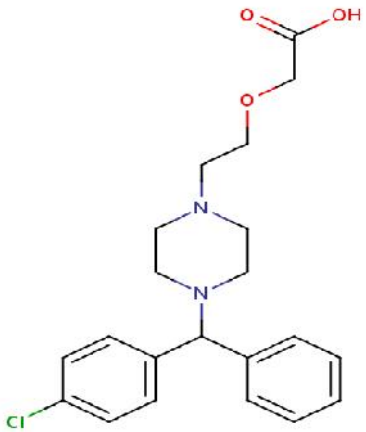

Figure 1: Levocetirizine

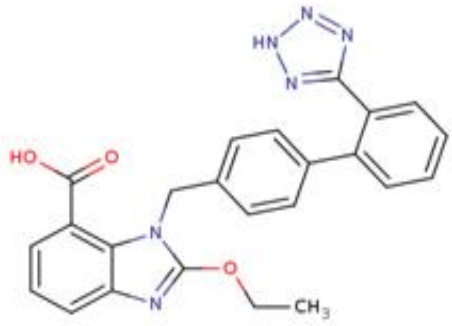

Figure 2: Candesartan cilexetil

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available.

2. Materials and Methods

Apparatus: The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2690, PDA detector with Empower-software version-2.

Reagents and Materials: The solvents used were Methanol, Ortho phosphoric acid, Acetonitrile, Tri Ethyl Amine of HPLC Grade and HPLC Water.

Selection of detection wavelength:

The sensitivity of method that uses UV- Vis detector depends upon the proper selection of wavelength. An ideal wavelength is that gives maximum absorbance and good response for both the drugs to be detected. Standard solutions of Levocetirizine and Candesartan cilexetil were scanned in the UV range (200-400nm) and the spectrums obtained were overlaid and the overlain spectrum was recorded. From the overlain spectrum, 230 nm was selected as the detection wavelength for the present study.

Selection of mobile phase

Initially the mobile phase tried was Methanol and water, Methanol, Acetonitrile and water in various proportions. Finally, the mobile phase was optimized to Degassed Methanol and Acetonitrile in the ratio of 90:10 V/V.

Chromatographic trials for Simultaneous Estimation of Levocetirizine and Candesartan cilexetil by RP- HPLC.

Trial-1 Chromatographic conditions

Flow rate : 1.0ml/min
Mobile Phase : Degassed Acetonitrile 100% v/v
Column : Inertsil - C18, ODS column
Detector wavelength: 254 nm
Column temp. : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 4.092 min for LVCTRZN and 4.550 min for CNDSRTN

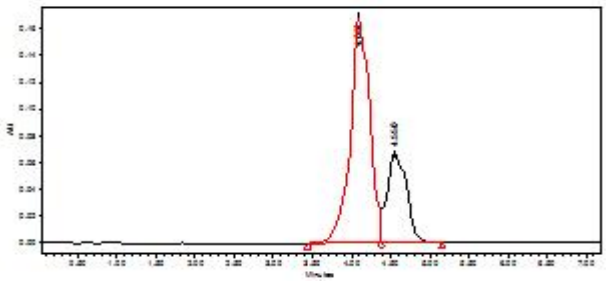

Figure 3: Chromatogram of Trial-1

Observation: In this, trial two peaks are merged and not separated completely.

Trial-2 Chromatographic condition

Flow rate : 1 ml/min
Mobile Phase : Degassed Acetonitrile and methanol (90:10 v/v)
Column : Inertsil -C18, ODS column
Detector wavelength : 254nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 3.757 min for LVCTRZN and 4.0 min for CNDSRTN.

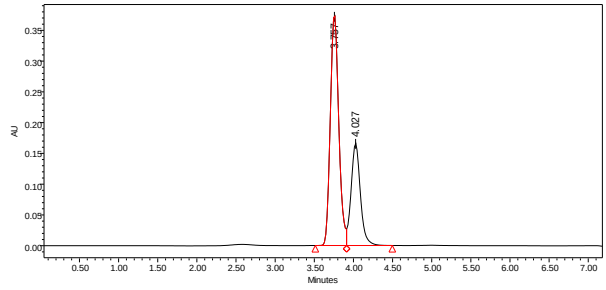

Figure 4: Chromatogram of trial-2

Observation: In this trial two peaks are separated completely but peak shapes are not good.

Trial-3 Chromatographic condition

Flow rate : 1.0ml/min
Mobile Phase : Degassed Acetonitrile and methanol (80:20 v/v)
Column : Inertsil - C18, ODS column
Detector wavelength : 254 n.m
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 1.747 min for LVCTRZN and 2.821 min for CNDSRTN.

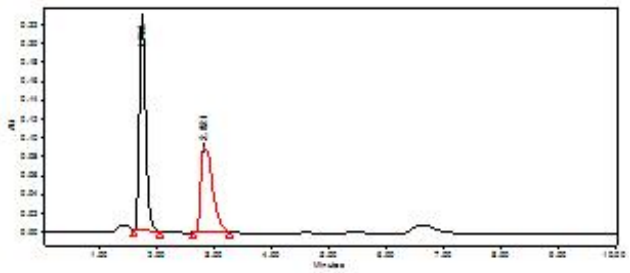

Figure 5: Chromatogram of trial-3

Observation:
In this trial both Levocetirizine and Candesartan cilexetil were eluted but there is no proper resolution. Still more trials were required for better resolution in peaks.

Trial -4 Chromatographic conditions (OPTIMISED METHOD):

Flow rate : 1.0ml/min
Mobile Phase : Methanol and Acetonitrile (90:10 v/v)
Column : Inertsil - C18, ODS column (250 x 4.6 mm, 5 µ)
Detector wavelength : 230 nm
Column temp : Ambient
Injection volume : 20 µl
Run time : 10min
Retention time : 2.780 min for LVCTRZN and 3.481 min for CNDSRTN

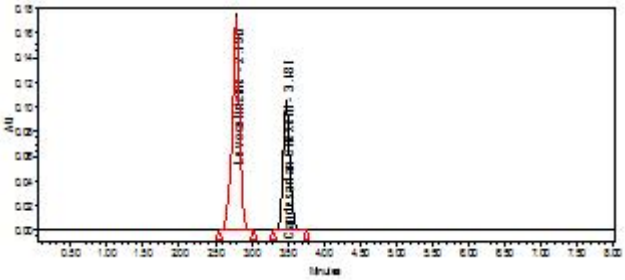

Figure 6: Chromatogram of trial-4 (Optimized Method)

Observation: The separation was good and peak shape was good so we conclude that no trials are required for separation of both the drugs.

Procedure

Mobile Phase: Degassed Methanol and Acetonitrile in the ratio of 90:10 V/V

Preparation of stock solution:

The Stock solution was prepared by dissolving 20.0 mg of accurately weighed Levocetirizine and 25.0 mg Candesartan cilexetil in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicated for 20 min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicated for 10 min.

Preparation of working standard solution:

The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Levocetirizine and Candesartan cilexetil, sonicated and filtered through 0.45µ membrane.

Preparation of sample drug solution for pharmaceutical formulations:

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 20 mg Levocetirizine and 25 mg Candesartan cilexetil was weighed and dissolved in the 70 mL mobile phase with the aid of ultra sonication for 20 min. The content was diluted to 100 mL with mobile phase to furnish a stock test solution. The stock solution was filtered through a 0.45 µm Nylon syringe filter and 10.0 mL of the filtrate was diluted into a 50.0 mL volumetric flask to give a test solution containing 40 µg/mL Levocetirizine and 50 µg/mL Candesartan cilexetil.

3. Results and discussions

Method Validation Parameters

Specificity

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

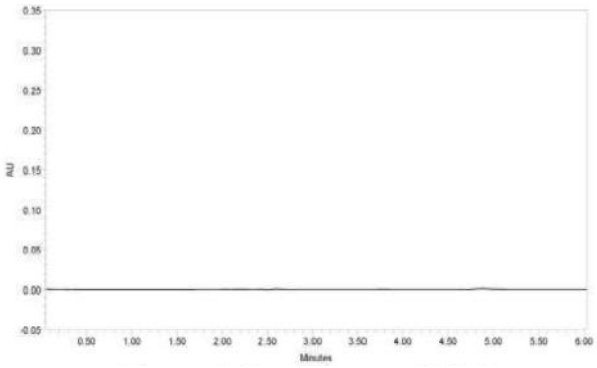

Figure 7: Chromatogram of Blank

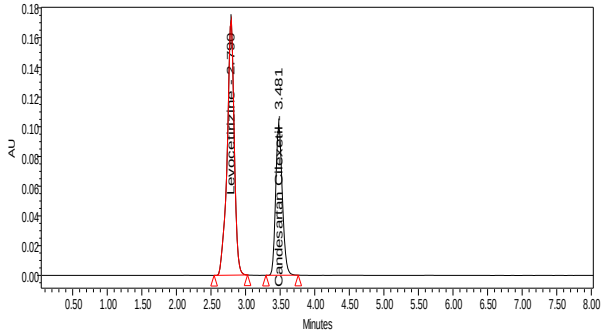

Figure 8: Chromatogram of Sample

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Serial dilutions of Levocetirizine and Candesartan cilexetil (20-80 ppm) were injected into the column and detected at a wavelength set at 230 nm. The calibration curve was obtained by plotting the concentration vs. peak area.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

Range

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range 20-80 ppm for Levocetirizine and Candesartan cilexetil respectively

Accuracy: Accuracy of the method was determined by recovery experiments. There are mainly 2 types of recovery studies are there.

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration. Percentage method: For these assay method samples are prepared in three concentrations of 50%, 100%, and 150% respectively.

Acceptance criteria:

The mean % recovery of the Levocetirizine and Candesartan cilexetil at each level should be not less than 95.0% and not more than 105.0%.

Assay procedure: 20µL of the standard and sample solutions of Levocetirizine and Candesartan cilexetil were injected into the HPLC system and the chromatograms were recorded. Amount of drug present in the Tablets were calculated using the peak areas.

Precision

Method precision also called as repeatability/Intra-day precision indicates whether a method gives consistent results for a single batch. Method precision was demonstrated by preparing six test solutions at 100% concentration as per the test procedure & recording the chromatograms of six test solutions. The % RSD of peak areas of six samples was calculated. The method precision was performed on Levocetirizine and Candesartan cilexetil formulation.

Acceptance criteria

The % RSD for the area of sample injections results should not be more than 2.

Selection of solvent

Solutions of Levocetirizine and Candesartan cilexetil were prepared by dissolving in mobile phase and UV spectrum of each was recorded by scanning between 200-400 nm. Both the drugs show good response at 230 nm.

Validation of the method

Linearity

Levocetirizine and Candesartan cilexetil: Serial dilutions of Levocetirizine and Candesartan cilexetil (20-80 ppm) were injected into the column and detected at a wavelength set at 230 nm. The calibration curve was obtained by plotting the concentration vs. peak area and the correlation coefficient was found to be 0.999 and 0.999 respectively.

Recovery studies

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. To an equivalent quantity of formulation powder a known quantity of standard Levocetirizine and Candesartan cilexetil were added at 50%, 100% and 150% level and the contents were re-analyzed by the proposed method.

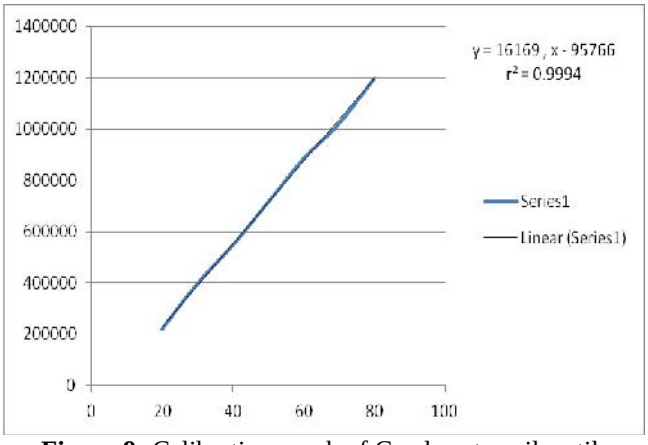

Figure 9: Calibration graph of Candesartan cilexetil

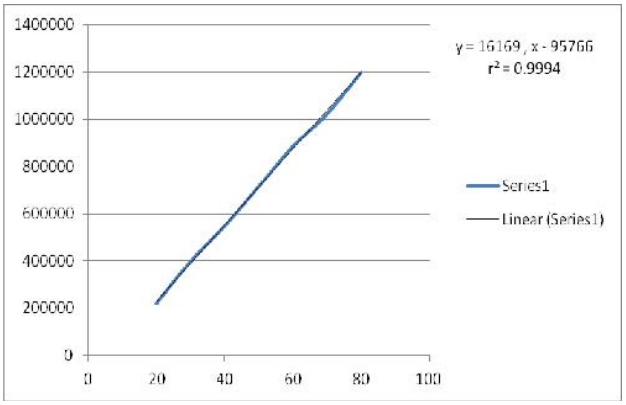

Figure 10: Calibration graph of Levocetirizine

Table 1: Calibration data of Levocetirizine

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	33025
20	572087	y-Intercept	91183
30	887800	Correlation Coefficient	0.999
40	1239364		
50	1570861		
60	1869524		
70	2234112		
80	2546863		

Table 2: Calibration data of Candesartan cilexetil

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	16169
20	219695	y-Intercept	95766
30	398090	Correlation Coefficient	0.999
40	547437		
50	715694		
60	885479		
70	1022457		
80	1199855		

Table 3: Showing accuracy results for Levocetirizine

%Conc. (at specification level)	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	20	19.95	99.81%	99.91%
100%	40	39.96	99.91%	
150%	60	60.04	100.06%	

Table 4: Showing accuracy results for Candesartan cilexetil

%Conc. (at specification level)	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	20	19.98	99.9%	99.9%
100%	40	40.05	100.1%	
150%	60	60.02	100.03%	

Robustness:

Table 5: System Suitability Results for Levocetirizine

S. No	Flow rate (ml/min)	System suitability results	
		Standard Area	Tailing Factor
1	0.8	1241755	1.13
2	1	1250579	1.14
3	1.2	1249382	1.07

Table 6: System Suitability Results for Candesartan cilexetil

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	Tailing Factor
1	0.8	548197	1.078
2	1	550726	1.076
3	1.2	550129	1.077

Precision:

Table 7: Data of Repeatability (Method precision) for Levocetirizine

	Injection	Peak Areas	% Assay
Conc 40 ppm	1	1243389	98.6
	2	1264984	99.02
	3	1248352	98.12
	4	1256493	98.31
	5	1239664	98.81
	6	1243411	98.36
Statistical Analysis	Mean	1250579	98.48
	SD	10222.12	0.352647
	% RSD	0.817391	0.35

Table 8: Data of Repeatability (Method precision) for Candesartan cilexetil

	Injection	Peak Areas	% Assay
Conc 40 ppm	1	547265	98.55
	2	553782	98.88
	3	551981	99.40
	4	551495	99.30
	5	547437	100.53
	6	549117	98.28
Statistical Analysis	Mean	550726	99.278
	SD	2422.819	0.827236
	% RSD	0.439932	0.83

Table 5: LOD and LOQ

Drug name	LOD(µg)	LOQ(µg)
Levocetirizine	0.33	1.01
Candesartan cilexetil	0.32	0.98

4. Conclusion

A new method was established for simultaneous estimation of Levocetirizine and Candesartan cilexetil by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Levocetirizine and Candesartan cilexetil by using Inertsil-ODS C_{18}(250 x 4.6

mm, 5 µ) flow rate was 1 ml/min, mobile phase ratio was Methanol: Acetonitrile (90:10 v/v), detection wavelength was 230 nm. Precision and recovery studies were also found to be with the range. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Levocetirizine and Candesartan cilexetil in Tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. Hence the suggested RP-HPLC method can be used for routine analysis of Levocetirizine and Candesartan cilexetil in API and Tablet dosage form.

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