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**RESEARCH ARTICLE**

# Development and Validation of RP - HPLC Method for the Simultaneous Estimation of Candesartan Cilexetil and Levocetirizine Hydrochloride

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**Abstract:** In the present work, a rapid, accurate and precise RP-HPLC method was developed for simultaneous estimation of candesartan cilexetil(CAN) with levocetrizine hydrochloride(LEV) using a physical mixture of these two drugs. A new method was developed using phenominex $C_8$ column (250 mm x 4.6 mm, 5 µ) as stationary phase and mobile phase consists of acetonitrile: Buffer (Heptane sulphonic acid) in ratio 80:20 (pH 4.4) with a flow rate of 1 mL/min and UV detection was performed at 230 nm. The retention time of LEV and CAN were found to be 2.8±0.02 min and 4.8±0.02 min respectively. The method was validated by using various validation parameters like linearity, accuracy, precision, specificity, stability and robustness. Linearity was observed in the concentration range of 1-30 µg/mL for LEV and CAN with good regression response of 0.999. The LOD of LEV and CAN was observed to be 100 µg/mL and 50 µg/mL, the LOQ of LEV and CAN was observed to be 600 µg/mL and 300 µg/mL respectively. Accuracy of the method was performed by recovery studies and the percentage recoveries obtained for LEV and CAN were 100.8% and 99.69% respectively. Precision studies were carried out and %RSD was found to be 0.91 and 1.44 for LEV and CAN for system precision and 1.55 and 1.54 for LEV and CAN for method precision respectively. All the validation parameters were found to be well within the acceptance criteria. Hence the method can be used for routine estimation of LEV and CAN in bulk drug and pharmaceutical formulations both in individual and in combination.

**Keywords**: RP-HPLC, Candesartan cilexetil, Levocetrizine hydrochloride

## Introduction

Candesartan chemically known as (1RS)-1-[[(cyclohexyloxy) carbonyl] oxy] ethyl 2-ethoxy-1-[[2'(1*H*-tetrazol-5-yl)biphenyl-4-yl]-1*H*-benzimidazole -7-carboxylate[1]. It is an angiotensin-receptor blocker (ARB) that may be used alone or with other agents to treat hypertension[1]. It is administered orally as a prodrug, candesartan cilexetil which rapidly gets converted to its active metabolite candesartan during absorption in the gastrointestinal tract. candesartan lowers blood pressure by antagonizing the renin-angiotensin-aldosterone

system (RAAS); it competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) subtype and prevents the rise in blood pressure, by decreasing the effects of angiotensin II[3-6]. Levocetrizine hydrochloride chemically known as [2-[4-[(*R*)-(4-chlorophenyl) phenyl methyl]-1-piperazinyl] ethoxy]-acetic acid hydrochloride[2]. It is a third-generation non-sedative antihistamine, developed from the second-generation antihistamine cetrizine. It is pharmacologically active *R*-enantiomer of cetrizine, orally active, potent and selectively long acting H1-histamine receptor antagonist. Levocetrizine works by blocking histamine receptors. It does not inhibit the actual release of histamine from mast cells, but prevents its binding to its receptors. This in turn prevents the release of other allergy chemicals and increased blood supply to the area, and provides relief from the typical symptoms of hay fever. It is said to be more effective with fewer side effects than the second-generation drugs. It is used in the treatment of perennial and seasonal allergic rhinitis and chronic idiopathic urticaria. The structures of LEV and CAN shown in Figure 1 and 2 respectively[3,4].

These two drugs are being prescribed and consumed by the patients at a time as per the survey of prescriptions. Their combination as a single dosage form is not available but needs their estimation when present together in blood. Literature review reveals that various methods have been reported for analysis of LEV by UV Spectrophotometry method[8] LEV with other drugs by HPLC method[9-16], LEV by LC-MS method[17], LEV by HPTLC method[18] and CAN by HPLC method[19,20], CAN by HPLC method[21-25], CAN by LC-MS method[26], CAN by HPTLC method[27]. But no data have been reported about the simultaneous estimation of LEV with CAN in bulk and tablet dosage form. Hence a new analytical method developed for the simultaneous estimation of LEV and CAN by HPLC method and the method was validated as per ICH guidelines.

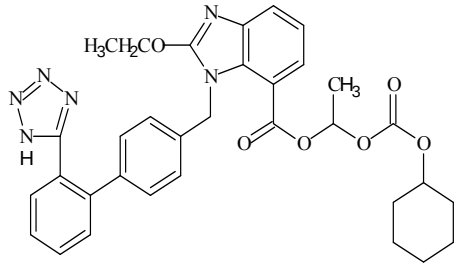



**Figure 1.** Structure of candesartan cilexetil

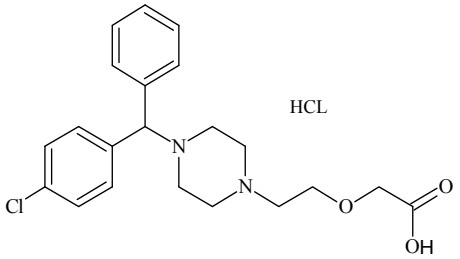



**Figure 2.** Structure of levocetrizine hydrochloride

## Experimental

An SHIMADZU-SPD20A HPLC with a LC-20AT pump, SPD-M20A PDA detector, 20 µL Rheodyne injector and HPLC system was well equipped with LC Solution-20AT software for data processing. The column used was PHENOMENEX – $C_8$ (250 mm x 4.6 mm, 5 µm) for analysis.

### *Chemical reagents*

Acetonitrile used was of HPLC grade of Rankem, Milli Q water was used for the preparation of the mobile phase. Heptane sulphonic acid and sulphuric acid used were of AR grade of SD fine chemicals. Candesartan cilexetil and levocetrizine hydrochloride gift samples were obtained from Micro Labs Bangalore.

**Method development**

*Preparation buffer solution*

Accurately weighed 126 mg of heptane sulphonic sodium salt was dissolved in few mL of milli Q water and then the volume was made up to 200 mL with milli Q water. pH of resulting solution was adjusted to 4.4 with 0.2 M sulphuric acid and it was filtered through 0.45 µm nylon filter paper.

*Preparation of mobile phase*

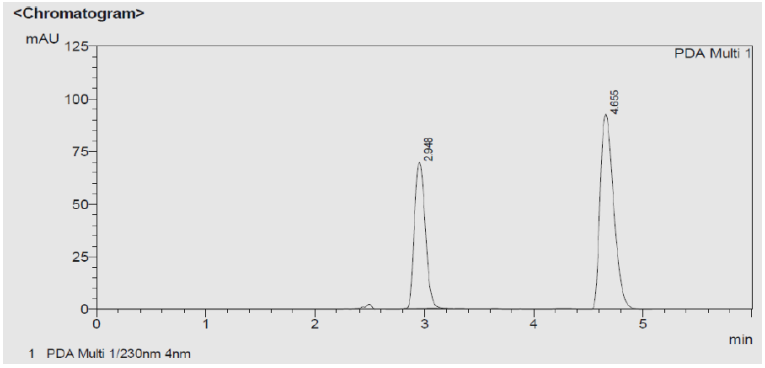
The buffer of 1 mM heptane sulphonic acid sodium salt and aceyonitrile in the ratio 20:80 v/v was used as mobile phase. The prepared mobile phase was filtered through 0.45 µm nylon filter paper and it was ultrasonicated for 10 minutes to degas the mixture.

*Preparation standard stock solution*

10 mg of LEV and CAN is weighed into 10 mL volumetric flask and dissolved in few mL of mobile phase and final volume is made up to 10 mL with mobile phase (stock A concentration of 1000 µg/mL mixture). From above mixture, 1 mL was pipetted into clean 10 mL volumetric flask and volume was made up to mark with mobile phase (stock B concentration of 100 µg/mL of mixture). From the standard stock solution B, 1 mL solution was pipetted in to 10 mL volumetric flask and diluted to 10 mL with mobile phase to gave the working standard solution of 10 µg/mL. The resulting solution was filtered, sonicated for five minutes and injected into HPLC system.

*System suitability studies*

Working standard solution mixture of 10 µg/mL each was injected into HPLC system and the retention times of CAN and LEV under these conditions were found to be 2.94 and 4.95 min respectively. The chromatogram and system suitability parameters were shown in Figure 3.




| PDA | | Qualitative results | | | | | |
|---|---|---|---|---|---|---|---|
| ID# | Name | Ret. Time | Area | Height | Tailing factor | Resolution | Theoretical plate |
| 1 | Levoctz | 2.948 | 483692 | 69641 | 1.256 | 0.000 | 3447.507 |
| 2 | Candesartan | 4.655 | 892335 | 92681 | 1.483 | 7.735 | 5979.360 |

**Figure 3.** Chromatogram of LEV and CAN (10 µg/mL each)

*Method validation*

The developed new analytical HPLC method for simultaneous estimation of LEV and CAN was validated in accordance with ICH guidelines[6].

### *Linearity*

10 mg of LEV and CAN is weighed into 10 mL volumetric flask and dissolved in few mL of mobile phase and final volume is made up to 10 mL with mobile phase (stock A concentration of 1000 µg/mL mixture). From the above mixture, 1 mL of solution was pipetted out into clean 10 mL volumetric flask and volume is made up to mark with mobile phase (stock B concentration of 100 µg/mL of mixture). Working standard solutions of various concentrations were made by diluting standard stock solution B of mixture separately in a series of 10 mL volumetric flask with a mobile phase and linearity studies were performed by injecting each concentration. Calibration graphs were plotted on the basis of replicate (n=6) analysis of each calibration solutions. Linear correlations were obtained over the range 1-30 µg/mL with correlation coefficients of ($r^2$) 0.999 and 0.999 for LEV and CAN respectively.

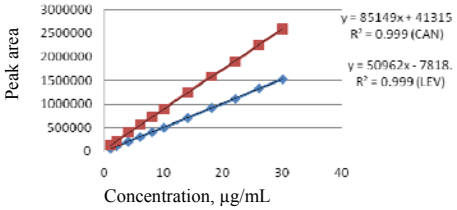



**Figure 4.** Linearity curve for LEV and CAN (in mixture)

### *Limit of detection (LOD) and limit of quantitation (LOQ)*

The parameters LOD and LOQ were determined on the basis of signal to noise ratio. Detection limit was defined as the lowest concentration level resulting in a peak of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a peak with precision (% RSD) and accuracy (% assay) with in ±10%.

### *Precision*

The precision of the method was carried out by replicate (n=6) analysis of standard solution preparations mixture with concentration of 10 µg/mL. The precision was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days. The intra-day and inter-day variation was calculated in terms of % RSD and the results are given in Table 1.

**Table 1.** Data of precision studies

| Replicates | Retention Time, min | | *Area | | Conc. obtained µg/mL | | % Found | |
|---|---|---|---|---|---|---|---|---|
| | LEV | CAN | LEV | CAN | LEV | CAN | LEV | CAN |
| 1 | 2.83 | 4.81 | 488201 | 883145 | 9.71 | 9.9 | 97.1 | 99.06 |
| 2 | 2.81 | 4.82 | 491408 | 880592 | 9.82 | 9.87 | 98.2 | 98.76 |
| 3 | 2.83 | 4.86 | 484022 | 893265 | 9.67 | 10.02 | 96.7 | 100.24 |
| 4 | 2.83 | 4.82 | 492151 | 899256 | 9.78 | 10.09 | 97.8 | 100.94 |
| 5 | 2.84 | 4.81 | 495581 | 861519 | 9.9 | 9.65 | 99 | 96.54 |
| 6 | 2.82 | 4.85 | 485033 | 889688 | 9.69 | 9.98 | 96.9 | 99.82 |
| Average | 2.826667 | 4.828333 | 489399.3 | 884577.5 | 9.69 | 9.9183 | 96.9 | 99.226 |
| Std dev | 0.0103279 | 0.02137 | 4455.02 | 13165.7 | 0.1505 | 0.1538 | 1.5059 | 1.5348 |
| RSD | 0.0036537 | 0.004426 | 0.009103 | 0.014884 | 0.0155 | 0.0155 | 0.0155 | 0.0154 |
| %RSD | 0.3653758 | 0.4426 | 0.9103 | 1.4884 | 1.5541 | 1.5507 | 1.5541 | 1.5467 |

**Replicates of 6 injections*

### *Accuracy*

The study was performed by taking a fixed amount of sample mixture (Individual marketed tablet triturated solutions) and varied amounts of standard drugs (working standards) were added, such that 80%, 100% and 120% of analysis levels at the ratio of 1:1 of drugs is maintained. Each level was injected 6 times. These solutions were then analyzed for recovery studies. Results for determination of accuracy are presented in Table 2.

**Table 2.** Data of Accuracy (Recovery studies)

| Levels | Vol taken into 10 mL VF | | Conc. taken µg/mL | *Peak Area | | Conc. Obtained µg/mL | | % Found | |
|---|---|---|---|---|---|---|---|---|---|
| | Std. soln 100 µg/mL | Sample mix. soln 100 µg/mL | | LEV | CAN | LEV | CAN | LEV | CAN |
| 80% | 0.3 mL | 0.5mL | 8 | 401241 | 712722 | 8.05 | 7.91 | 100.6 | 98.96 |
| 100% | 0.5 mL | 0.5mL | 10 | 510428 | 890125 | 10.19 | 9.98 | 101.9 | 99.88 |
| 120% | 0.7 mL | 0.5mL | 12 | 601985 | 1062752 | 11.99 | 12.03 | 99.95 | 100.2 |

**Average of 3 readings*

### *Specificity*

The selectivity of the method was checked by injecting solutions of two drugs. It was observed that two sharp peaks for LEV and CAN were obtained at retention times of 2.94 and 4.95 min respectively, noise of mobile phase didn't interfere with LEV and CAN retention times.

### *Stability*

To demonstrate the stability of both standard solutions during analysis, both solutions were analyzed over a period of 48 h. The results showed that for solutions, the retention times and peak areas of CAN and LEV remained almost unchanged (RSD<2.0%) indicating that no significant degradation occurred within this period, *i.e*. both solutions were stable for at least 48 h, which was sufficient to complete the whole analytical process. Results for stability studies are presented in Table 3.

**Table 3.** Data for stability of analytical solution

| Time interval | Conc taken µg/mL | LEV | | | CAN | | |
|---|---|---|---|---|---|---|---|
| | | Area | Conc Obtained, µg/mL | % Found | Area | Conc. obtained µg/mL | % Found |
| 0 | 10 | 487033 | 9.73 | 97.38 | 888973 | 9.97 | 99.74 |
| 1 | 10 | 481169 | 9.62 | 96.23 | 875108 | 9.81 | 98.12 |
| 2 | 10 | 499793 | 9.98 | 99.89 | 897073 | 10.06 | 100.6 |
| 4 | 10 | 482617 | 9.65 | 96.52 | 887025 | 9.95 | 99.51 |
| 6 | 10 | 481037 | 9.62 | 96.21 | 873745 | 9.79 | 97.96 |
| 12 | 10 | 514802 | 10.28 | 102.8 | 882455 | 9.89 | 98.98 |
| 24 | 10 | 515436 | 10.29 | 102.9 | 868983 | 9.74 | 97.41 |
| 48 | 10 | 497601 | 9.94 | 99.46 | 880257 | 9.87 | 98.72 |
| Average | | | | 98.92 | | | 98.88 |

### *Ruggedness and robustness*

The robustness of the method was determined by making slight changes in the chromatographic conditions (buffer pH±0.1, flow rate±0.2 min, mobile phase ratio ±2 mL).

The ruggedness of the method was determined by using different instrument and different column of similar type. Again there was no marked change in the chromatograms. These results indicated that the method was rugged and robust with regard to these conditions.

*System suitability*

System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared standard stock solution of CAN and LEV. In addition, standard deviation of CAN and LEV standards were evaluated by injecting mixed standard of both CAN and LEV (10 µg/mL).

## Results and Discussion

The proposed method, which is a new HPLC method for the simultaneous estimation of LEV and CAN in combination or individually, were found to be simple, rapid, accurate, precise, specific and economical. Since the linearity range was 1-30 µg/mL for LEV and CAN, with good regression response of 0.9995. The LOD of LEV and CAN was observed to be 100 µg/mL and 50 µg/mL, the LOQ of LEV and CAN was observed to be 600 µg/mL and 300 µg/mL respectively. The percentage recoveries obtained for LEV and CAN were 100.8% and 99.69%. The %RSD for precision studies for LEV and CAN were found to be 0.91 and 1.44 for system precision and 1.55 and 1.54 for method precision respectively. This method can be used to estimate either of these drugs individually or in combination when present together.

**Table 4.** Summarized results of validation parameters

| S.No | Parameter | Acceptance criteria | | LEV | CAN |
|---|---|---|---|---|---|
| 1 | Linearity | • Percentage fitting curve should be more than 99.7 % | | 99.9% | 99.9% |
| | | • Regression coefficient ($r^2$) not less than 0.999 | | 0.999 | 0.999 |
| | | • Beer's range | | 1-30 µg/mL | 1-30 µg/mL |
| | | • Regression equation | | y=85149x+ 41315 | y=50962x-7818.3 |
| 2 | LOD | - | | 100 µg/mL | 600 µg/mL |
| 3 | LOQ | - | | 50 µg/mL | 300 µg/mL |
| 4 | System Precision | % RSD should be less than 2.0 % | | 0.91 | 1.44 |
| 5 | Method Precision | % RSD should be less than 2.0 % | | 1.55 | 1.54 |
| 6 | Accuracy | Recovery range between 98-102% | | 99.95-101.9% | 98.96-100.2% |
| 7 | Robustness | Change in mobile phase ratio | 75: 25 | 97.8% | 96.9% |
| | | | 80: 20 | 100.8% | 100.1% |
| | | | 85: 15 | 98.4% | 100.7% |
| | | Change in Flow rate | 0.8 mL/min | 99.7% | 98.1% |
| | | | 1.0 mL/min | 102.1% | 100.5% |
| | | | 1.2 mL/min | 97.8% | 98.6% |
| | | Change in Wave length | 220 nm | 97.9% | 98.1% |
| | | | 230 nm | 101.8% | 101.9% |
| | | | 240 nm | 98.4% | 98.5% |

*Contd…*

| | | | | | |
|---|---|---|---|---|---|
| | | Change in pH of mobile phase | pH 4.2 | 99.1% | 101.5% |
| | | | pH 4.4 | 101.8% | 102% |
| | | | pH 4.6 | 97.7% | 100.4% |
| | | | % assay should be 95-105 % | | |
| 5 | Ruggedness | Change in analyst | Analyst1 | 99.5% | 97.9% |
| | | | Analyst2 | 99.7% | 100.6% |
| | | Change in Day | Day 1 | 100.1% | 100.4% |
| | | | Day 2 | 99.6% | 98.4% |
| | | | Day 3 | 100.6% | 99.9% |
| | | Change in Column | $C_8$ | 99.4% | 99.6% |
| | | | $C_{18}$ | 97.54% | 97.5% |
| | | % assay should be 95-105 % | | | |
| 8 | Specificity | Non interference of placebo and blank in analysis | | Noise of mobile phase didn't interfere with LEV and CAN retention times (Complies) | |

## Conclusion

A validated RP-HPLC method has been developed for the determination of LEV & CAN in pure and in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and the conclusion that could be used for this method is rapid and reliable determination of LEV and CAN for individual or simultaneously in pharmaceutical formulation.

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