Method Development and validating the Detection and Determination of Cinacalcet in bulk and its formulation using RP-HPLC

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ABSTRACT

Cinacalcet (INN), a calcium mimetic drug that mimics the activity of calcium in the body. It expresses itself by activation of calcium-sensing receptor allosterically in different organs and tissues. The secretion of parathyroid hormone is regulated principally by calcium-sensing receptors that are present on the surface of the parathyroid gland. Cinacalcet is used in the treatment of hyperparathyroidism, which is the usual consequence of parathyroid cancers and CKF. There had been a rapid increase in the number of drugs that are adding to each class of drugs. These drugs are formulated into newer formulations either in a single or multi-drug dosage forms. These newer marketed formulations demand a new investigation for the estimation of the drug in the formulations. The existing analytical techniques for those drugs are available in the research literature, but not all the methods are stable and economical to use. The objective of this work was to develop an analytical RP-HPLC method for the estimation of cinacalcet in bulk and tablet formulation. The emphasis was given to the short time of analysis, and simplicity in the method. RP-HPLC analysis of the drug satisfies the peak integrity, suitability, recovery of the drug. LOQ and LOD of the drug were achieved with high sensitivity. The data shows the precision of the method and the accuracy of the method. Overall, the data suggest that the proposed analytical method can be used to analyse the drug in the formulation. This method can be recommended for the routine analysis of the drug in its dosage form.

INTRODUCTION

Cinacalcet (INN), a calcium mimetic drug that mimics the activity of calcium in the body. It expresses itself by activation of calcium-sensing receptor allosterically in different organs and tissues (Goodman, 2002). The secretion of parathyroid hormone is regulated principally by calcium-sensing receptors that are present on the surface of the parathyroid gland. The present drug lowers the parathyroid hormone levels by elevating the calcium-sensing receptors sensitivity. This elevation causes the activation of extracellular calcium which inhibits the PTH secretion (Torres, 2004).
the PTH is generally associated with the decrease of the serum calcium level. Cinacalcet is used in the treatment of hyperparathyroidism, which is the usual consequence of parathyroid cancers and CKF (Chronic Kidney Failure) (Wada et al., 2000), (Figure 1).

There had been a rapid increase in the number of drugs that are adding to each class of drugs. These drugs are formulated into newer formulations either in a single or multi-drug dosage forms. These newer marketed formulations demand a new investigation for the estimation of the drug in the formulations. The existing analytical techniques for those drugs are available in the research literature, but not all the methods are stable and economical to use. Few other methods are time-consuming too. Numerous drugs were estimated using HPLC, LC-MS (Sreelakshmy et al., 2011; Tiwari and Sathe, 2010). The objective of this work was to develop an analytical RP-HPLC method for the estimation of cinacalcet in bulk and tablet formulation.

MATERIALS AND METHODS

Chemicals used

Potassium Dihydrogen Phosphate, Acetonitrile and Orthophosphoric acid were used for the above study. All the chemicals and reagents were purchased from S.D. fine chem which are of analytical grade.

Instruments used

UV-Visible Spectrophotometer

Pharmaspec-1700 model of UV-VISIBLE Spectrophotometer manufactured by SHIMADZU was used for the study. Software used to analyse the peaks is U.V. Probe 2.0.

HPLC

Model of HPLC

LC-2010CHT manufactured by SHIMADZU attached with an autosampler. The detector used was a PDA SPD-M20A Prominence-Diode Array Detector and UV-Visible. The column used was a Phenomenex Kromasil column with 5μ 100 RC18 (250 mm x 4.6 mm, i.e., 5 microns) measurements. Software used to analyse the peaks is LC-solution.

Mobile Phase

Acetonitrile and Methanol are taken in 60:40 v/v mixed and were adjusted using a pump which is operated by software. The degassing is done, and the solvents used were of HPLC grade. Different solvents were used to store solvent systems.

Preparation of Standard solution

1mg of accurately weighed pure drug, Cinacalcet was dissolved in 5ml of the diluent solution and ultrasonicated for about 30mins. In another volumetric flask, 10mg of the impurity A-Cinacalcet was weighed and dissolved in 10ml of diluents. 0.5ml of this solution was pipetted out and labelled as a working solution. The final volumes were made with diluents and used for further analysis (Varsha et al., 2011).

Preparation of sample solution

An accurately weighed quantity of oral suspension powder equivalent to 10mg of the drug was dissolved in 60ml of methanol. This mixture was sonicated for 30min to ensure proper dissolution. This solution was filtered through a filter paper, and the residue was washed with methanol. The solution was diluted to the mark with methanol. 10ml of standard stock solution was transferred to a 100ml volumetric flask. The final volume was adjusted to 10 μg/ml with methanol and used for further study (Jat et al., 2012; Rathinaraj et al., 2010).

The chromatographic conditions

The flow rate was maintained at 1ml/min. The temperature was maintained at 30°C. The injection volume and runtime were maintained at 10μL and 10min. The detection was done at 262nm.
Determination of $\lambda_{max}$

The standard solution made of pure Cinacalcet was injected in the HPLC, and U.V. spectrum between of 200 to 400 nm was determined with the help of software, L.C. solution (Kumar and Gowda, 2012).

Method Validation

Linearity and range

The plotting of the calibration curve was performed with concentration ranges of the cinacalcet solution of 50% 75% 100% 125% 150%. These concentrations were made using standard cinacalcet drug. These solutions were vialed and auto sampled. 20$\mu$l samples of the solutions were injected into the autosampler by maintaining the chromatographic conditions. The calibration curves were obtained by plotting the peak area vs the concentration of the drug. The regression constant from the slope was determined (Nataraj et al., 2012; Souri and Amalou, 2010).

Accuracy (% Recovery)

The drug recovery was calculated to determine the accuracy and recovery by adopting the standard addition method. Samples of known concentration of the drug were added to the previously analysed samples of the powder. The quantity of the cinacalcet was analysed by considering the values of the regression equation from the calibration curve. The experiments were repeated for 6 times.

Precision

Method precision (% Repeatability)

The sample injection was done 6 times repeatedly into the instrument. The results were reported in terms of % of the variance which should not exceed 2%.

Day precision
Table 2: Accuracy Results

| Level of spike | Weight of sample | Area of Sample | Quantity added in μg/ml | Quantity found in μg/ml | Recovery percentage % | Mean percentage % |
|---------------|------------------|----------------|-------------------------|-------------------------|------------------------|-------------------|
| 50%          | 37.50            | 608783         | 49.550                  | 49.71                   | 100                    | 100               |
| 50%          | 37.50            | 610503         | 49.550                  | 49.68                   | 100                    |                   |
| 50%          | 37.50            | 607132         | 49.550                  | 49.70                   | 100                    |                   |
| 50%          | 37.50            | 607132         | 49.550                  | 49.65                   | 100                    |                   |
| 50%          | 37.50            | 601708         | 49.550                  | 49.77                   | 100                    |                   |
| 50%          | 37.50            | 607089         | 49.550                  | 49.71                   | 100                    |                   |
| 100%         | 75.00            | 1208783        | 99.100                  | 99.55                   | 100                    |                   |
| 100%         | 75.00            | 1210503        | 99.100                  | 99.58                   | 100                    |                   |
| 100%         | 75.00            | 1204001        | 99.100                  | 99.48                   | 100                    |                   |
| 150%         | 112.50           | 1835361        | 148.650                 | 149.41                  | 101                    | 100               |
| 150%         | 112.50           | 1837303        | 148.650                 | 149.34                  | 101                    |                   |
| 150%         | 112.50           | 1831982        | 148.650                 | 149.39                  | 101                    |                   |
| 150%         | 112.50           | 1835361        | 148.650                 | 149.45                  | 101                    |                   |
| 150%         | 112.50           | 1837303        | 148.650                 | 149.29                  | 101                    |                   |

Table 3: Repeatability Results

| Sl No | Sample Name | Peak Name                    | R.T. | Area     |
|-------|-------------|------------------------------|------|----------|
| 1     | Precision 1 | Cinacalcet Hydrochloride     | 3.725| 1208703  |
| 2     | Precision 2 | Cinacalcet Hydrochloride     | 3.703| 1204101  |
| 3     | Precision 3 | Cinacalcet Hydrochloride     | 3.746| 1207232  |
| 4     | Precision 4 | Cinacalcet Hydrochloride     | 3.706| 1206332  |
| 5     | Precision 5 | Cinacalcet Hydrochloride     | 3.712| 1201808  |
| 6     | Precision 6 | Cinacalcet Hydrochloride     | 3.711| 1217189  |

Table 4: Intermediate Precision Results

| Sl No | Sample Name | Peak Name                    | R.T. | Area     |
|-------|-------------|------------------------------|------|----------|
| 1     | INT-Precision 1 | Cinacalcet Hydrochloride | 3.739| 1208783  |
| 2     | INT-Precision 2 | Cinacalcet Hydrochloride | 3.717| 1210503  |
| 3     | INT-Precision 3 | Cinacalcet Hydrochloride | 3.742| 1204001  |
| 4     | INT-Precision 4 | Cinacalcet Hydrochloride | 3.701| 1207132  |
| 5     | INT-Precision 5 | Cinacalcet Hydrochloride | 3.715| 1201708  |
| 6     | INT-Precision 6 | Cinacalcet Hydrochloride | 3.701| 1207089  |

The intra and inter-day precision were evaluated in 3 different concentrations of the drug. The intra-day precision was calculated by analysing samples thrice in one day, and inter-day was calculated by analysing samples thrice over three days. The results were reported as % variance.

Robustness

Robustness was estimated by making minor changes in the mobile phase ratios and injection volumes. The column temperature and the flow rates were also changed by a small extent to find the robustness. It was analysed at three concentrations, and the results were expressed in % variance.

LOD & LOQ

The limits of detection (L.O.D) and the limits of quantification (L.O.Q) of this method were esti-
Table 5: System suitability results

| Parameter                  | Value  |
|----------------------------|--------|
| Rt-(Minutes)               | 3.7    |
| Resolution -Rs             | 3.4    |
| Theoretical plates-TP      | 3265   |
| Tailing factor-Tf          | 1.0    |
| Asymmetric factor-Af       | 0.67   |
| Capacity factor-K’         | 6.5    |

Table 6: Solution stability results

| Sl  | Sample Name | Peak Name                   | R.T.  | Area      |
|-----|-------------|-----------------------------|-------|-----------|
| 1   | Acid        | Cinacalcet Hydrochloride    | 3.705 | 3183248   |
| 2   | Base        | Cinacalcet Hydrochloride    | 3.688 | 3924866   |
| 3   | Peroxide    | Cinacalcet Hydrochloride    | 3.731 | 4526402   |
| 4   | Heat        | Cinacalcet Hydrochloride    | 3.703 | 4835827   |
| 5   | Sunlight    | Cinacalcet Hydrochloride    | 3.716 | 4926787   |

mated visually based on trial and error.

Selectivity and Specificity

The selectivity of this developed method was analysed by a resolution factor which corresponds to the drug peak within the nearest resolving peaks and also to all the other available peaks. The data of peak purity confirms the selectivity with the help of a PDA detector. To assess the method selectivity, a placebo was prepared with oral suspension powder without cinacalcet and the same is compared with the prepared cinacalcet standard to investigate the selectivity of the method. The chromatograms of standard and the placebo were prepared. The comparisons were made between the retention time Rt, Purity and Resolution factor.

System suitability

Resolution (Rs), the capacity factor (K’), asymmetry factor (As), retention time-(RT), theoretical plates (Tp), and tailing factor-(Tf) was reported in the E.P. by using L.C. software. HPLC was calibrated with the initial mobile phase with 6 injections of the common standard solution. To establish the suitability of the system to the analysis, 6 samples were prepared using the standard solutions and were analysed (ICH Guideline, 1994; Pervaiz et al., 1995).

Solution stability

The stability of the solution of the drug was assayed in the method by storing the solution in a tightly sealed container for 24 hrs. Samples from this stock solution were analysed at regular intervals of 6, 18 and 24hrs. The results obtained from this test were compared with the freshly prepared solutions.

RESULTS AND DISCUSSION

All the results of the studies in the RP-HPLC of the drug were documented for the Accuracy, Precision, Linearity, LOD and LOQ.

Linearity and range

The linearity of the analytical method was investigated at 7 different concentrations using methanol as a diluent, and the prepared concentrations ranged from 50-150%. The results showed a perfect relationship between peak area vs concentration of the analysed sample. The calibration curve was determined, and the AUC was estimated using the detector. The liner graph was plotted with an r² value of 0.9972, which gave the best linearity. The results were demonstrated in Table 1.

Accuracy (% Recovery)

The drug was recovered at many concentrations which indicate the accuracy of this method. The pre measured quantity of the drug is taken and mixed with the already analysed solution. The same process was repeated over 3 repeated days and to determine the precision in the inter-day validation. The resultant % of the variance of this method was estimated to be <2% along with a good percentage of the recovery. The results were tabulated in Table 2 and Figure 2.

Precision (% Repeatability)

The precision of the analysis was determined by repeated injection of the drug solution for 6 times. The results were given in Table 3 and were expressed as % variance that did not exceed
Table 7: Robustness Results

| Sl No | Sample Name | Peak Name              | R.T.  | Area     | USP Trailing | USP Plate Count |
|-------|-------------|------------------------|-------|----------|--------------|-----------------|
| 1     | Flow 1      | Cinacalcet Hydrochloride | 4.410 | 6108891  | 1.56         | 3357            |
| 2     | Flow 2      | Cinacalcet Hydrochloride | 3.222 | 4487968  | 1.68         | 3032            |
| 3     | Temp 1      | Cinacalcet Hydrochloride | 3.690 | 5179221  | 1.57         | 3994            |
| 4     | Temp 2      | Cinacalcet Hydrochloride | 3.702 | 5177191  | 1.50         | 3195            |

2% (Madhukar et al., 2012; Dhaneshwar et al., 2008).

Inter-day and Intra-day precision
The precision of the inter-day and intra-day variations were analysed at different concentrations were documented in Table 4. The variance was not significant, which supports there are an intra and inter-day precision of the proposed method of analysis.

Limit of detection and quantitation
The LOD and LOQ were determined for the drug. The least concentration of the drug and LOQ were determined by measuring accuracy and precision (Figure 3).

System suitability
The measurement of the suitability of the system is an embedded module of the HPLC analytical method-development to determine the system was suitable for this analysis. The results were tabulated in Table 5. The results were in acceptance of the CDER guidelines (Figure 4).

Solution stability
The percentage of the variance of the drug solution was found to be under limits when tested for the stability study. The data obtained in the study were tabulated in Table 6. This analysis showed that the drug solutions were stable, and the results showed that the sample responded well, and the data was within limits of less than 2%.

Robustness
The robustness of the experiment was determined by changing a few parameters like mobile phase, Flow rate, Column and Injection volume of the sample. The method was performed at various concentrations of 0.6, 0.8 and 1.0 \( \mu g/ml \). The values were tabulated in Table 7.

CONCLUSIONS
The drug, cinacalcet, was estimated using the analytical method that is proposed in the study. The method was studied for its simplicity, accuracy, precision and specificity. The proposed analytical method is suitable for the routine analysis of the drug in the dosage form. The simplicity of the analysis was emphasised due to the lack of sophisticated instruments like LC-MS. The emphasis was given to the short time of analysis, and simplicity in the method. RP-HPLC analysis of the drug satisfies the peak integrity, suitability, recovery of the drug. LOQ and LOD of the drug were achieved with high sensitivity. The data shows the precision of the method and the accuracy of the method. Overall, the data suggest that the proposed analytical method can be used to analyse the drug in the formulation. This method can be recommended for the routine analysis of the drug in its dosage form.

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Conflict of Interest
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