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# METHOD DEVELOPMENT AND VALIDATION FOR IMPURITY METHOD OF CINACALCET HYDROCHLORIDE TABLETS (STABLITY INDICATING) BY HPLC TECHNIQUE

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**ABSTRACT**

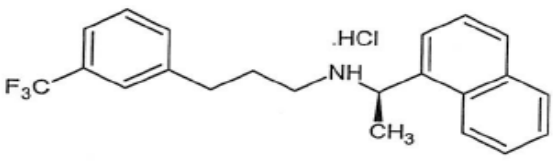
Cinacalcet is a calcimimetic agent and acts on calcium sensing receptor of the parathyroid. Also it increases sensitivity of calcium-sensing receptors on parathyroid glands to extracellular calcium, resulting in decreased serum parathyroid hormone (PTH) and calcium concentrations. The chemical name of Cinacalcet Hydrochloride is (R)-N-(3-(3-(trifluoromethyl) phenyl)propyl)-1-(1-naphthyl) ethyl amine Hydrochloride and it molecular weight is 357.4 g/mol and molecular formula, $C_{22}H_{22}F_3N$. The calcium sensing receptor on the surface of the chief cell of the parathyroid gland is the principal regulator of parathyroid hormone secretion. It acts by directly lowering parathyroid hormone levels by increasing the sensitivity of the calcium sensing receptors to activation by extracellular calcium, resulting in the inhibition of PTH secretion. The reduction in PTH is associated with a concomitant decrease in serum calcium levels2. Since, the method for determination of Cinacalcet related substances in pharmaceutical products is not described in current pharmacopoeias, the aim of this work was to develop and validate a precise, accurate and robust method. Separation was achieved on a RP C-18 column (50 x 3.0 mm, 2.5 µm particle size) along with pre column, column temperature kept at 40°C. Gradient method developed to achieve the separation. The mobile phase A contains 100% Buffer solution (pH 5.00) and Mobile phase B contains mixture of Acetonitrile and water in the ratio of 95:5 V/V, The preparation of buffer was dissolved in 1.8g of Disodium hydrogen phosphate in 1000 ml of water. pH was adjusted to 5.00 with phosphoric acid. Flow rate of 1.0 ml/min. Injection volume 5µL and UV detection wavelength at 223nm. The method was validated by following ICH guidelines and validation parameters showed that method used as stability indicating for determination of related compound of Cinacalcet hydrochloride tablets.

**Key Words**: Cinacalcet hydrochloride, HPLC, Method development and Validation.

## INTRODUCTION

Analytical method has to ensure good separation of active compounds and their degradation products. These compound, active and placebo, have different properties, and they have to be separated, and this method has to be developed as stability-indicating method. The aim of this study was to develop HPLC analytical method to determine related substance of Cinacalcet in Cinacalcet Hydrochloride tablets 30mg, 60mg and 90mg and to validate the same analytical method as per ICH guidelines (European pharmacopeia, 2014).

**Cinacalcet Hydrochloride**

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## MATERIAL AND METHOD

Cinacalcet Standards is having 99.06% purity. N-Oxide Impurity [(R)-N-(3-(3-(trifluoromethyl) phenyl) propyl)-1-(1-naphthyl) ethyl amine N-oxide] was identified as degradation impurity and Tetrahydro Cinacalcet Impurity were identified as process impurity. Disodium hydrogen phosphate, Acetonitrile, methanol were used HPLC grade chemicals/solvents and orthophosphoric was pure grade used for the experiment. Milli-Q water was used to prepare mobile phase and diluents (Sorbera ET AL., 2002).

### *Apparatus and chromatographic conditions*

Waters alliance HPLC instrument equipped with UV or PDA detector and Empower 2 software, column waters X Bridge Shield RP18, 2.5µm, 50 x 3.0mm, column temperature 40°C. The mobile phase A contains 100% Buffer solution (pH 5.00) and Mobile phase B contains mixture of Acetonitrile and water in the ratio of 95:5 V/V, The preparation of buffer was dissolved in 1.8g of Disodium hydrogen phosphate in 1000 ml of water. pH was adjusted to 5.00 with phosphoric acid, filtered and degassed, Elution was performed with a linear gradient (time, (min) / % solution B): 0/37, 5/84, 6/84, 6.1/37, 7.3/37, at flow rate 1.0 mL/minute. Retention time for Cinacalcet was 2.4 minutes. Run time is 7.3 min. UV detection was performed at a wavelength of 223 nm. Injection volume was 5 µl.

### *Diluent preparation*

Dilute 1.2g of 85% Orthophosphoric acid in 1000 ml of water. Adjust the pH to 3.00 ± 0.05 with 1N Sodium hydroxide solution.

### Standards and sample preparation

***Standard stock solution: (About 100µg/mL Cinacalcet)***

Weighed and transferred accurately about 22.0 mg of Cinacalcet hydrochloride reference standard into a 200ml volumetric flask, added 20ml of methanol and 20ml of diluent, sonicate for atleast 5 minutes. Shake by orbital shaker for 10 minutes and diluted to volume with diluent, mixed well.

***Standard solution: (About 0.50µg/mL Cinacalcet)***

Transferred 1mL of standard stock solutions into a 200ml volumetric flask and added 5ml of methanol and dilute to volume with diluent and mix well. Filtered a portion of the solution through 0.20µm PTFE filter (pall) by discarded the first 2ml of the filtrate.

***Quantification Limit solution: (About 0.05µg/mL Cinacalcet)***

Transferred 100µl of standard stock solution into 200ml volumetric flask. Added 10ml of methanol and diluted to volume with diluent and mix well. Filtered a portion of the solution through 0.20 µm PTFE filter (pall), by discarded the first 2ml of the filtrate.

***Tetrahydro Cinacalcet Impurity stock solution: (About 200 µg/mL of Tetrahydro Cinacalcet)***

Weighed about 2.0 mg of Tetrahydro Cinacalcet impurity standard into a 10ml volumetric flask. Added 5 ml of acetonitrile and sonicate for 10 minutes. Dilute to volume with acetonitrile and mix well.

***N-Oxide impurity stock solution: (About 200 µg/mL of N-Oxide)***

Weighed about 2.0 mg of N-Oxide impurity standard into a 10ml volumetric flask. Added 5 ml of acetonitrile and shaken by mechanical means for 10 minutes. Diluted to volume with acetonitrile and mix well.

***Resolution solution: (About 0.5µg/mL of Cinacalcet, 0.5µg/mL of Tetrahydro Cinacalcet and 0.5ppm of N-Oxide)***

Transferred 500µl of Standard stock solution-2, 250µl of Tetrahydro Cinacalcet impurity stock solution and 250 µl of N-Oxide impurity stock solution into a 100ml volumetric flask. Add 10ml of methanol. Dilute to volume with diluent and mix well. Filtered a portion of the solution through 0.20µm PTFE filter (pall), by discarding the first 2ml of the filtrate.

***Test solution for Cinacalcet hydrochloride tablets 30mg, 60mg and 90mg: (About 100µg/mL of Cinacalcet)***

Average weight was determined by using 20 tablets. Weighed a portion of the tablet powder equivalent to about 20mg of Cinacalcet and quantitatively transferred to 200ml volumetric flask. Added about 20ml of methanol and 20ml of diluent, sonicate for atleast 5 minutes. Shaken by orbital shaker for about 10 minutes. Diluted to volume with diluent and mixed well. Filtered the solution through 0.20µm pall PTFE filter, discarding the first 2ml of the filtrate.

### *Validation of HPLC method*

The proposed method was validated with the aspect of system suitability test, specificity, linearity and range, accuracy, precision, Detection Limit, Quantitation Limit, Robustness and stability of analytical solutions according to the ICH guidelines (International Conference on Harmonization, 2005).

### *Specificity*

To determine the specificity of the method spiked sample, Tetrahydro Cinacalcet impurity stock solution and 250 µl of N-Oxide impurity individual impurity of Tetrahydro Cinacalcet impurity and N-Oxide impurity solution, placebo solution, sample solution were prepared and injected into a PDA system. No peaks detected at the retention time of Cinacalcet in the chromatograms of diluents and placebo solution. The impurity peaks also well separated from the Cinacalcet peak and each other. The

purity angle (0.178) is less than the purity threshold (0.623) for Cinacalcet peak and there is no tick mark in the purity flag column for Cinacalcet peak in spiked test solution. Hence, the method is acceptable with respect to specificity (Manikandan K *et al*., 2013).

***Forced degradation***

A forced degradation study was conducted to demonstrate that the method is stability indicating. Separate portions of drug product, drug substance and placebo were exposed to the following stress condition to induce degradation.

Acid Stress-Samples were stressed with 0.2M HCl at 70°C for 3 hours.

Base Stress - Samples were stressed with 0.2M NaOH at 70°C for 2 hours.

Oxidative Stress-Samples were stressed with 3% hydrogen peroxide ($H_2O_2$) at 60°C for 2 hour.

Thermal Stress- Samples were exposed to heat at 70°C for about 72hrs.

Photolytic stress- Samples were exposed to UV light for 14days.

Stressed samples were injected into the HPLC system with photo diode array detector

The chromatograms of the stressed test samples were evaluated for peak purity of Cinacalcet peak using Waters Empower networking software. For all forced degradation test solutions, the purity angle is less than the purity threshold for the Cinacalcet peak and in placebo solution, no peak was observed at the retention time of Cinacalcet in all stress conditions.

Degradation of molecule was observed in all stress condition and it was observed less than 4%. Also, the purity of Cinacalcet hydrochloride was unaffected by the presence of its impurities and degradation products, hence it confirms the stability-indicating power of the developed method

% Assay value calculated for all stress samples against the reference standard of Cinacalcet hydrochloride. Mass balance results were calculated for all stressed samples and found to be more than 98%.

***Linearity***

Linearity for peak area of Cinacalcet and N-Oxide impurity in relation to their respective concentration was determined. The best-fit line through an un weighed least square linear regression was generated. The result is shown in Figure 2 for Cinacalcet and Figure 3 for N-Oxide.

Slope(S):40802.4219, Intercept (I): 175.6490, % I: 0.9

Slope(S):40430.4379 Intercept (I): -0.5443, % I: 2.7

The regression coefficient, $r^2$ for Cinacalcet: 0.9999, N-Oxide impurity: 0.9996.The percent deviation of the y-intercept from the origin for Cinacalcet: 0.9%, N-Oxide impurity: 2.7%. The observed results reveal excellent correlation coefficient. No apparent non-linearity is observed. This shows that the linearity of the method is acceptable (Padhi D and Harris R, 2009).

**Limit of Quantitation (QL) and limit of Detection (DL)**

To determine the Quantitation limit and detection limit for Cinacalcet and N-Oxide impurity obtained from linearity curve, samples were prepared and injected into HPLC to get the signal to noise ratio of about 10(QL) and 3(DL). The results obtained are given in Table 1.

***Precision***

To determine the precision of the method, six test solutions of Cinacalcet tablets 90mg were prepared by spiking the N-Oxide Impurity. The acceptance criteria (% RSD should be not more than 20.0 for impurities greater than Quantitation Limit) has been met. Hence, the method is acceptable with respect to method precision and results are shown in Table-II.

***Accuracy***

To determine the accuracy of the method, three test solutions of 90mg strength in each spike level (QL level, 50%, 100% and 150% for N-Oxide impurity) were prepared. The acceptance criteria (each individual and average recovery should be from 80% to 120% of theoretical value) were met. This shows that the method is acceptable with respect to the accuracy and results shown in Table -3.

***Robustness***

In robustness experiment, Effect of variation in flow rate from 0.9 ml/min and 1.1 ml/min, Effect of variation in mobile phase composition B ± 2% variation of organic, change in the column temperature of 38°C and 42°C and change in the pH of 4.8 and 5.2 were performed with a test solution of 90mg strength, standard solution and Resolution solution. As the resolution between Cinacalcet and Tetrahydro Cinacalcet impurity was more than 2.0, %RSD of standard is less than 2.0 and Difference in % impurities from the unchanged chromatographic condition for unknown impurities and specified impurities results were less than 20.0%, hence method is robust with respect to flow rate from 0.9mL to 1.1mL/min, column temperature variation from 38°C to 42°C, change in Mobile phase B composition ± 2%, pH of buffer in mobile phase from 4.8 to 5.2 (6. Practical HPLC Method Development).

**RESULTS AND DISCUSSION**

The present study was aimed at developing a sensitive, precise and accurate HPLC method for the analysis of related substances of Cinacalcet Hydrochloride tablets in pharmaceutical dosage forms. For this, pH 5.0 buffer solution (Mobile phase A) and A mixture of acetonitrile and water in the ratio of 95:5 v/v, mix well (Mobile phase B) was found to be the most suitable mobile phase as the chromatographic peaks obtained with this

system were better defined and related substances of Cinacalcet tablets. Under the above-mentioned conditions, Retention time obtained for Cinacalcet was about 2.4minutes, N-Oxide Impurity and Tetrahydro Cinacalcet impurity were 5.0 minutes and 2.7 minutes respectively. The impurity peak areas were reproducible as indicated by low coefficient of variation were found (United States Pharmacopoeia, 2009).

A good linear relationship for N-Oxide impurity ($r^2$ = 0.9998) and Cinacalcet ($r^2$ = 0.9999) were observed between the concentrations and respective peak areas. The regression curve was constructed by linear regression fitting and its mathematical expression. The % impurities in the tablet were quantified using the proposed analytical method. This reveals that the method is quite precise. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. It can be concluded that the proposed HPLC method is sensitive and reproducible for related substances of Cinacalcet in Cinacalcet tablets 90mg. No peaks detected at the retention time of Cinacalcet in the chromatograms of diluents and placebo solution. The related substances peaks also well separated from the Cinacalcet peak. The purity angle is less than the purity threshold and there is no tick mark in the purity flag column for Cinacalcet peak in spiked test solution. Hence, the method is acceptable with respect to specificity. The method was duly validated by evaluation of the required parameters (Shaik.Saida *et al*., 2014; Ravinder *et al*., 2009).

**Table 1. QL and DL results**

| Compound name | Detection limit(DL) | | Quantification limit(QL) | |
|---|---|---|---|---|
| | Concentration (ppm) | S/N ratio | Concentration (ppm) | S/N ratio |
| Cinacalcet | 0.02 | 6 | 0.05 | 13 |
| N-Oxide impurity | 0.02 | 4 | 0.05 | 10 |

**Table 2. Method precision results**

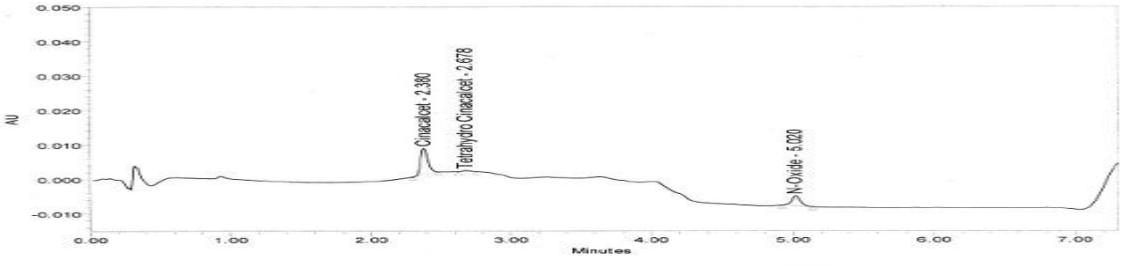
| Name of the solution | % of N-Oxide impurity |
|---|---|
| Test solution 1 | 0.506 |
| Test solution 2 | 0.518 |
| Test solution 3 | 0.520 |
| Test solution 4 | 0.501 |
| Test solution 5 | 0.491 |
| Test solution 6 | 0.511 |
| Average (%) | 0.508 |
| SD | 0.0109 |
| RSD (%) | 2.1 |

**Table 3. Accuracy results**

| Concentration Levels[#] | Mean | RSD |
|---|---|---|
| QL | 94.2% | 3.2 |
| 50% | 98.1 | 1.1 |
| 100% | 99.7 | 0.6 |
| 150% | 98.4 | 1.7 |

[#] Amount of N-Oxide impurity spiked to 0.5%

**Fig. 1. separation between impurities and Cinacalcet**




| | Name | RT | Area | % Area | USP Resolution |
|---|---|---|---|---|---|
| 1 | Cinacalcet | 2.380 | 28250 | 67.06 | |
| 2 | Tetrahydro Cinacalcet | 2.678 | 1318 | 3.13 | 3.0 |
| 3 | N-Oxide | 5.020 | 12556 | 29.81 | 22.2 |

**Fig. 2. Calibration curve of Cinacalcet**

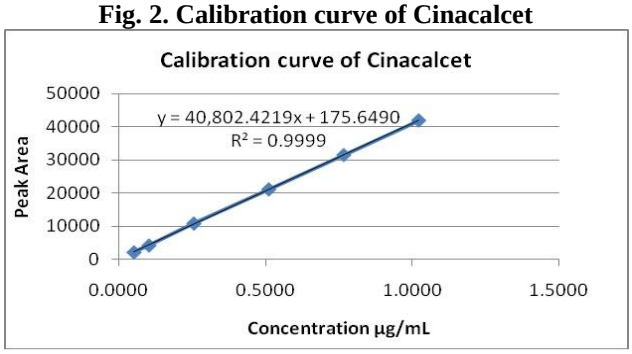



**Fig. 3. Calibration curve of N-Oxide Impurity**

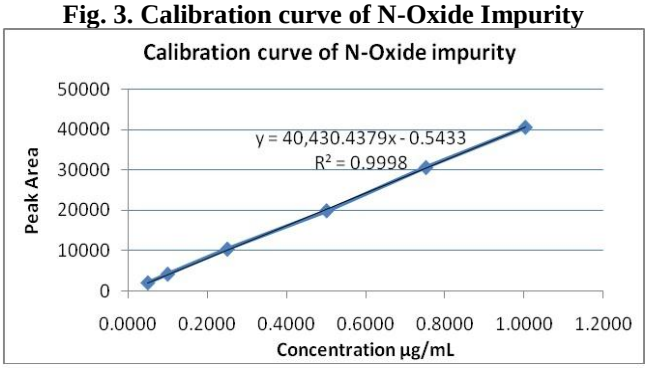
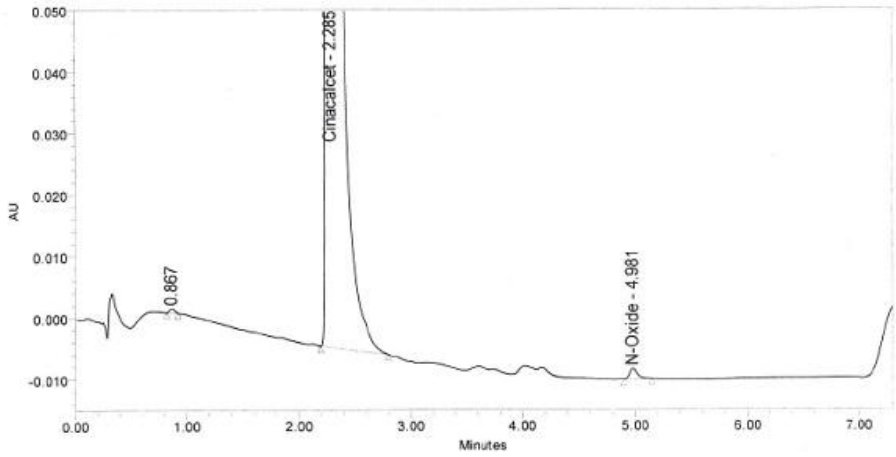



**Fig. 4. Spiked test solution with 0.5% N-Oxide impurity**




## CONCLUSION

The proposed method is sensitive, precise, accurate, robust, rugged and economic. Hence this HPLC method has been developed and validated for determination of related substances of Cinacalcet in Cinacalcet Hydrochloride tablets with gradient elution. Validation parameters have proved that developed analytical method can be used as stability indicating method to determine related substances of Cinacalcet in Cinacalcet Hydrochloride tablets 30mg, 60mg and 90mg.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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