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Validated RP-HPLC Method for the Estimation of Cinacalcet in Bulk and Tablet Dosage form

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ABSTRACT

A Simple, selective, accurate, precise and linear RP-HPLC method was developed and subsequently validated for estimation of cinacalcet in bulk & tablet dosage form. Gradient elution at a flow rate of 0.8ml/min was used for separation of drugs in reversed-phase mode using Waters HPLC 22695 model on a INERTSIL ODS C_{18} column (150 x 4.6 mm; 5µ) at a ambient temperature. Mobile phase consisted of water: methanol: acetonitrile (20:60:20). The UV detection wavelength was 235nm 20 µl was injected. The retention time for cinacalcet was 3.7min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The % recovery was within the range between 99.73% and 99.85%. The method was validated as per the ICH guidelines.

Key words: cinacalcet, RP-HPLC, validation

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INTRODUCTION

Cinacalcet is a drug that acts as a calcimimetic (i.e. it mimics the action of calcium on tissues) by allosteric activation of the calcium-sensing receptor that is expressed in various human organ tissues. Cinacalcet is used to treat hyperparathyroidism (elevated parathyroid hormone levels), a consequence of parathyroid tumors and chronic renal failure. Brand name of cinacalcet is "SENSIPAR". Its empirical formula is $C_{22}H_{22}F_3N$•HCl with a molecular weight of 393.9 g/mol (hydrochloride salt) and 357.4 g/mol (free base). The hydrochloride salt of cinacalcet is described chemically as N-[1-(R)-(-)-(1-naphthyl) ethyl]-3-[3(trifluoromethyl) phenyl]-1-aminopropane hydrochloride. Literature survey revealed that very few analytical methods have been reported for the determination of Cinacalcet in pure drug, pharmaceutical dosage forms and in biological samples using liquid chromatography. The aim of the present work is to develop and validate a simple, fast and reliable isocratic RP-HPLC method with UV detection for the determination of cinacalcet in bulk and in tablet dosage forms.

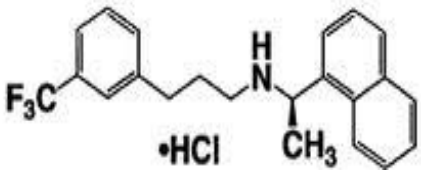


Figure 1: Structure of cinacalcet

MATERIALS AND METHODS

Chemicals & reagents

HPLC grade acetonitrile, water, methanol

Chromatographic conditions

- STATIONARY PHASE: Inertsil ODS C18 column (250x4.6mm, 5µm in particle size) .
- MOBILE PHASE: water: methanol: acetonitrile(20:60:20).
- FLOW RATE : 0.8ml/min
- INJECTION VOLUME : 20 µL
- DETECTION: the elution was detected at 235nm using PDA detector.

Standard and Sample preparation

Cinacalcet standard and marketed formulation equivalent to 10mg was accurately weighed and transferred into a 10ml volumetric flask containing HPLC grade Methanol as the diluent. It was sonicated, dissolved completely and made volume up to the mark with the same solvent. 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with HPLC grade Methanol. The contents were mixed well and filtered through 0.45µm nylon

filter paper to get this stock solution (1mg/ml)

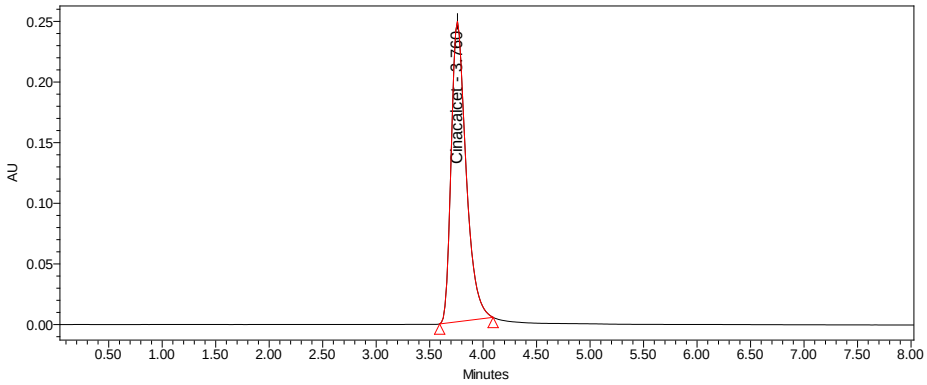
RESULTS AND DISCUSSION

Selection of the detection wavelength

The UV spectrum of CINACALCET was scanned in the region between 200 and 400 nm and shows max At 235nm.

Method development

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug cinacalcet is non-polar. Non polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of water, methanol and Acetonitrile was selected as mobile phase and the effect of composition of mobile phase on the retention time of cinacalcet was thoroughly investigated. The concentration of water, methanol & acetonitrile were optimized to give symmetric peak with short run time (figure 2). A short run time and the stability of peak asymmetry were observed in the ratio of 20:60:20 of water, methanol and acetonitrile. It was found be optimum mobile phase concentration.



Name	Retention Time	Area	% Area	Height	USP Tailing	Symmetry Factor	USP Plate Count
Cinacalcet	3.760	2384016	100.00	247418	1.411942	1.411942	9589.060065

Figure. 2: Typical chromatogram of cinacalcet standard

METHOD VALIDATION

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

Linearity

The calibration curve obtained by plotting peak area against concentration showed linearity in

the concentration range of 20-80 µg/ml Linear regression data for the calibration curves are given in Table 1.

Table 1: Linear regression data for the calibration curve

Concentration (PPM)	Mean peak area
20	2098389
30	3074458
40	4125697
50	5324156
60	6229069
70	7387207
80	8490280

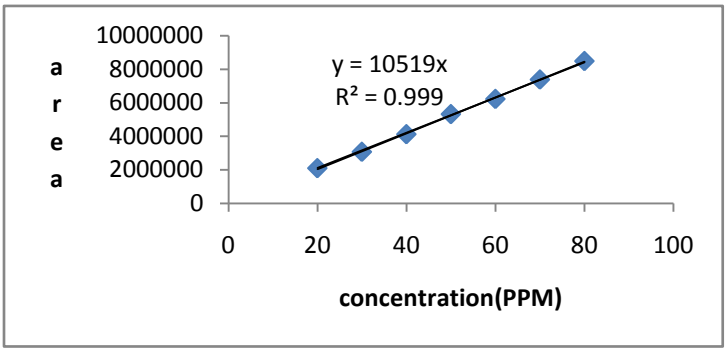


Figure 3: calibration curve for linearity

Accuracy

The % mean recovery obtained for cinacalcet was 101% .The %RSD is less than 2, results were given in Table 2

Table 2: Accuracy data for proposed method

Spiked level of drug (%)	Amount of drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery
50	20	20.29	105.0
100	40	40.2	101.0
150	60	60.7	98.0
% RSD	0.14	0.22	

Table 3 : Precision of the proposed HPLC method

Conc. of cinacalcet (40 µg/ml)	Peak area of cinacalcet	
	Intra-day	Inter-day
Injection-1	2384016	2549666
njection-2	2307740	2562855
Injection-3	2384016	2582387
Injection-4	2386448	2545475
Injection-5	2372398	2684016
Average	2366924	2584880
Standard Deviation	3353.4	5725.3

Precision

Results for repeatability expressed as %RSD. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the %RSD values, which indicates that the proposed method was reproducible, results were showed in Table 3.

Detection limit and quantification limit

LOD for cinacalcet was 0.35 µg/ml respectively, while LOQ was 0.69 µg/ml

Robustness

There was no significant change in the peak areas and retention times of cinacalcet, when the composition of mobile phase ±1ml and flow rate was varied by ±0.2 ml. The results are showed in Table 4.

Table 4: Results of robustness for proposed method

Factor	Level	Retention time	Asymmetry
A: Flow rate (ml/min)			
0.6	-0.2	3.90	1.34
0.8	0	3.75	1.32
1.0	+0.2	2.89	1.28
%RSD		0.3	0.7
B: % of methanol (ml)			
59	-1	3.9	1.34
60	0	3.75	1.32
61	+1	3.10	1.28
%RSD		0.2	0.7

Specificity

No interference from any of the excipients was found at retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram received by the standard solution at the wavelengths applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

Table 5: System suitability parameters

Parameters	Cinacalcet”
Linearity (µg/ml)	20.-80
Correlation coefficient	0.999
Theoretical plates	9589
Tailing factor	1.41
LOD (µg/ml)	0.35
LOQ (µg/ml)	0.69

System suitability

The acceptance criteria are % RSD of peak areas and retention time less than 2%, theoretical

plates numbers (N) at least 4500 per each peak and tailing factors less than 1.5 for cinacalcet the results are shown in the Table 5.

Quantification of cinacalcet tablet dosage form

The proposed method was applied to the estimation of cinacalcet dosage form(SENSIPAR). The results of the assay 99.73±0.29%, of label claim of the injection. The assay results showed that the method was selective for the estimation of cinacalcet without interference from the excipients used in the injection form. The results are shown in the Table 6.

Table 6:Results of sample analysis for proposed method

Brand name	Analyte	Label claim (mg)	% Analyte estimated (mean±SD)	%RSD
SENSIPAR	CINACALCET	10	99.73±0.29	0.185

CONCLUSION

A validated RP-HPLC method has been developed for the determination of CINACALCET in TABLET form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 8 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of cinacalcet in pharmaceutical dosage form. The limit of detection for cinacalcet was found to be 0.35 µg/ml and the limit of quantification was found to be 0.69 µg/ml. It proves the sensitivity of method.

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