Original Article

# A validated ultra high-pressure liquid chromatography method for separation of candesartan cilexetil impurities and its degradents in drug product

*Abstract*

**Introduction:** A selective, specific, and sensitive "Ultra High-Pressure Liquid Chromatography" (UPLC) method was developed for determination of candesartan cilexetil impurities as well asits degradent in tablet formulation. **Materials and Methods:** The chromatographic separation was performed on Waters Acquity UPLC system and BEH Shield RP18 column using gradient elution of mobile phase A and B. 0.01 M phosphate buffer adjusted pH 3.0 with Orthophosphoric acid was used as mobile phase A and 95% acetonitrile with 5% Milli Q Water was used as mobile phase B. Ultraviolet (UV) detection was performed at 254 nm and 210 nm, where (CDS-6), (CDS-5), (CDS-7), (Ethyl Candesartan), (Desethyl CCX), (N-Ethyl), (CCX-1), (1 N Ethyl Oxo CCX), (2 N Ethyl Oxo CCX), (2 N Ethyl) and any unknown impurity were monitored at 254 nm wavelength, and two process-related impurities, trityl alcohol and MTE impurity, were estimated at 210 nm. Candesartan cilexetil andimpurities were chromatographed with a total run time of 20 min. **Results**: Calibration showed that the response of impurity was a linear function of concentration over the range limit of quantification to 2 µg/mL ($r2 \geq 0.999$) and the method was validated over this range for precision, intermediate precision, accuracy, linearity, and specificity. For the precision study, percentage relative standard deviation of each impurity was <15% ($n$=6). **Conclusion:** The method was found to be precise, accurate, linear, and specific. The proposed method was successfully employed for estimation of candesartan cilexetil impurities in pharmaceutical preparations.

**Key words:** Degradent, impurities, method validation, ultra high-pressure liquid chromatography – candesartan cilexetil

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## INTRODUCTION

Candesartan cilexetil, a prodrug, is hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective $AT_1$ subtype angiotensin II receptor antagonist. Candesartan cilexetil, a nonpeptide, is chemically described as (±)-l-Hydroxyethyl 2-ethoxy-l-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester). Its empirical formula is $C_{33}H_{34}N_6O_6$.

It is practically insoluble in water and sparingly soluble in methanol, and soluble in acetonitrile. Candesartan cilexetil is a racemic mixture containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group.

In liquid chromatography, the analysis time can be reduced by using small columns packed with sub-2 µm particles. In addition, with sub-2 µm particles, due to the higher efficiency and smaller retention volume, sensitivity is also improved, compared with conventional High performance liquid chromatography (HPLC). Ultra High-Pressure Liquid Chromatography (UPLC), which uses 1.7-µm particles at a maximum operating pressure of 1,000 bar (compared with conventional

HPLC on 3.5- and 5-µm particles at 400 bar), has proved to be a suitable analytical technique with the advantages of high efficiency and resolution at greater linear velocities and reduced solvent consumption.

In order to improve the sensitivity and selectivity of the chromatographic determination of candesartan cilexetil impurities, a simple reversed-phase UPLC method, with UV detection at 254 nm and 210 nm, has been developed, where all 12 impurities have been separated in a single analytical column with a run time of 20 min. In our study, Water ACQUITY UPLC has been successfully used for the quantitative estimation of (CDS-6), (CDS-5), (CDS-7), (Ethyl Candesartan), (Desethyl CCX), (N-Ethyl), (CCX-1), (1 N Ethyl Oxo CCX), (2 N Ethyl Oxo CCX), (2 N Ethyl) at 254 nm and (Trityl Alcohol), (MTE Impurity) at 210 nm. A reduction in separation time has been achieved, without compromising separation quality, compared with other traditional Liquid Chromatography (LC) methods.

Candesartan cilexetil and its impurities' chemical structure are as shown in Figure 1a-m. Candesartan

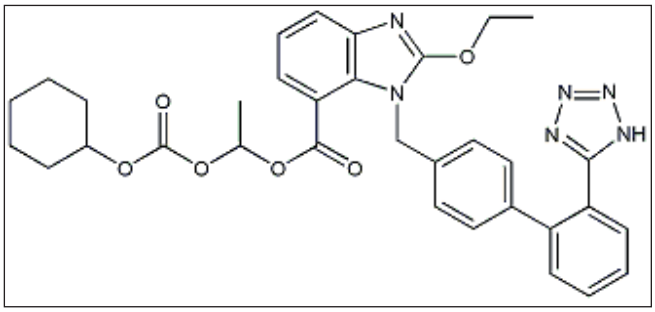

**Figure 1a:** Structures of candesartan cilexetil and its impurities (a) Candesartan Cilexetil. (±)-l-Hydroxyethyl 2-ethoxy-l-[p-(o-1H-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate

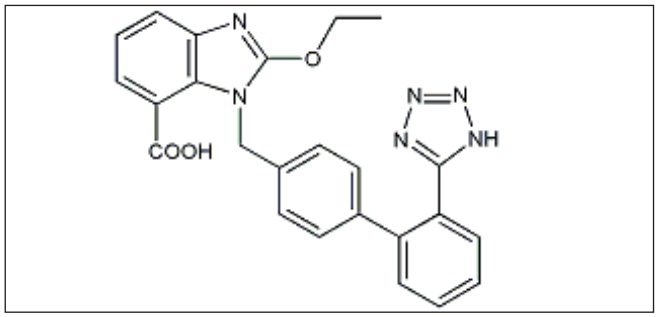

**Figure 1b:** Structures of candesartan cilexetil and its impurities (b) CDS-6. 2-Ethoxy-1-[[(2'-(1H-tetrazol-5-yl)biphenyl)-4-yl-]methyl] benzimidazole-7-carboxylic acid

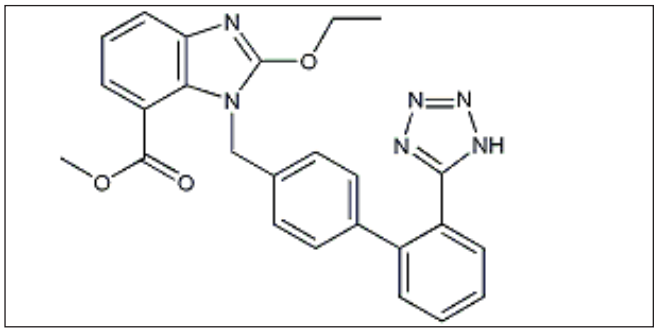

**Figure 1c:** Structures of candesartan cilexetil and its impurities (c) CDS-5. Methyl-2-ethoxy-1-[[(2'-(1H-tetrazol-5-yl) biphenyl)-4-yl-] methyl] benzimidazole-7-carboxylate

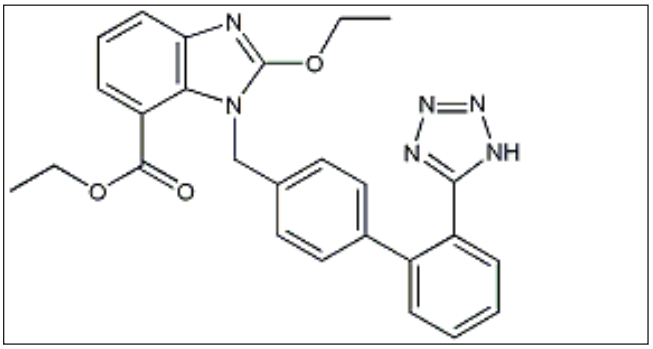

**Figure 1d:** Structures of candesartan cilexetil and its impurities (d) Ethyl Candesartan. Ethyl-2-ethoxy-1-[[(2'-(1H-tetrazol-5-yl) biphenyl)-4-yl-]methyl] benzimidazole-7-carboxylate

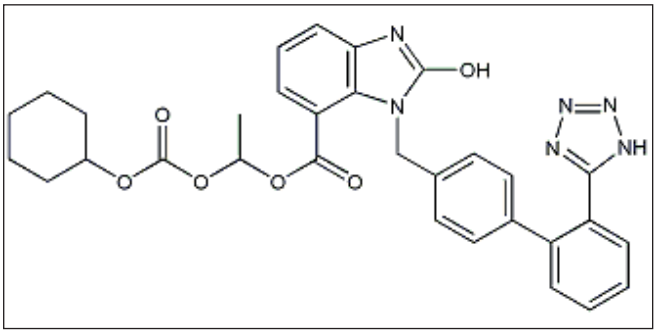

**Figure 1e:** Structures of candesartan cilexetil and its impurities (e) Desethyl CCX. (RS)-1-[[(cyclohexyloxy)carbonyl]oxy] ethyl 2-oxo-3-[[2'-(1H-tetrazol-5-yl)biphenylyl]methyl]-2,3-dihydro-1H-benzimidazole-4-carboxylate

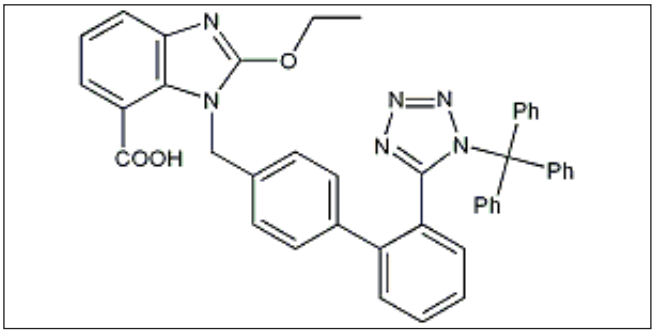

**Figure 1f:** Structures of candesartan cilexetil and its impurities (f) CDS-7 (ENH). 2-Ethoxy-1-[[2'-(1-tri phenylmethyl-1H-tetrazol-5-yl) biphenyl-4-yl] methyl]benzimidazole-7-carboxylic acid

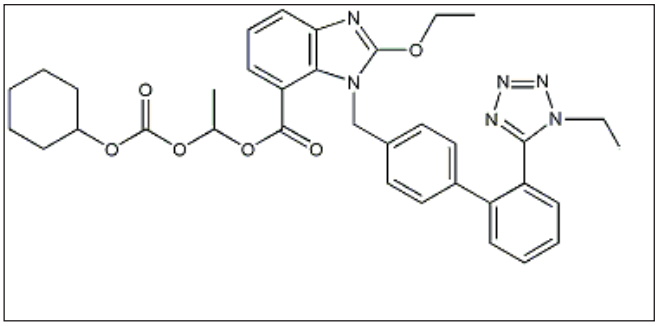

**Figure 1g:** Structures of candesartan cilexetil and its impurities (g) N-ETHYL IMPURITY. (RS)-1-[[(cyclohexyloxy)carbonyl]oxy] ethyl 2-ethoxy-1-[[2'-(2-ethyl-2H-tetrazol-5-yl)biphenylyl]methyl]-1H-benzimidazole-7-carboxylate

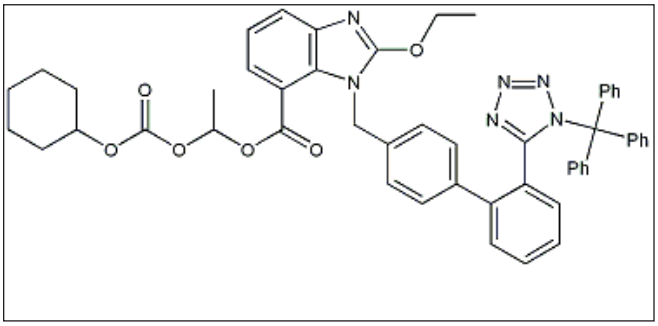

**Figure 1h:** Structures of candesartan cilexetil and its impurities (h) CCX-1. (±)-1-(cyclohexyloxycarbonyloxy) ethyl 2-ethoxy-1-[[2'-(1-tri phenyl methyl-1H-tetrazol-5-yl)biphenyl-4-yl]methyl] benzimidazole-7-carboxylate

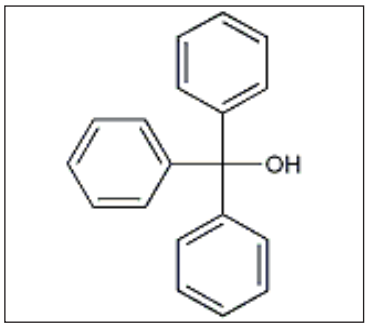

**Figure 1i:** Structures of candesartan cilexetil and its impurities (i) Trityl Alcohol. Tri phenyl methanol

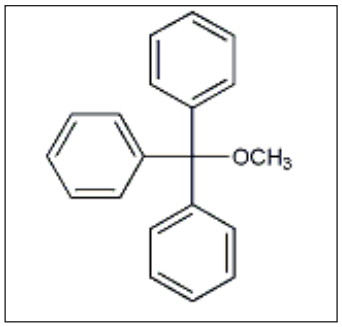

**Figure 1j:** Structures of candesartan cilexetil and its impurities (j) MTE IMPURITY. Tri phenyl methyl Methyl Ether

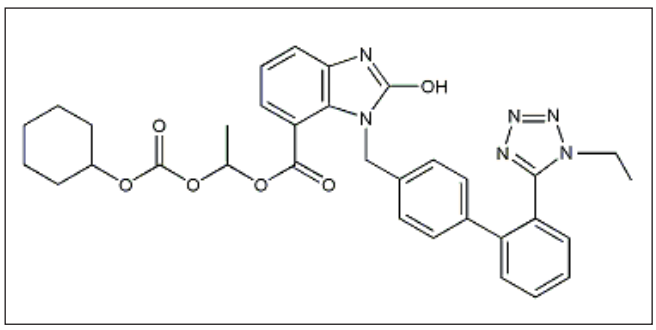

**Figure 1k:** Structures of candesartan cilexetil and its impurities (k) 1N-ETHYL OXO Candesartan Cilexetil (rigioisomers). 1-[(cyclohexyloxy) carbonyl] oxy}ethyl 2-oxo-1-{[2'-(1-ethyl-1H-Tetrazol-5-yl)biphenyl-4-yl] methyl}-1H-benzimidazole-7-carboxylate

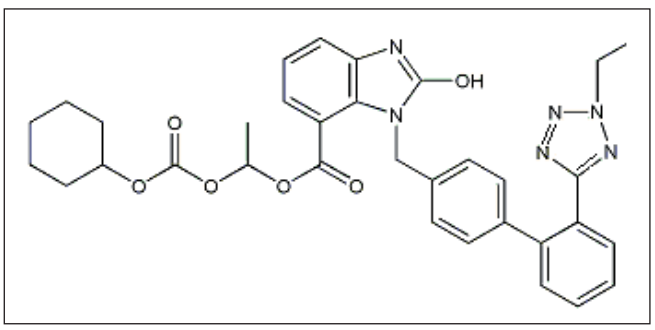

**Figure 1l:** Structures of candesartan cilexetil and its impurities (l) 2N-ETHYL OXO Candesartan Cilexetil (rigioisomers). 1-{[(cyclohexyloxy)carbonyl]oxy}ethyl 2-oxo-1-{[2'-(2-ethyl-1H-Tetrazol-5-yl)biphenyl-4-yl] methyl}-1H-benzimidazole-7-carboxylate

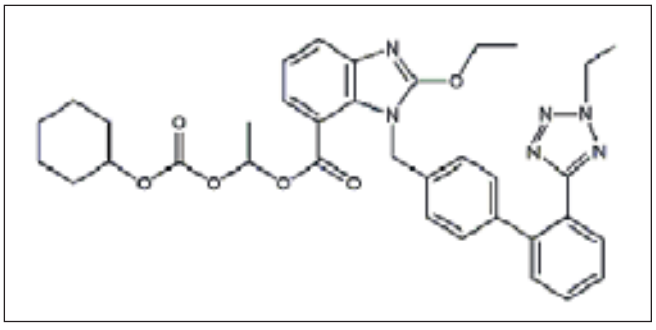

**Figure 1m:** Structures of candesartan cilexetil and its impurities (m) 2N-ETHYL IMPURITY. 1-{[(cyclohexyloxy) carbonyl]oxy} ethyl 2-ethoxy-1-{[2'-(2-ethyl-1H-tetrazol-5-yl)biphenyl-4-lyl]methyl}-1H-benzimidazole-7-carboxylate

cilexetil undergoes base hydrolysis to impurity CDS-6, acid hydrolysis to impurity Des Ethyl CCX, and thermal degradation to impurities Des Ethyl CCX, 1 N Ethyl Oxo CCX, 2 N Ethyl Oxo CCX, 2 N Ethyl, N Ethyl.

Single analytical approaches are available for the related substances of candesartan cilexetil in tablet formulations and drug substances.[1] A method for the isolation of degradation products is also available.[2] A number of assay methods for determination of candesartan cilexetil in pharmaceutical formulations and human plasma are also available.[3-7] A number of assay methods for determination of candesartan

cilexetil in combination pharmaceutical formulations are also available.[8,9] However; all the above-mentioned methods are orientated to the determination of the active pharmaceutical compound. Nowadays, the pharmaceutical industry is forced to assess a strict control of impurities when manufacturing drug substances and drug products. Determination of impurities during the development of separation methods is one of the main and difficult tasks for pharmaceutical analysts, especially if more and more impurities of closely related structure require determination. Methods are available for the estimation of candesartan cilexetil with spectrofluorimetry.[10]

To the best of our knowledge, none of the currently available analytical methods can separate all the known related compounds and degradation impurities of candesartan cilexetil dosage form. Attempts were made to develop a stability-indicating UPLC method for the estimation of related substance of candesartan cilexetil in solid orals (tablets).

The published candesartan cilexetil impurity method demonstrates analysis of estimation of candesartan cilexetil impurities in presence of placebo with detection wavelength at 254 nm and 210 nm. This paper deals with the forced degradation of candesartan cilexetil tablets under stress condition like acid hydrolysis, base hydrolysis, oxidation, heat, and UV light. This paper also deals with the validation of the developed method for the accurate quantification of impurities of candesartan cilexetil.

## EXPERIMENTAL PROCEDURE

### Chemicals and reagents

Candesartan cilexetil and its impurities was available from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Acetonitrile (HPLC—grade) was from J.T. Baker, USA. Potassium dihydrogen phosphate, hydrochloric acid, and hydrogen peroxide were from Merck (Darmstadt, Germany). Water was purified by a Millipore (Bedford, MA, USA) Milli-Q water-purification system and passed through a 0.22 μm membrane filter (Durapore; Millipore, Dublin, Ireland) before use.

Standard and test samples were prepared in water and acetonitrile in ratio of 80:20 as diluent.

### Equipment

UPLC analysis was performed with a Waters (Milford, MA, USA) Acquity UPLC system equipped with a binary solvent manager, sample manager, column-heating compartment, and photodiode array detector. This system was controlled by Waters Empower software.

An ACQUITY UPLC™ BEH Shield $RP_{18}$ column, 100 mm × 2.1 mm, 1.7 μm (Waters, Milford, MA, USA) was employed for chromatographic separation. All samples were centrifuged by Thermo Scientific multifuge machine. The specificity study was conducted by using heating oven, photostability chamber, and heating mantle (Thermo Lab, India).

### Standard and sample preparation

The impurity stock solution was prepared by dissolving an accurately weighed amount of impurity in diluent, resulting in a concentration of 100 μg/mL of each impurity.

The identification solution was prepared by dissolving 25 mg of candesartan cilexetil mixed with 1 mL of impurity stock solution and diluted to 50 mL in diluent.

The working standard solution of candesartan cilexetil was prepared by dissolving an accurately weighed amount of candesartan cilexetil working standard in diluent, resulting in a concentration of 500 μg/mL. Then, the above solution was further diluted in diluent to get a final solution of 2.5 μg/mL.

The test solution was prepared by dissolving an accurately weighed portion of the powder (20 tablets crushed with mortar and pestle), equivalent to 50 mg of candesartan cilexetil, in 80 mL diluent. After sonicating for around 20 min, the volume was made up to 100 mL, resulting in a concentration of 500 μg/mL. The above solution was centrifuged at 10,000 rpm for 5min in order to eliminate insoluble excipients. The supernatant liquid was used for chromatographic analysis.

### Chromatography

The analytes were separated on an ACQUITY UPLC™ BEH Shield $RP_{18}$ column, 100 mm × 2.1 mm, 1.7 μm column, at column oven temperature of 25°C with a gradient run program at a flow rate of 0.35 mL/min. 0.01 M phosphate buffer, adjusted pH 3.0 with orthophosphoric acid was used as mobile phase A, and Milli Q Water and acetonitrile in 5:95 v/v ratio was used as mobile phase B. The separation was achieved by gradient elution and the beginning ratio of mobile phase was A - B 50:50 (V/V); constant

at the same ratio for 2 min. Then, the ratio was changed linearly to 30:70 (V/V) within 10min; 0:100 (V/V) within 14 min; constant at the same ratio up to 16 min The system came back to the initial ratio at 17 min and continued at the same ratio up to 20 min. The mobile phase was filtered through a 0.22-µm Millipore filter before use. UV detection was performed at 254 nm and 210 nm. The sample injection volume was 1 µL in partial loop with needle overfills with 10-µL loop.

**Method validation**

The method was validated for specificity, precision, accuracy, sensitivity, and linear range as per the International Conference on Harmonization (ICH) guidelines.[11]

*System suitability*

Inject diluents as blank, identification solution for peak identification, and resolution between MTE impurity and CDS-7 peak at 210 nm; three standard solutions and calculate %Relative standard deviation (% RSD).

*stability*

The study was conducted to demonstrate the effective separation of candesartan cilexetil and its impurities. Also, the study was intended to ensure the effective separation of degradation peaks of formulation ingredients at the retention time of candesartan cilexetil and its impurities. Separate portions of drug product and ingredients were exposed to the following stress conditions to induce degradation.

The placebo (excipients without active) solution was prepared by dissolving an accurately weighed portion of the powder equivalent to 50 mg of candesartan cilexetil in 80 mL diluent. After sonicating for around 20 min, the volume was made up to 100 mL.

The drug product was subjected to base hydrolysis using 0.5 N Sodium hydroxide, acid hydrolysis with 0.5 N Hydrochloric acid at room temperature for 2 h, and neutral hydrolysis with water at 50°C for 16 h. Oxidation study was performed with 10% hydrogen peroxide solution at 25°C for 16 h. On photostability study, the drug product was sufficiently spread on Petri plates (1-mm thick layer), exposed to sunlight and UV light (1.2 million lux h) at ambient conditions for 7 days. Humidity study was performed separately by exposing the drug product to humidity at 25°C, 90% RH, for 7 days. Thermal degradation study was performed by heating the drug product at 105°C for 24 h. Similarly, placebo samples were prepared like the drug product by exposing formulation matrices without drug substance.

Physically stressed (photolytic, heat and humidity-stressed sample and placebo prepared as per section 2.3 under sample preparation) while chemically stressed sample prepared by dissolving an accurately weighed portion of the powder (20 tablets crushed with mortar and pestle), equivalent to 50 mg of candesartan cilexetil in 80 mL diluents, after sonicating for around 20 min, add 5 mL reagent (0.5 N Hydrochloric acid, 0.5 N Sodium hydroxide, water and 10% peroxide), kept for above conditions, kept at room temperature to attain room temperature, neutralized it (for acid or base sample), and the volume was made up to 100 mL resulting in a concentration of 500 µg/mL.

Stressed samples were injected into the UPLC system with photodiode array detector by the following test method conditions.

*Precision*

The precision of the test method was evaluated by using six samples of candesartan cilexetil tablet test preparation, spiking 0.2% of target concentration (500 µg/mL) with impurities blend solution to get the concentration of 1.0 µg/mL of each impurity and analyzed as per test method. The %RSD of area percent for each impurity was calculated. Intermediate precision was also studied using a different column and performing analysis on a different day.

*Accuracy*

To confirm the accuracy of the proposed method, recovery studies were carried out by standard addition technique. Samples were prepared in triplicate by spiking impurities in test preparation at the level of limit of quantification (LOQ), 50%, 100%, and 150% (a nominal concentration of about 0.125 µg/mL to 1.5 µg/mL) of the standard concentration.

*Sensitivity*

Sensitivity of the method was established with respect to limit of detection (LOD) and LOQ for candesartan cilexetil peak and its impurities (i.e. (CDS-6), (CDS-5), (CDS-7), (Ethyl Candesartan), (Desethyl CCX), (N-Ethyl), (CCX-1), (1 N Ethyl Oxo CCX), (2 N Ethyl Oxo CCX), (2 N Ethyl) at 254 nm and (Trityl Alcohol), (MTE Impurity) at 210 nm.). Series of concentration of drug solution and its impurities were injected; LOD and LOQ was established by visual method.

LOD and LOQ were experimentally verified by injecting six replicate injections of each impurity at the concentration obtained from the above formula.

*Linearity of detector response*

A series of solutions of candesartan cilexetil impurities in concentrations ranging from LOQ level to 200% (2.0 µg/mL) of standard concentration were prepared and injected into the UPLC system.

**Application of developed method**

The method suitability was verified by analyzing five different strengths of finished product in-house formulated product: 20 tablets (each containing 32 mg, 16 mg, 8 mg, 4 mg and 2 mg of candesartan cilexetil, respectively) were crushed using mortar and pestle and intimately mixed. Quantity equivalent to 50 mg of drug was weighed accurately and dissolved in 100 mL of diluent by 20 min sonication. The solution was centrifuged and injected. The developed method is suitable for stability sample analysis.[12, 13]

**Method development and optimization**

Retention time of impurity has been identified

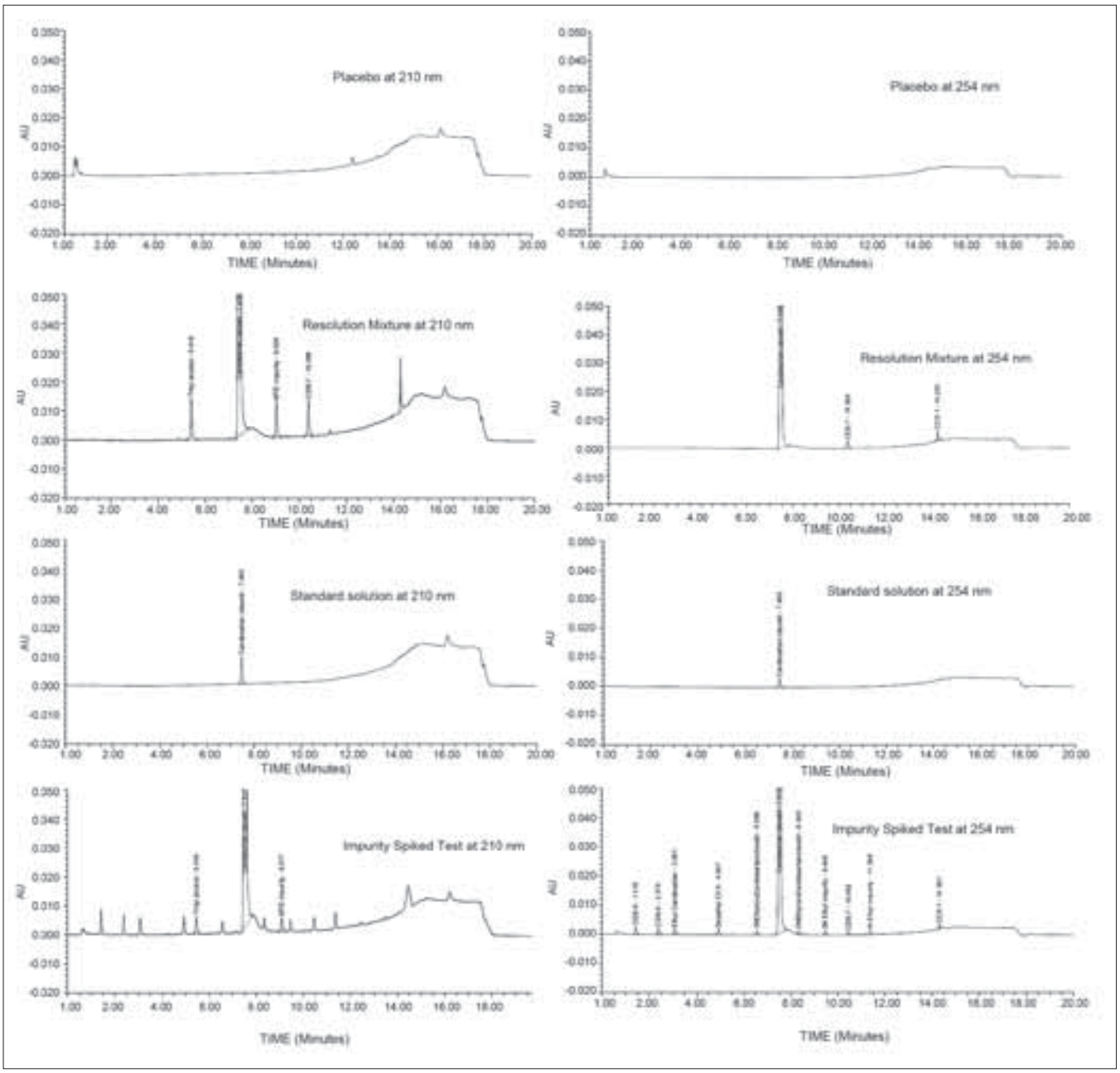


**Figure 2:** Typical chromatograms of candesartan cilexetil at 210 nm and 254 nm (placebo, resolution mixture, standard solution and test spiked with impurities) at optimized chromatographic conditions

by injecting impurity stock in chromatographic condition as per section 2.3. During development of the method, it has been observed that during accelerated stability studies (40°C 75% RH) impurities Des Ethyl CCX, 1 N Ethyl Oxo CCX, 2 N Ethyl Oxo CCX, 2 N Ethyl, N Ethyl are formed.

A reversed-phase chromatographic technique was developed to quantitate candesartan cilexetil and it impurities at 254 nm and 210 nm. The presence of nonaqueous solvents in the mobile phase, such as methanol and acetonitrile, was studied. While using methanol in the mobile phase B, impurity CCX-1 retention time increased significantly. Hence, acetonitrile was chosen as organic modifier. In mobile phase B, 95% acetonitrile is required to elute impurity CCX-1.

The C18 column was first evaluated as stationary phase for the separation of candesartan cilexetil and its impurities. Sensitivity of the method is also improved, compared with conventional HPLC method, by reducing the particle size of the stationary phase. Selectivity, sensitivity, resolution, and speed of chromatographic separation were optimized for the UPLC method. Comparing the signal-to-noise ratio of candesartan cilexetil shows that the proposed method has better sensitivity. The present UPLC method offers well resolution within 20 min. The retention times of candesartan cilexetil at 7.9, CDS-6 at 1.41, CDS-5 at 2.37, Ethyl Candesartan at 3.08, Desethyl CCX at 4.93, Trityl Alcohol at 5.45, 1 N Ethyl Oxo CCX at 6.56, 2 N Ethyl Oxo CCX at 8.34, MTE impurity at 9.07, 2 N Ethyl at 9.44, CDS-7 at 10.45, N-Ethyl at 11.36, CCX-1 at 14.30, respectively, under the chromatographic conditions described. Chromatograms obtained from placebo, resolution mixture, and test spiked with impurities mixture solution are shown in Figure 2.

**Table 1: System suitability**

| Parameter | Value |
|---|---|
| The tailing factor for candesartan cilexetil peak in standard preparation | 1.0 |
| Resolution between MTE impurity and CDS-7 peak at 210 nm | 3.3 |
| %RSD of candesartan cilexetil peak in three standard preparations | 0.1 |

## RESULTS AND DISCUSSION

UPLC system has been proved to be a promising tool for separation of candesartan cilexetil and its impurities. Use of small (1.7 μm) particles of stationary phase enabled optimization of UPLC for both peak selectivity and analysis speed. Candesartan cilexetil and its impurities were well separated with good peak shape and resolution. No interfering peaks were observed in blank and placebo, indicating that signal suppression or enhancement by the product matrices was negligible. Use of UPLC resulted in a reduction in run-time to 20 min, without compromising the efficiency, compared with a run-time of approximately 60 min on traditional LC analysis of candesartan cilexetil impurities. UPLC method will reduce acetonitrile consumption (at least 80%) without compromising productivity and performance.

After satisfactory method development,

**Table 2: Specificity**

| Stress condition | % Impurity degradation |
|---|---|
| Refluxed with 0.5 N HCl solution for about 1/2 hours at 25°C | 7.65 |
| Refluxed with 0.5 N NaOH solution for about 1/2 hours at 25ºC | 3.96 |
| Refluxed with 10% hydrogen peroxide for about 16 hours at RT | 1.53 |
| Refluxed with Water for about 16 hours at 50°C | 0.41 |
| Exposed to sunlight/UV for about 1.2 million lux h/200 watt h/$m^2$ | 0.34 |
| Dry heating done at 105°C for about 24 h | 13.5 |

**Table 3a: Regression and precision data**

| Parameter | CDS-6 | CDS-5 | Ethyl candesartan | 1-N Ethyloxo CCX | 2-N Ethyloxo CCX | 2-N Ethyl |
|---|---|---|---|---|---|---|
| LOD (μg/mL) | 0.015 | 0.025 | 0.03 | 0.04 | 0.035 | 0.045 |
| LOQ (μg/mL) | 0.06 | 0.085 | 0.09 | 0.11 | 0.1 | 0.135 |
| Correlation coefficient | 0.9999 | 0.9999 | 1.0000 | 1.0000 | 0.9999 | 1.0000 |
| Bias at 100% response | 5 | 4 | 3 | 4 | 1 | 0 |
| Precision (%RSD) | 0.7 | 0.5 | 0.4 | 1.0 | 0.8 | 0.6 |
| Intermediate precision (%RSD) | 1.7 | 1.8 | 2.0 | 1.6 | 2.9 | 4.0 |
| Precision at LOQ (%RSD) | 3.2 | 2.3 | 4.1 | 3.6 | 2.7 | 1.6 |

**Table 3b: Regression and precision data**

| Parameter | Trityl alcohol | MTE impurity | Desethyl CCX | N-Ethyl | CDS-7 | CCX-1 | Candesartan cilexetil |
|---|---|---|---|---|---|---|---|
| LOD (µg/mL) | 0.015 | 0.015 | 0.035 | 0.045 | 0.03 | 0.04 | 0.04 |
| LOQ (µg/mL) | 0.04 | 0.05 | 0.13 | 0.14 | 0.1 | 0.15 | 0.14 |
| Correlation coefficient | 1.0000 | 0.9999 | 1.0000 | 1.0000 | 0.9999 | 0.9999 | 1.0000 |
| Bias at 100% response | 0 | 2 | 1 | 1 | 4 | 3 | 0 |
| Precision (%RSD) | 0.6 | 0.6% | 0.4% | 0.8% | 0.9% | 1.0% | 0.5% |
| Intermediate precision (%RSD) | 0.7 | 0.8% | 2.6% | 1.5% | 2.2% | 3.3% | 3.7% |
| Precision at LOQ (%RSD) | 0.0% | 0.0% | 2.0% | 4.2% | 2.9% | 3.6% | 1.9% |

**Table 4: Evaluation of accuracy**

| Amount spiked | CDS-6 (%) | CDS-5 (%) | Ethyl candesartan (%) | 1-N Ethyloxo CCX (%) | 2-N Ethyloxo CCX (%) |
|---|---|---|---|---|---|
| LOQ | | | | | |
| %Recovery | 101.5 | 99.3 | 101.3 | 90.3 | 98.1 |
| %RSD | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| 50% | | | | | |
| %Recovery | 100.7 | 100.1 | 101.9 | 98.5 | 99.1 |
| %RSD | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 100% | | | | | |
| %Recovery | 100.2 | 100.7 | 102.2 | 99.5 | 102.8 |
| %RSD | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| 150% | | | | | |
| %Recovery | 99.8 | 101.4 | 104.2 | 100.7 | 103.9 |
| %RSD | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

| Amount spiked | 2-N Ethyl | Trityl alcohol | MTE impurity | Desethyl CCX | N-Ethyl |
|---|---|---|---|---|---|
| LOQ | | | | | |
| %Recovery | 98.8 | 90.1 | 91.0 | 99.1 | 93.8 |
| %RSD | 0.8 | 0.8 | 0.8 | 0.8 | 0.8 |
| 50% | | | | | |
| %Recovery | 95.6 | 99.9 | 96.9 | 98.6 | 95.3 |
| %RSD | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 100% | | | | | |
| %Recovery | 97.5 | 99.0 | 97.0 | 98.1 | 94.6 |
| %RSD | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| 150% | | | | | |
| %Recovery | 97.7 | 99.8 | 97.9 | 98.8 | 96.0 |
| %RSD | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

| Amount spiked | CDS-7 | CCX-1 | Candesartan cilexetil |
|---|---|---|---|
| LOQ | | | |
| %Recovery | 94.5 | 96.0 | 100.5 |
| %RSD | 0.8 | 0.8 | 0.8 |
| 50% | | | |
| %Recovery | 94.2 | 94.9 | 108.2 |
| %RSD | 1.0 | 1.0 | 1.0 |
| 100% | | | |
| %Recovery | 96.6 | 97.1 | 101.9 |
| %RSD | 0.4 | 0.4 | 0.4 |
| 150% | | | |
| %Recovery | 97.3 | 98.4 | 102.9 |
| %RSD | 0.3 | 0.3 | 0.3 |

%RSD values calculated with three sample recovery at each level

it was subjected to method validation as per ICH guidelines.[11] The method was validated to demonstrate that it is suitable for its intended purpose by standard procedure to evaluate adequate validation characteristics. The result of system suitability parameter was found to be complying

with acceptance criteria: Relative standard deviation standard area of replicate injection is not more than 5.0%, resolution between CDS-6 and MTE Impurity at 210 nm is more then 2.0, and relative retention time of impurity peak should comparable as shown in Table 1. The results of the specificity study ascertained the separation of degradation peaks from candesartan cilexetil peak, and the spectral purity of all exposed samples was found spectrally pure. Data of degradation studies is shown in Table 2. The %RSD of replicate determination percent area was found to be <5% in both precision and intermediate precision, which indicates that the method is precise. The data of precision studies is shown in Tables 3a and b. The results obtained from the recovery study were found within the range of 90% to 110% (LOQ to 150%), which indicates that method is accurate, and data for the same is shown in Table 4. Sensitivity of the method was verified and the method was found to be linear, accurate, and precise at LOQ. The data of LOD and LOQ studies is given in Tables 3a and b. The calibration curves of all impurities were obtained by plotting the peak area of individual impurity versus concentration over the range of about 0.02–2 µg/mL and were found to be linear ($r$=0.999). The data of regression analysis of the calibration curves is shown in Table 3. The impurity content in the in-house formulations was found to be satisfactory.

## CONCLUSION

Although liquid chromatography is a versatile technique for the analysis of drug in complex matrices such as biological or pharmaceuticals, a number of analytical approaches have been previously described to determine candesartan cilexetil in biological materials and pharmaceutical preparations. However, this is the first study reporting a validated reversed phase method for quantification of all impurities as well as degradents in candesartan cilexetil formulation. The simple UPLC method developed in this study makes it suitable for separation and estimation of impurities without interference from excipients and other related substances present in the pharmaceutical matrices. The analytical performance and the results obtained from analysis of two different formulations demonstrated that the method is reliable and sufficiently robust. In conclusion, the high sensitivity, good selectivity, accuracy, and reproducibility of the UPLC method developed in this study makes it suitable for quality control analysis of complex pharmaceutical preparations containing candesartan cilexetil and its impurities. The reduction of acetonitrile consumption is one of the best solutions to the current global acetonitrile shortage and will safeguard against future risk.

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