Determination and Validation of HPTLC Method for Cinacalcet Hydrochloride

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Abstract
Cinacalcet Hydrochloride (CIN) is a new calcimimetic agent indicated for the use in hypercalcemia. The present work is aimed at development and validation of a novel and simple high-performance thin-layer chromatographic (HPTLC) method for the analysis of Cinacalcet Hydrochloride (active pharmaceutical ingredient, API). In the method, Aluminum-backed silica gel 60 F254 plates (10 × 10 cm) were used as stationary phase and chloroform: acetonitrile (6:4, v/v) as the mobile phase, which showed compact bands of Cinacalcet HCl (RF 0.30 ± 0.02). Quantitative analysis was carried out by densitometry at a wavelength of 282 nm. Linear regression analysis for the calibration spots showed good correlation with regression coefficient $r^2 = 0.9994$ in the range of 40 - 160 ng/band. The developed method suitability for quantification of CIN was learned by validating it as per the ICH guidelines. CIN detection limit was 0.48 ng/band and the quantification limit was 1.59 ng/band. The proposed method was found to be linear ($r^2 = 0.999$), precise (%RSD < 2% for intraday and intermediate precision), accurate, specific, and robust. Further, the developed method was validated and found suitable for stress induced studies, since presence of degradants has no effect on CIN estimation. The proposed method was found to be simple, sensitive, precise, accurate and reproducible for the estimation of CIN.

Keywords
Cinacalcet Hydrochloride, HPTLC, Method Validation, Densitometry, Forced Degradation

1. Introduction
Cinacalcet hydrochloride (CIN), chemically
N-[1-(R)-(−)-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl]-1-aminopropane
is new calcimimetic agents, which acts on a calcium-sensing receptor of the parathyroid gland. CIN is a principal negative regulator of parathyroid hormone release enhances its selectivity to activation by extracellular calcium, thereby decreases the parathyroid hormone levels [1] [2]. CIN has been approved for the treatment of secondary hyperthyroidism in patients undergoing dialysis with chronic kidney disease, and is also indicated for the treatment of hypercalcemia in parathyroid carcinoma [3].

There are reported methods for the analysis of CIN in pure and pharmaceutical dosage forms including high-performance liquid chromatography (HPLC) [4] [5] [6] [7] [8], UV-Visible spectrophotometer [9] and liquid chromatography-mass spectrometry [10] [11]. However, Cinacalcet hydrochloride is analyzed by various techniques; no method has been reported for determination of CIN by using HPTLC. Hence, this HPTLC method was developed which is simple, accurate, and precise for the determination of bulk drug.

Features like effectiveness, accuracy, economy, low sample utilization and time effectiveness have made TLC-densitometry as a routine technique in the analysis [12] [13] [14]. Versatile traits of the techniques persuaded us to develop a sensitive, accurate, precise and robust HPTLC method for the assessment of Cinacalcet. The present study describes, for the first time, the development of a highly sensitive and simple HPTLC method with UV detection for the assessment of Cinacalcet. The developed method was validated in accordance with the International Conference on Harmonization (ICH) guidelines [15].

2. Materials and Methods

2.1. Reagents and Chemicals

Cinacalcet HCl (Purity 98.5%) was provided as a gift sample by Aurabindo Pharma Ltd. Hyderabad. All other reagents used were of analytical grade. Acetonitrile, chloroform, methanol were purchased from Sigma Aldrich chemical Pvt Ltd, Bengaluru. Silica gel 60 F254 HPTLC plates (10 × 10 cm with 0.2 mm layer thickness) were purchased from Merck Limited, Germany. Standard volumetric flasks were used for the preparation of all the dilutions. Millipore water and Whatmann filter paper grade I were used in the whole experimental work.

2.2. Apparatus and Chromatographic Conditions

A CAMAG HPTLC system with Linomat V semi automatic applicator (CAMAG, Muttenz, Switzerland) equipped with CAMAG TLC scanner 3, operated through win CATS software (version 1.4.3) along with accessories like CAMAG twin trough glass chamber (20 cm × 20 cm), and CAMAG syringe of 100 μL capacity was used in the present study. The samples and standard solutions were spotted on plates as 8 mm width bands at a constant application rate. The developed mobile phase consisted of Chloroform: Acetonitrile (6:4 v/v). The linear ascending chromatogram was developed to a distance of 75 mm at room temperature, in a CAMAG twin trough glass chamber already saturated with mobile phase.
vapor was 30 min. Subsequent to the development, HPTLC plates were dried in air. Densitometric scanning was performed at 282 nm by using CAMAG TLC scanner 3. The slit dimension was kept at 6 mm × 0.3 mm and scanned at a speed of 10 mm/s.

2.3. Method Development

2.3.1. Preparation of Stock and Working Standard Solution
Stock solution A (1000 μg/mL) of CIN was prepared by mixing 10.0 mg CIN standard and 7 mL of methanol in a 10 mL volumetric flask and sonicated it for 2 min for solubilization, followed by made up to the volume of 10 mL with methanol, the solutions were cooled to room temperature. Stock Solution B (100 μg/mL) of CIN was prepared by diluting 1 ml of stock solution A to 10 ml with methanol followed by sonication for 2 min. The solution was allowed to cool to room temperature. Further 1 mL of stock solution B is diluted to 10 mL (10 μg/mL) to produce concentrations of 40, 60, 80, 100, 120, 140 and 160 ng/band.

2.3.2. Development of the Optimum Mobile Phase
By using reference standard drug, multiple trials were performed with different solvent systems, with an aim to develop accurate HPTLC method with good resolution. From the trials, the mobile phase composed of chloroform: acetonitrile (6:4, v/v) resulted into sharp and symmetrical peaks with Rf 0.30 ± 0.02. At room temperature, chamber saturation with mobile phase for 30 min resulted into well defined peaks.

2.3.3. Selection of Detection Wavelength
After chromatographic development, bands were scanned over 200 - 400 nm. Within the scanned range, CIN has shown good absorption at 223 nm and 282 nm. However, sharp peak at 223 nm led to a broad change in the area, hence 282 nm was selected as detection wavelength (Figure 1).

2.4. Method Validation

2.4.1. Linearity
From the CIN standard solution of 10 μg/mL, CIN of concentrations 40, 60, 80, 100, 120, 140, 160 ng/band were applied by applying 4, 6, 8, 10, 12, 14, 16 μL of CIN standard solution (10 μg/mL) as separate bands on HPTLC plate, respectively. The plate was developed, dried and analyzed at 282 nm by densitometry. The densitograms were recorded and mean CIN peak areas (Y-axis) were plotted against the corresponding concentration (X-axis).

2.4.2. Precision
The standard CIN at three concentration levels (60, 80, 100 ng/spot) were selected to study the precision of the developed chromatographic procedure. Intra-day and inter-day analyses were performed to check the repeatability and reproducibility of the method. Intra-day precision analysis of the selected three concentrations was performed in hexaplicate on the same day. Intermediate precision
of the method was studied by hexaplicate analysis of the same concentrations on three different days. Intra-day and inter-day variation of the methods was determined by %RSD value.

2.4.3. Limit of Detection and Quantification (LOD, LOQ)
LOD was calculated as three times to the noise level (LOD = 3 × σ/s, where σ is the standard deviation of the peak areas of the drug and s is the slope of corresponding calibration plot); LOQ was calculated as 10 times the noise level (LOQ = 10 × σ/s)

2.4.4. Accuracy
Accuracy of developed method was assessed by performing percentage recovery (%). To determine the accuracy of the method, pre-analyzed sample solutions (100 ng) were spiked with 50%, 100%, 150% of CIN. The spiked samples were analyzed by proposed method for five times.

2.4.5. Robustness
Robustness was evaluated by analyzing the sample solutions at altered conditions like small change in detection wavelength, saturation time of development chamber, volume of mobile phase and composition of mobile phase. The effects on the results were examined by calculation of RSD (%).

2.5. Specificity
The specificity of the method for API, part from degradant was determined by exposing drug solution to stress conditions like acidic (0.1 M HCl), basic (0.1 M NaOH) and photo induced degradation. The resulting solutions were analyzed.
for CIN and its unknown degradants generated by stress induced degradation. Specificity was performed by using a stock solution of 100 μg/mL in methanol. This stock solution was made by adding 10 mg of CIN to 50 mL of methanol and sonicated for 5 min, and then the volume was made up to 100 ml by using methanol.

### 2.5.1. Acid Induced Degradation

A 5 ml of the stock solution was added to 5 ml of 0.1 M HCl. The resultant solution was refluxed for 30 min at 80°C in the dark conditions and the refluxed solution a sample volume corresponding to 400 ng per band was spotted on to the plate and the chromatograms were developed as described.

### 2.5.2. Alkali Induced Degradation

A 5 ml of the stock solution was added to 5 ml of 0.1 M NaOH. The resultant solution was refluxed for 30 min at 80°C in the dark conditions, from the refluxed solution a sample volume corresponding to 400 ng per band was spotted on to the plate and the chromatograms were developed as described.

### 2.5.3. Photochemical Degradation

A 5 ml of the stock solution was added to 5 ml of methanol, this solution was exposed to direct sunlight for 3 consecutive days (GMT: 9:00 - 17:00 h; total 24 h). A solution corresponding to 400 ng per band was applied on to the plate, and the chromatograms were developed as described.

### 4. Results and Discussion

#### Validation of the Method

1) Linearity

A series of seven spot of CIN from prepared standard solutions of CIN were applied in the concentration range of 40 - 160 ng/spot. Densitometric scanning was recorded at λ_max of 282 nm. The linearity was performed in triplicate and the correlation coefficient was 0.9994 for Cinacalcet ([Figure 2](#)). Linear regression data were shown in Table 1.

2) Precision

Intraday and intermediate precision results of the method were shown in Table 2. The precision of the method was expressed in percentage relative standard deviation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cinacalcet hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (ng·spot⁻¹)</td>
<td>40 - 160</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 5.912X + 20.831</td>
</tr>
<tr>
<td>Correlation coefficient (r² ± SD)</td>
<td>0.999 ± 0.0002</td>
</tr>
<tr>
<td>Slope ± SD</td>
<td>5.912 ± 0.044</td>
</tr>
<tr>
<td>Intercept ± SD</td>
<td>20.83 ± 1.335</td>
</tr>
</tbody>
</table>

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Table 2. Precision study of proposed stability-indicating HPTLC method.

<table>
<thead>
<tr>
<th>Conc. (ng/spot)</th>
<th>Intraday precision (n = 6)</th>
<th>Intermediate precision (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean. ± SD</td>
<td>%RSD</td>
</tr>
<tr>
<td>60</td>
<td>367.72 ± 2.87</td>
<td>0.78</td>
</tr>
<tr>
<td>80</td>
<td>480.19 ± 2.21</td>
<td>0.46</td>
</tr>
<tr>
<td>100</td>
<td>593.90 ± 5.81</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Figure 2. Linearity by area for cinacalcet hydrochloride.

deviation (%RSD). The developed method intraday precision and intermediate precision values were found as 0.78 and 1.27 respectively. As per ICH guidelines %RSD value limit for precision was <2%, hence the method developed was proved to be precise.

3) Limit of detection and Limit of quantification

The LOD and LOQ with signal to noise ratio of 3:1 and 10:1 were considered and the LOD and LOQ values of CIN were found to be 0.48 and 1.59 ng/spot, respectively.

4) Accuracy

Accuracy of the method was determined and the results were shown in Table 3. The low %RSD values were indicative of the accuracy of the method. The % recovery of CIN at tested levels was found to be in the range of 99.51 - 99.84%. The difference between the measured value and true value was in the range of 0.06 - 0.49%.

5) Robustness

Robustness results of the proposed method were presented in Table 4. For
Table 3. Accuracy of method in term of % recovery (n = 3).

<table>
<thead>
<tr>
<th>Spiked amount</th>
<th>Amount detected ± SD</th>
<th>Mean % recovered</th>
<th>% RSD</th>
<th>% bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ng (50%)</td>
<td>149.265 ± 0.683</td>
<td>99.51</td>
<td>1.50</td>
<td>0.49</td>
</tr>
<tr>
<td>100 ng (100%)</td>
<td>199.880 ± 1.109</td>
<td>99.94</td>
<td>1.60</td>
<td>0.06</td>
</tr>
<tr>
<td>150 ng (150%)</td>
<td>249.600 ± 0.556</td>
<td>99.84</td>
<td>1.55</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 4. Robustness studies (n = 3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CIN (100 ng·spot⁻¹)</th>
<th>SD</th>
<th>%RSD</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of mobile phase (mL)</td>
<td></td>
<td>13</td>
<td>0.83</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.53</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>0.79</td>
<td>0.46</td>
</tr>
<tr>
<td>Saturation time of development chamber (min)</td>
<td></td>
<td>15</td>
<td>4.03</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>3.97</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>5.33</td>
<td>1.13</td>
</tr>
<tr>
<td>Change in detection wavelength (nm)</td>
<td></td>
<td>254</td>
<td>4.61</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>258</td>
<td>3.60</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260</td>
<td>5.68</td>
<td>1.19</td>
</tr>
<tr>
<td>Composition of mobile phase</td>
<td>+0.1 mL acetonitrile</td>
<td>0.85</td>
<td>0.28</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>−0.1 mL acetonitrile</td>
<td>0.62</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>+0.1 mL chloroform</td>
<td>0.58</td>
<td>0.44</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>−0.1 mL chloroform</td>
<td>0.55</td>
<td>0.39</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Each altered parameter, peak areas standard deviation and %RSD values were found to be less than 2%, hence the method was found to be robust. Evident variation was observed in the result, when mobile phase composition was altered however the difference was insignificant.

6) Specificity

The specificity of the developed method was determined by estimating the accuracy and specificity of the analyte in the presence of degraded components of the analyte under different stress conditions like HCl (0.1 M), NaOH (0.1 M) and photo-degradation in day light. Results of the stress induced degradation studies were illustrated in Table 5. An insignificant degradation of the analyte was seen under all the tested stress conditions. However, the developed method has shown well separated peaks of the drug and its degradants. Densitograms of the CIN under acidic, basic and photo degradation stress conditions were shown in Figures 3(a)-(c), respectively; standard CIN was shown in Figure 3(d). From this, it is evident that the pure drug peak and degradants did not overlap and influence the estimation of CIN pure drug. Hence, the developed method was suitable and selective for the analysis.
5. Conclusion

The drug, Cinacalcet hydrochloride’s stability indicating that HPTLC densitometric determination method was developed and validated according to ICH guidelines. The developed method was simple, sensitive, precise, accurate and
reproducible. Statistical analysis of the method proved that the developed method was precise, accurate and reproducible. The method was rapid and simple in terms of usage of laboratory solvents, buffers and technology involved, in comparison with other pharmacopeial methods of analysis. The developed method was sensitive up to a level of quantifying 1.59 ng of CIN. In present study, stability of the CIN was established using ICH-recommended stress conditions. The drug was found to be stable in acid, base and photo degradation conditions. The developed method was validated for precision, accuracy, specificity, and robustness. Thus the method can be employed for routine quality control and stability studies of Cinacalcet hydrochloride.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References


