Specific and Stability Indicating Assay Method of Cadesartan Cilexetil in Presence of Process and Degradation Impurities

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Abstract:
Candesartan is used for the treatment of hypertension and works as angiotensin II receptor antagonists. In the presented paper a stability indicating HPLC method is described for the determination of assay of Candesartan Cilexetil, in presence process related impurities (Trityl alcohol and MTE) and degradation impurities. The degradation impurities are (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). The stability indicating property of the method was further established by stress studies in different degradation mediums. The method was validated using Kromasil C-18 reversed phase HPLC column, UV-Vis detection at 257 nm. Mobile phase consisted of 0.01 M of ammonium acetate buffer 45% of pH 4.5 and 55% of Acetonitrile. The method is validated for its specificity, precision, accuracy, linearity and ruggedness. Response of Candesartan is linear from 80.0 to 120.0 µg/ml which is 80% and 120 % of sample concentration level, Correlation coefficient was found 1.000.

Keywords: Reverse phase, HPLC, UV-Vis detection, C18 column, Degradation study.

Introduction:
Candesartan is chemically known as 2-ethoxy-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-1,3-benzodiazole-6-carboxylic acid and the empirical formula is C_{24}H_{20}N_{6}O_{3}, structure is given in figure-1. Its molecular weight is 440.45. The Candesartan is commercially available as Candesartan Cilexetil chemically known as 1-cyclohexyloxy) carbonyl oxy] ethyl 2-ethoxy-1-[{2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl[methyl]-1H-benzimidazole-7-carboxylate and the empirical formula is C_{33}H_{34}N_{6}O_{6}. Its molecular weight is 610.67 and the chemical structure is given below. Candesartan is a angiotensin II receptor antagonist used mainly for the treatment of hypertension. As all angiotensin II receptor antagonists, Candesartan is indicated for the treatment of hypertension. Results from the CHARM (Candesartan in heart failure-Assessment of reduction in mortality and morbidity program) study in the early 2000s demonstrated the morbidity and mortality reduction benefits of Candesartan therapy in congestive heart failure. Candesartan works by making the blood vessels relax and widen. This helps to lower the blood pressure and make it easier to purge blood to all parts of the body. Candesartan is also available in a combination formulation with

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a low dose thiazide diuretic, invariably hydrochlorothiazide, to achieve an additive antihypertensive effect \[1\].

Literature survey revealed that several chromatographic and non-chromatographic methods are reported, some pharmaceutical manufacturers are producing the drug and quality of drug is monitored with in-house developed analytical methods. Some significant contributions are available as, Vishnu P.Chaudhari has developed an UV method for the determination of Candesartan Cilexetil and Amlodipine besilate in tablet dosage forms by selecting two wavelengths (291nm and 360nm) and zero absorption method\[2\]. Similarly Bhadke Tejaswani has developed UV method at (251nm and 273nm) for Candesartan combination with Hydrochlorothiazide tablet dosage form\[3\]. Ayisha Khalid has developed flow injection spectrophotometric and spectrofluorimetric method for determination of Candesartan in different formulations and the same method has been compared with HPLC method. In this method few surfactants like CTAB(Cetyl trimethyl ammonium bromide) and SDS (Sodium dodecyl sulphate) were used\[4\]. Incilay Suslu et al, developed a method for the determination of Candesartan Cilexetil by Square-wave adsorptive stripping voltametric in pharmaceutical formulations\[5\]. An isocratic reversed-phase liquid chromatographic method has been developed by D. V. Subba Rao for quantitative determination of Candesartan cilexetil, used to treat hypertension, in the bulk drug and in pharmaceutical dosage forms. The method is also applicable to analysis of related substances. Chromatographic separation was achieved on a 250 mm × 4.6 mm, 5 μm particle, CN column with a 50:50 (v/v) mixture of phosphate buffer, pH 3.0, and acetonitrile as mobile phase. The flow rate was 1.0 mL min\(^{-1}\) and the detection wavelength was 210 nm.\[6\]

The pharmacodynamic properties of Candesaratan was studied in human by Claudius Malerzyk with the help of HPLC and RRA (Radio receptor assay).\[7\] Five potential degradation impurities in Candesartan cilexetil tablets were identified, Isolated and characterized by Arivozhi mohan\[8\].

No references are available for the analytical process which is specific even in presence of process and degradation impurities.

Some references available where different chemical entities were used as an internal standards for estimation of Candesartan Cilexetil assay in bulk and formulation. Bromhexine\[9\] and Paracetamol \[10\] are known to be used as an internal standards. The other reference is also available to analyze the Candesartan Cilexetil in presence the combination drugs and excipients.\[11\]

Derivative Spectroscopy UV analytical methods\[12\] are also available for Candesartan Celextil assay estimation but these methods are having limited utility and unable to indicate the impurities present with the product. Many references are available in the literature for the development of impurities and some researchers have reported analytical methods for determination of impurities of Candesartan.

There are studies available on UPLC \[13\], where the impurities were separated.
Present study involves the development of a method for the estimation of assay of Candesartan cilexetil in presence of its process impurities and degradation impurities in API using high performance liquid chromatography (HPLC) with ultraviolet detector and it is subsequently validated as per USP and ICH guidelines. In present study no additional internal standards are required to be used. The ratio selected for buffer and acetonitrile made the separation of impurities and its elusion possible, so that it can be clearly marked against its retention time in chromatograms for assessment. The proposed method is simple, accurate, reproducible, stability indicating and suitable for routine use.

MATERIALS AND METHODS:

Chemicals and Reagents:
Acetonitrile and Methanol of HPLC grade were used of Rankem. Ultra pure Millipore water was used. Ammonium Acetate (AR grade), Glacial acetic acid (HPLC grade), Tetrahydrofuran (HPLC grade), Hydrochloric acid (AR grade), Sodium hydroxide (AR grade), Hydrogen peroxide (AR grade) were obtained from Merck, In house prepared Candesartan Cilexetil working standard, Sample and impurities standards were used.

Instrumentation and Chromatographic conditions:

The HPLC system (alliance, waters) consisting of UV / PDA detector with Empower software was used. Analytical balance (Mettler Toledo) was used. HPLC analysis was conducted using a Kromasil C18, 250mm x 4.6 mm, 5µm particle size column. Mobile phase constituted of 0.01M Ammonium Acetate solution, pH adjusted to 4.5 with Acetic acid, buffer 45 % and Acetonitrile 55%, Methanol was used as diluents. Isocratic chromatographic method with 10 min run time was used with UV detection at 257 nm, flow rate 1.5 ml/min., column oven temperature 30°C and injection volume 20µl.

Blank was prepared by mixing 5 ml of Tetrahydrofuran with 45 ml of methanol in a 50 ml volumetric flask, for Preparation of standard solution 50 mg of Candesartan Cilexetil standard was weighed and transferred in 50 ml volumetric flask 5 ml of Tetrahydrofuran was added Sonicated to dissolve, and further diluted stepwise to get a final concentration 100 µg/ml. And sample solutions were prepared similarly as standard solution. (final concentration 100 µg/ml). To establish system suitability criteria the the theoretical plate for Candesartan peak should not be less than 3500, the tailing factor should not be more than 2.5 and Relative standard deviation of six replicate injections of standard solution should not be more than 5.0% were followed during different days of analysis.

METHOD VALIDATION:

Method validation studies were performed using in-house produced sample of Candesartan Cilexetil, Standard and impurities. ICH Guideline for Validation of Analytical Procedures Q2 (R1) was followed for validation study [14, 15]. Different validation parameters were studied e.g. Specificity and selectivity, Linearity and range, Precision and Accuracy etc. Sample solution was kept at room temperature and analyzed at different time intervals; it was
found that sample solution can be used at least up to 15 hours of preparation. Forced degradation study was performed to prove specificity and to establish the stability indicating property of the method.

RESULT AND DISCUSSION

Specificity and Selectivity:
Blank, sample solution and a sample spiked with all known impurities were injected in HPLC and the peak purity of analyte peak was checked. No any interfering peak was found in blank at the retention time of Candesartan. The peak purity of Candesartan peak passes. Data is shown in table-1 and peak purity plot is given in figure-2.

Forced Degradation (Stress study):
Specificity and Stability indicating property of the developed method was further proved by forced degradation study. Sample solutions were prepared and exposed with acid, alkali, peroxide, thermal, photolytic and humidity, these exposed samples injected in HPLC and assay values were calculated.

For Acid hydrolysis sample was treated with diluted Hydrochloric acid (0.1M) and kept at 80°C for about 30min to accelerate acid hydrolysis. Finally sample was analyzed and assay value was estimated.

For Alkali hydrolysis sample was treated with 5 ml diluted Sodium hydroxide (1M), kept at room temperature for 5 minutes. Finally sample was analyzed and assay value was estimated.

For Oxidative degradation sample was treated with 5 ml diluted Hydrogen peroxide (5%v/v) and kept at 80°C for about 120 min to accelerate oxidation of Candesartan Cilexetil. Finally sample was analyzed and assay value was estimated.

Thermal degradation sample was kept at 80°C for 7 days and assay value was estimated. For Photolytic degradation sample was exposed to Fluorescence light for 15 days, analyzed for assay. For Humidity degradation sample was exposed to 100 % RH for 15 days and analyzed for assay.

The peak purity of analyte peak passes and results for different degradation conditions are tabulated in table-2. Representative chromatograms are attached in figure-3.

The peak purity of the analyte passes in different degradation conditions. Hence, the method is stability indicating.

Linearity and range:
Five different concentrations of sample solution from 80 µg/ml to 120 µg/ml of Candesartan were prepared and injected in HPLC, Correlation coefficient, slop and intercept were calculated and data is provided in Table-3 and represented graphically in figure-4. Correlation coefficient, slop and intercept were found 1.000, 22330 and 31930 respectively.

Precision and Accuracy:
System precision (system repeatability), method precision (n=6) and intermediate precision (n=6) was studied in sample at the 100 % of sample concentration, % relative standard deviation of results was calculated. Ruggedness of the method was established by comparing the results obtained on different days by different analyst on different instrument and HPLC column, accuracy of method was established by linearity and precision experiments, data given in Table-4.

CONCLUSION:
Presented method was found to be sensitive enough with linearity in the concentration range of 80–120 µg/ml. Method is specific as peak purity plot indicates no interference in the Candesartan peak, accuracy of method was inferred from linearity and precision study, the precision values are within acceptable limits at different samples and the data in table-4 indicated that the method is precise and rugged. Representative chromatogram is presented in figure-2 and figure-3 and the different values of validation data; linearity, precision, ruggedness, accuracy, limit of quantification and limit of detection are given in table-4.

The presented work describes that developed reverse phase HPLC method for the determination of Candesartan is Specific, Rugged, Linear, Accurate, Precise in presence of its process and degradation impurities. And the method can be applied for quantification of Candesartan cilexetil API.

ACKNOLEDGEMENT
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### TABLE 1: PEAK PURITY DATA OF SAMPLE AND SPIKED SAMPLE.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Purity Angle</th>
<th>Purity Threshold</th>
<th>Peak purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unspiked sample</td>
<td>0.024</td>
<td>1.010</td>
<td>pass</td>
</tr>
<tr>
<td>Spiked Sample</td>
<td>0.029</td>
<td>1.008</td>
<td>pass</td>
</tr>
</tbody>
</table>

### TABLE 2: FORCED DEGRADATION DATA OF CANDESARTAN IN DIFFERENT CONDITIONS

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Assay (% w/w)</th>
<th>% Degradation</th>
<th>Purity Angle</th>
<th>Purity Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>99.8</td>
<td>-</td>
<td>0.031</td>
<td>1.013</td>
</tr>
<tr>
<td>Acid degradation</td>
<td>87.5</td>
<td>12.3</td>
<td>0.032</td>
<td>1.042</td>
</tr>
<tr>
<td>Alkali degradation</td>
<td>74.9</td>
<td>24.9</td>
<td>0.032</td>
<td>1.033</td>
</tr>
<tr>
<td>Peroxide degradation</td>
<td>99.4</td>
<td>0.4</td>
<td>0.038</td>
<td>1.036</td>
</tr>
<tr>
<td>Thermal degradation</td>
<td>98.9</td>
<td>0.9</td>
<td>0.023</td>
<td>1.012</td>
</tr>
<tr>
<td>Photolytic degradation</td>
<td>99.2</td>
<td>0.6</td>
<td>0.024</td>
<td>1.007</td>
</tr>
<tr>
<td>Humidity degradation</td>
<td>99.2</td>
<td>0.6</td>
<td>0.025</td>
<td>1.009</td>
</tr>
</tbody>
</table>

### TABLE 3: LINEARITY DATA OF CANDESARTAN

<table>
<thead>
<tr>
<th>Concentration (μg/mL)</th>
<th>Area Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>80.0</td>
<td>1825858</td>
</tr>
<tr>
<td>90.0</td>
<td>2035261</td>
</tr>
<tr>
<td>99.9</td>
<td>2259614</td>
</tr>
<tr>
<td>109.9</td>
<td>2480827</td>
</tr>
<tr>
<td>120.0</td>
<td>2718395</td>
</tr>
</tbody>
</table>

Slope: 22330  
Intercept: 31930  
Correlation coefficient: 1.000
TABLE 4: Validation Report of HPLC Method For The Determination of Candesartan

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linearity</strong></td>
<td>0.80–120 µg/ml</td>
<td>$R^2 = 1.000$</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>22330</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td>31930</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) System Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD (n=6)</td>
<td>100 µg/ml (standard)</td>
<td>0.60 %</td>
</tr>
<tr>
<td>(b) Method precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD (n=6)</td>
<td>99.7 % w/w</td>
<td>0.10 %</td>
</tr>
<tr>
<td>(c) Intermediate precision by recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD (n=6)</td>
<td>99.6 % w/w</td>
<td>0.20 %</td>
</tr>
<tr>
<td>(d) Ruggedness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD (n=12)</td>
<td>99.7 % w/w</td>
<td>0.15 %</td>
</tr>
<tr>
<td><strong>Stability of solution</strong></td>
<td>100 µg/ml</td>
<td>15 hours</td>
</tr>
</tbody>
</table>

n = number of determinations.

Fig.1: Structure of Candesartan Cilexetil

Fig.2: Peak purity plot of Candesartan in spiked sample.
Fig. 3: Chromatogram of stress study
Fig. 4: Linearity plot of Candesartan.