Development and Validation of Stability Indicating RP-HPLC Method for the Estimation of Cinacalcet Hydrochloride in Bulk and Their Formulations

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Abstract: A Simple, selective, accurate, precise, linear, and stability-indicating RP-HPLC method was developed and validated for the estimation of Cinacalcet hydrochloride in bulk and tablet dosage forms. Chromatographic separation was achieved on X-Terra Symmetry C18 (4.6 x 150mm; 5 μm) with mobile phase containing Phosphate buffer: Acetonitrile (40:60 v/v) pH adjusted to 3.0 ±0.05 with diluted ortho-phosphoric acid. The flow rate was maintained at 0.9 mL/min. The eluent was monitored at 282 nm. Moreover, the retention time of Cinacalcet was 2.8 minutes. The method was validated for linearity, accuracy, precision, and robustness as per ICH guidelines. The developed method was found linear between 25-150 μg/ml, and the linear regression coefficient was 0.999. The % RSD values are less than 2 % indicating the accuracy and precision of the method. The percentage of recovery was obtained from 98-102%. The system suitability parameters were found to be within the limit. Forced degradation studies were conducted under various conditions. The proposed method is simple, rapid, precise, and accurate. It can be used for the quantitation of Cinacalcet hydrochloride in bulk and commercial pharmaceutical dosage forms.

Keywords: Cinacalcet Hydrochloride, Method development, Validation, Stability-indicating, ICH Guidelines, RP-HPLC.

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1. Introduction

Cinacalcet hydrochloride is a calcimimetic used for the treatment of secondary hyperthyroidism in patients to reduce hypercalcemia. Chemically, cinacalcet hydrochloride is \((R)-N-[1-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl) phenyl]propan-1-amine\) (Fig.1). The molecular formula is \(C_22H_22F_3N\), and the molecular weight is 357.412 g/mol. Cinacalcet hydrochloride is the first of a new class of drugs, the calcimimetics, which acts by increasing the sensing receptors in the parathyroid gland [1-3]. It is also used in the treatment of hypercalcemia in patients with parathyroid carcinoma. Overdose may lead to tachycardia, severe hypotension, and convulsions. A literature survey revealed that very few analytical methods had been reported for the determination of Cinacalcet in pure drug, pharmaceutical dosage forms, and biological samples using liquid chromatography and tandem mass spectrometry [4-24]. The developed RP-HPLC method was simple, fast, accurate, and stability-
indicating. The developed method was useful for the determination of Cinacalcet hydrochloride in bulk and tablet dosage forms.

![Chemical Structure of Cinacalcet Hydrochloride](image)

**Figure 1.** Chemical Structure of Cinacalcet Hydrochloride.

2. Materials and Methods

2.1. Chemicals and reagents.

Cinacalcet hydrochloride reference standard was received as a gift sample from Dr. Reddy’s Laboratories, Hyderabad, India. All chemicals and reagents used for the analysis are HPLC grade.

2.2. Instrumentation.

HPLC analysis was performed on Waters HPLC e2695 series consisting of pump, autosampler, UV-Vis detector, with Empower 2 software.

2.3. Buffer preparation.

Weighed and transferred 7.0 grams of Potassium dihydrogen phosphate into a 1000 mL beaker, dissolved and diluted to 1000 mL with HPLC water. Adjusted the pH to 3.0±0.05 with orthophosphoric acid; *Diluent: Mobile phase.*


10 mg of Cinacalcet working standard was weighed accurately and transferred into a 100 mL volumetric flask, dissolved in 60 ml of diluent and diluted the volume up to the mark with diluent and mixed well. 1.5 mL of the standard stock solution was diluted to, in a 10 mL volumetric flask, and mixed well.

2.5. Sample preparation.

Weighed twenty Cinacalcet hydrochloride tablets, and the average weight was calculated. Accurately weighed and transferred the sample equivalent to 10 mg of Cinacalcet into a 100 mL volumetric flask. Added about 70 mL of diluent and sonicated to dissolve entirely and made the volume up to mark with diluent. Mixed well and filtered through a 0.45 µm filter. Further pipetted 1.5 mL of the above stock solution into a 10 mL volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

2.6. Assay of Cinacalcet HCL (% of label claim).

Assay value in mg/tablet (rounded off value)/Label Claim X 100.
3. Results and Discussion

3.1. Method development.

RP-HPLC method was developed, keeping in mind the system suitability parameters, i.e., tailing factor (T), number of theoretical plates (N), run time, and cost-effectiveness.

3.1.1. Optimized chromatographic conditions.

Chromatography was achieved on Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) with a mobile phase containing Phosphate buffer pH 3.0±0.05 (adjusted with orthophosphoric acid), and Acetonitrile in the ratio of 40:60 v/v at a flow rate of 0.9 mL/min. The eluent was monitored at 282 nm. The injection volume was 20 µl, and the column temperature was maintained at 30°C. The optimized, developed method resulted in the elution of Cinacalcet at a retention time of 2.8 min. Figure 2 represents the chromatogram of the standard solution.

![Figure 2. Typical chromatogram of the standard solution](https://biointerfaceresearch.com/)

The total run time was maintained as 8 minutes. System suitability tests are used to ensure the adequate performance of the chromatographic system. Retention time, the number of theoretical plates, and peak tailing factor (T) were evaluated for six replicate injections of the standard at working concentration. The results are given in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cinacalcet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.885</td>
</tr>
<tr>
<td>Number of Theoretical plates (N)</td>
<td>6632</td>
</tr>
<tr>
<td>Tailing factor (T)</td>
<td>1.05</td>
</tr>
</tbody>
</table>

In order to verify the applicability of the developed method to a commercial formulation, ‘CAPICET’ was analyzed at working concentration and shown in Figure 3.

![Figure 3. Typical chromatogram of the sample solution.](https://biointerfaceresearch.com/)

The analyte peak in the sample was identified by comparing the retention time with the standard drug. System suitability parameters were found within the acceptable limits. The
introduction of separated peak area was done, and drug concentration was determined by using
the peak area concentration relationship obtained in the standardization step. The assay of the
drug in the sample ranging between 98.0 and 102.0 %, which is the standard and acceptance
level in any pharmaceutical quality control laboratory. The results are given in Table 2.

Table 2. Assay results of Cinacalcet.

<table>
<thead>
<tr>
<th>TABLET</th>
<th>Label Claim (mg)</th>
<th>Amount found (mg/tablet)</th>
<th>% Label claim± S.D.</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capicet</td>
<td>30</td>
<td>30.01</td>
<td>99.85±0.1247</td>
<td>100.86</td>
</tr>
</tbody>
</table>

*average of three determinations

3.2. Method validation.

Validation of the developed method was performed in order to prove that the method
was suitable for its intended use. The method was validated according to the International
Conference on Harmonization (ICH) guidelines for validation of analytical procedures [25-26].
The method was validated for the system suitability, specificity, linearity, accuracy, precision,
ruggedness, robustness.

3.2.1. Specificity.

The specificity of the analytical method is its capability to measure analyte precisely
and particularly in the presence of other components that may be likely to be present in the
sample matrix. Chromatograms of blank (Figure 4) proves that the method was specific.

![Specificity Chromatogram of Cinacalcet.](image)

3.2.2. System precision.

Injected six replicate injections of the standard solution at working concentration
showed % RSD (Relative Standard Deviation) less than 2.0, which indicates the acceptable
reproducibility and thereby the precision of the system.

3.2.3. Method precision.

Method precision was determined under the tests of (i) repeatability (Intra-day
precision) and (ii) Intermediate precision/ Ruggedness/ Inter day precision performed at
working concentration by different analysts on different instruments with columns of different
lot numbers.

3.2.4. Repeatability (Intra-day precision).

The intraday precision was determined with six assay preparations of the Cinacalcet
test solution with respect to the valid working standard. The % RSD for six assay results was
less than 2 %, which indicates that the developed method is precise by the test of repeatability. The results were tabulated in table 3.

### 3.2.5. Intermediate Precision (Ruggedness / Inter day precision).

Intermediate precision was evaluated by performing method precision on different days by different analysts, showed % RSD less than 2, which indicates the method developed is precise or rugged. The results were tabulated in table 3.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Intraday precision</th>
<th>Inter day precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak Area</td>
<td>% Assay</td>
</tr>
<tr>
<td>1</td>
<td>3737080</td>
<td>102.1</td>
</tr>
<tr>
<td>2</td>
<td>3728170</td>
<td>102.0</td>
</tr>
<tr>
<td>3</td>
<td>3738936</td>
<td>102.5</td>
</tr>
<tr>
<td>4</td>
<td>3732249</td>
<td>102.4</td>
</tr>
<tr>
<td>5</td>
<td>3754858</td>
<td>102.4</td>
</tr>
<tr>
<td>6</td>
<td>3762639</td>
<td>102.8</td>
</tr>
<tr>
<td>AVG</td>
<td>3742322</td>
<td>102.4</td>
</tr>
<tr>
<td>STDEV</td>
<td>13495.33</td>
<td>0.28</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.36</td>
<td>0.28</td>
</tr>
</tbody>
</table>

### 3.2.6. Linearity.

The linearity plot was constructed with six concentrations at 25-150% levels (5, 10, 15, 20, 25, and 30 μg/mL of CIN). The calibration curve was constructed by plotting the concentration level of the drug versus the corresponding peak area. The results proved that an excellent correlation between peak area and concentration level of the drug within the concentration range and the results are given in Tables 4 to 5 and Figure 5. The correlation coefficient was found to be 0.999, which meets the method validation acceptance criteria. Hence, the method is said to be linear.

### 3.2.7. Accuracy.

Accuracy was determined through recovery experiments, by the determination of % mean recovery of the sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated, as shown in Table 6. The accepted limits of recovery are 98% - 102% and all observed data are within the required range, which indicates good recovery values, and hence the accuracy of the method developed.
Figure 5. Calibration graph of Cinacalcet

Table 6. Results of Accuracy studies for Cinacalcet

<table>
<thead>
<tr>
<th>Concentration Level (%)</th>
<th>Area</th>
<th>*% Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1832703</td>
<td>101.37</td>
</tr>
<tr>
<td>100</td>
<td>3588525</td>
<td>100.13</td>
</tr>
<tr>
<td>150</td>
<td>5379174</td>
<td>101.07</td>
</tr>
</tbody>
</table>

*Mean of three replicates

3.2.8. Robustness.

The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions like flow rate, wavelength, and temperature. System suitability data was found to be satisfactory during the variation of the analytical conditions. Results of system suitability show that the analytical method remained unaffected by slight but deliberate changes in the analytical conditions. The results were summarized in table 7.

Table 7. Evaluation data of robustness study of Cinacalcet

<table>
<thead>
<tr>
<th>Robust conditions</th>
<th>Rt (min)</th>
<th>Peak area</th>
<th>System-suitability parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Theoretical Plates</td>
</tr>
<tr>
<td>Temp 25°C</td>
<td>3.124</td>
<td>3495145</td>
<td>6574</td>
</tr>
<tr>
<td>Temp 30°C</td>
<td>2.881</td>
<td>3435628</td>
<td>6570</td>
</tr>
<tr>
<td>Temp 35°C</td>
<td>2.552</td>
<td>3691542</td>
<td>6715</td>
</tr>
<tr>
<td>Flow 0.9mL/min</td>
<td>3.261</td>
<td>3586472</td>
<td>6659</td>
</tr>
<tr>
<td>Flow 1.0mL/min</td>
<td>2.881</td>
<td>3435628</td>
<td>6570</td>
</tr>
<tr>
<td>Flow 1.1mL/min</td>
<td>2.456</td>
<td>3610472</td>
<td>6625</td>
</tr>
<tr>
<td>Less wavelength 280 nm</td>
<td>2.765</td>
<td>3345892</td>
<td>6587</td>
</tr>
<tr>
<td>wavelength 282 nm</td>
<td>2.881</td>
<td>3435628</td>
<td>6570</td>
</tr>
<tr>
<td>More wavelength 284 nm</td>
<td>2.881</td>
<td>3563544</td>
<td>6642</td>
</tr>
</tbody>
</table>

3.2.9. Forced degradation.

Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like the exposure of sample solution to acid (0.1 N HCl), base (0.1 N NaOH), peroxide (3 % H₂O₂), thermal (at 50°C for 24 hrs.) and UV( exposed to UV light for 7 days). In each degradation condition, no interference was observed with the analyte peak. Figure 6-10 represents the degradation of chromatograms and their purity plots. In each degradation study, it was observed that the purity threshold is greater than the purity angle. It indicates that the peak was said to be pure. The results are presented in Table 8.
Table 8. Results of Degradation studies for Cinacalcet

<table>
<thead>
<tr>
<th>Nature of the Sample</th>
<th>% Assay</th>
<th>% Degradation</th>
<th>Purity angle</th>
<th>Purity threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td>87.47</td>
<td>-12.53</td>
<td>0.114</td>
<td>0.232</td>
</tr>
<tr>
<td>Base</td>
<td>89.73</td>
<td>-10.27</td>
<td>0.232</td>
<td>0.546</td>
</tr>
<tr>
<td>Thermal</td>
<td>90.87</td>
<td>-9.13</td>
<td>0.223</td>
<td>0.643</td>
</tr>
<tr>
<td>Peroxide</td>
<td>92.9</td>
<td>-7.1</td>
<td>0.104</td>
<td>0.294</td>
</tr>
<tr>
<td>UV</td>
<td>90.75</td>
<td>-9.25</td>
<td>0.285</td>
<td>0.632</td>
</tr>
</tbody>
</table>
4. Conclusions

The developed method proved that less consumption of organic phase; hence the method was economical. A good linear relationship was found for the drug between concentration ranges of 5 to 30 μg/ml. The inter-day and intraday precision results were found satisfactory proved that the developed method is precise and reproducible because of the low % RSD (below 2%). Accuracy studies revealed that mean recoveries were between 98.0 and 102.0%, and indicative of the accurate method. No variation was observed in the system suitability results even after small, deliberate changes were made in the flow rate and temperature; hence the method was robust. Degradation studied is performed under different conditions like acid, base, peroxide, UV, and thermal. In each degradation study, it was observed that the purity angle is less than the threshold value. It indicated the no interference of degradants with the drug peak, so the peak was said to be pure. Degradation studies proved that the developed method was stability-indicating.

A stability-indicating and economic RP-HPLC method were developed and validated as per ICH guidelines in terms of specificity, accuracy, precision, linearity, ruggedness, robustness for the quantitative estimation of Cinacalcet in tablets.

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Conflicts of Interest

The authors declare no conflict of interest.

References