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Research Article

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Method Development and Validation and Degradation Studies for Cinacalcet Hcl Drug by RP-HPLC Method

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Abstract

The purpose of this study is to develop and validate a simple, rapid, sensitive, and precise, degradation studies for Cinacalcet Hcl drug by RP-HPLC method as per ICH guidelines. The HPLC analysis used a reversed phase Agilent Zorbax C_{18} (250X4.6,5µm) column, a mobile phase constituted of buffer solution and methanol (30:70). The buffer was composed of 1ml orftho phosphoric acid in 1000 ml of water and adjusts P^H 2.1 with ortho phosphoric acid. Column temperature is 30°c. This method in wavelength is detecting used for PDA detector and 10ml was injected. The retention time for cinacalet was 3.7min. The validation data showed that the method is sensitive, specific and reproducible for the determination of cinacalcet in the dosage form. The accuracy of the method was found to be 101%. Linearity is not less than 0.99. precission was found to be 99%. LOD and LOQ were found to be 2.913 and 9.709 ng $spot^{-1}$. Degradation studies in retention time are 3.7min.

Keywords: Cinacalet Hcl, HPLC, Agilent Zorbax $C_{18,}$ validation, degradation studies.

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1. Introduction

Cinacalcet Hcl (fig 1) 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 also indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma. Cinacalcet chemically called as (*R*)-*N*-[1-(1-naphthyl)ethyl]-3-[3-(trifluoromethyl)phenyl]proan-1-amine. Formula is $C_{22}H_{22}F_3N$, molecular weight 357.412g/mol[1]. Mechanisam action of the calcium-sensing receptors on the surface of the chief cell of the parathyroid gland is the principal regulator of parathyroid hormone secretion (PTH). Cinacalcet directly lowers 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 levels[2]. High-performance liquid chromatography (HPLC) using silica,

cyano, cation-exchange and C_{18} columns[3]. The proposed RP-HPLC method with RI detection has been validated using USP (4) and ICH (22) guidelines as references[4].

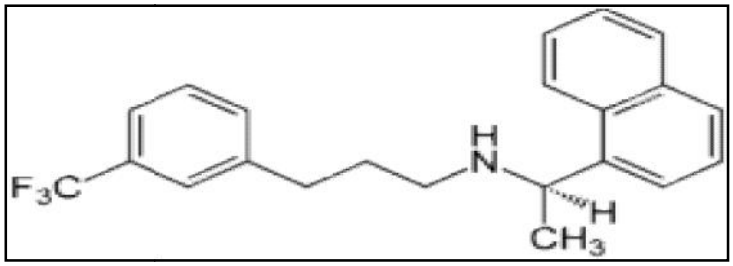

Figure.1

The literature survey reveals that several analytical methods have been reported for the quantification and determinat ion of the drug individually in human plasma by liquid chromatography/tandem mass spectrometry[5]. The present study describes, for the first time, the development of a highly sensitive and simple HPLC method with UV detection for the determination of Cinacalcet Hcl[6].

2. Materials and Methods

Cinacalcet hydrochloride was collected by MSN Laboratories, India.

Table.1

Name	Manufacture
HPLC	Water e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software
Ultra sonicator	Fastclean Ultrasonic cleaner
pH meter	Lab India
Electronic balance	Sartorius
HPLC column	Agilent Zorbax C_{18}

Ortho phosphoric acid is collected by Fischer scientific chemicals/ Analytical Grade, methanol was received by Rankem/HPLC Grade, Water Fischer scientific chemicals/HPLC Grade.

Buffer preparation:

Take 1ml of ortho phosphoric acid in 1000ml of water and adjust p^H 2.1 with ortho phosphoric acid.

Standard preparation:

25mg of drug in 25ml of volumetric flask dissolve and diluted to volume with methanol. Take 1ml of above standard diluted up to the mark with methanol.

Method development & Optimization:

Using a mobile phase consisting of different buffers and methanol at different concentrations and different mobile phase's pH values are attempted. The peak was observed that the shape and retention time of cincalcet Hcl was found to be broad compared to the buffer and methanol composition of mobile phase. After various trails in used different types of mobile phases used like buffer solution with methanol and buffer solution with acetonitrile. After selecting the best conditions based on peak performance, buffer solution and methanol ratio(30:70) and hplc using column is agilent zorbax c_{18},250x4.6,5µm, the run times of the proposed method was 25mins with isocratic solution. Column temperature is 30^oc, flow rate is 1.3ml/min, PDA detector is mainly used this purpose, after inject the standard solution volume was found to be 10µl. Retention times found were about 3.81minutes for cinacalcet Hcl, tailing factor is 1.76. It shows good shape of chromatogram with consistent peak. The proposed chromatographic conditions were found to be appropriate for quantitative determination. Refer figure 1 standard solution.

Method validation:

Cinacalcet standard taken to the 25mg was accurately weighed and transferred into a 25ml of volumetric flask containing HPLC grade Methanol s diluents. It was sonicated, dissolves completely and made volume up to the mark with the same solvent.

The method was validated in accordance with ICH guidelines. The parameters assessed were precision, accuracy, linearity, specificity, LOD & LOQ, robustness, degradations studies.

Degradation studies:

Acid: The cinacalcet Hcl standard was treated with 6N HCL at a concentration of 150mg/ml, the solution was heated at 70 ± 2^oc for a period of 2 h. Before carrying out the HPLC analysis, were suitably diluted and neutralized with 10N HCL.

Base: Base hydrolysis was performed in 1N NaOH at a standard concentration of 150mg/ml. the solution was then subjected to heating at 70±2°c for 5min and neutralized with 1N HCl.
Hydrogen peroxide: It was utilized for the oxidative degradation study. The standar substance was treated with 15% hydrogen peroxide solution 70±2°c for a period of 2 h.
Light: This studies were conducted by exposing the standard in solution (150mg/ml) and solid state to UV and fluorescent light separately. Samples were withdrawn after 72h and analyzed.
Heat: a thin layer of Cinacalcet Hcl was spread on a petridish and subjected to heat at 60±2°c in a dry heat oven for 72h.

3. Results and Discussion

Method development: optimizing chromatogram

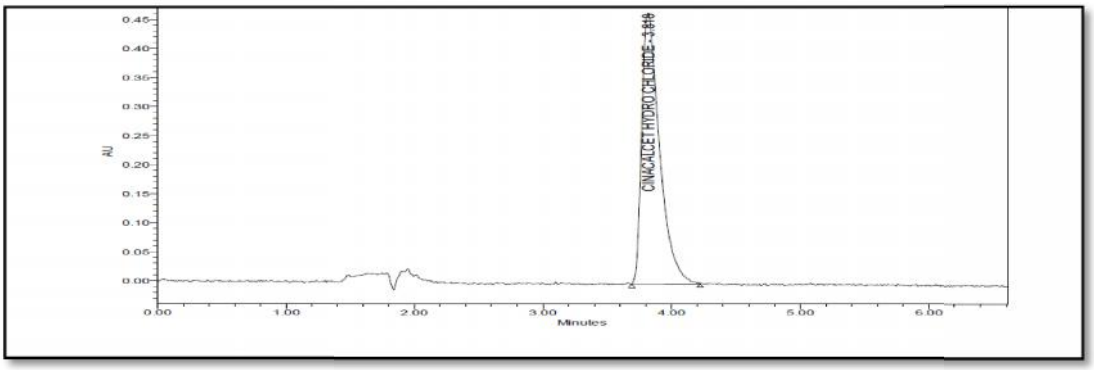

Figure. 2

Table. 2

Peak name	RT	Area	USP Plate count	USP Tailing
Cinacalcet Hcl	3.818	4460183	3755	1.76

Method validation:
Precision:

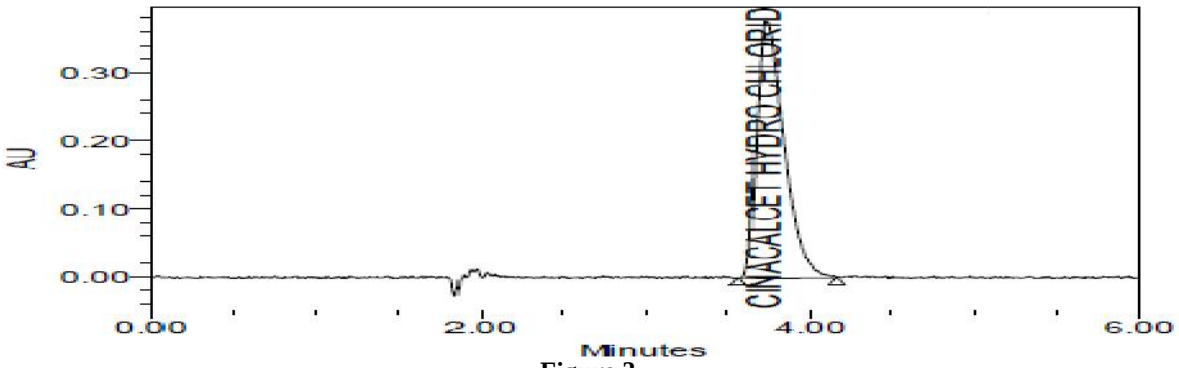

Figure.3

Table. 3

Sample name	Sample weight	RT	Area	% Assay
Precision 1	25	3.725	5130451	99
Precision 2	25	3.703	5133649	100
Precision 3	25	3.746	5131708	99
Precision 4	25	3.706	5132099	99
Precision 5	25	3.712	5130242	99
Precision 6	25	3.711	5138001	100
%RSD				0.06

Accuracy:

Table. 4

Spiked level	Sample weight	Amount of drug added(µg/ml)	Amount of drug found(µg/ml)	% Recovery
50	12.50	49.50	49.71	100
100	25	99	99.58	101
150	37.50	148.50	149.45	101

Specificity:

Blank

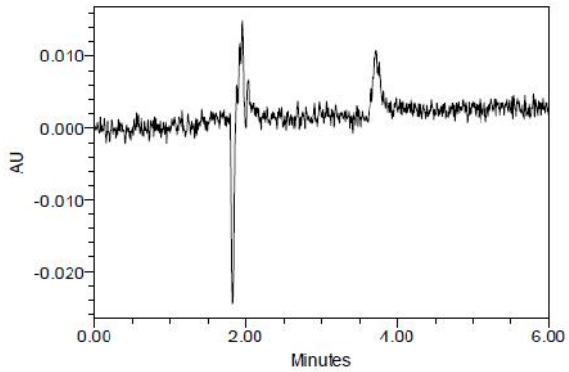

Figure. 4

Placebo

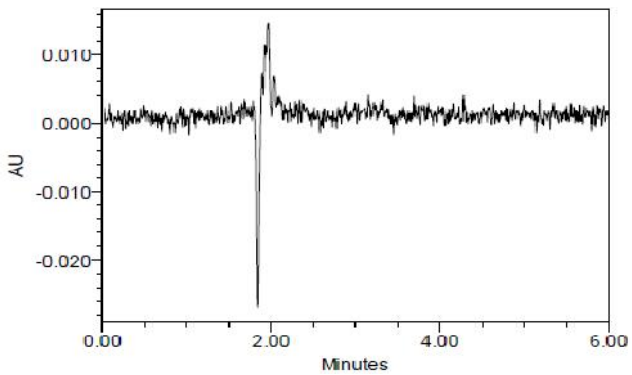

Figure. 5

Table. 5

Sample name	Peak name	RT
blank	Cinacalcet Hcl	3.700
placebo	Cinacalcet Hcl	3.700

Linearity:

Table. 6

Concentration	Mean peak area
50	2563722
75	3857928
100	5136164
125	6424293
150	7704684

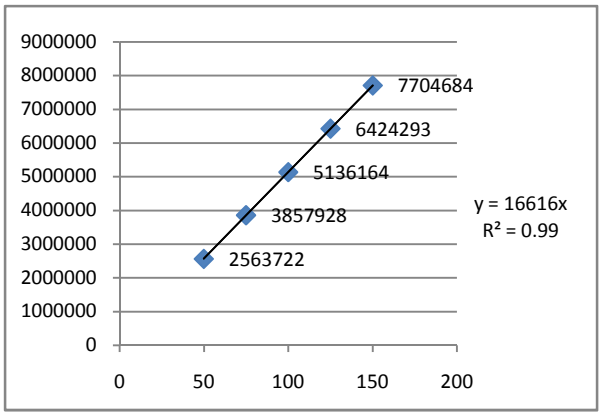

Figure.6

LOD:

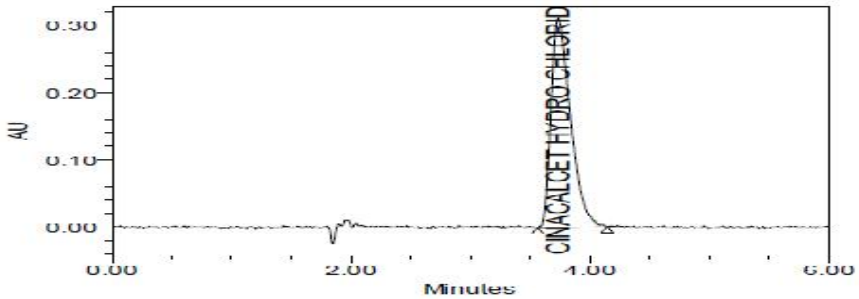

Figure. 7

LOQ:

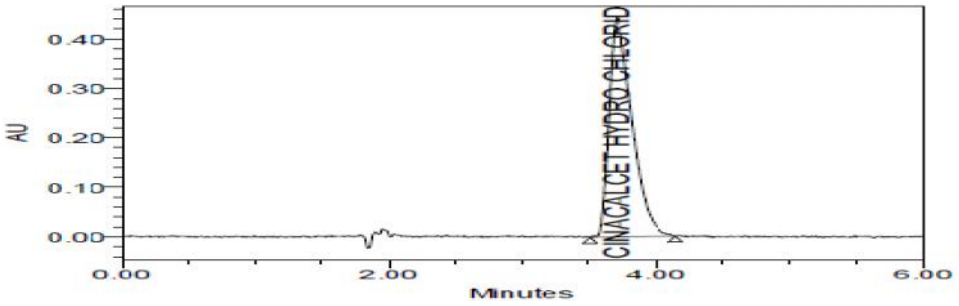

Figure. 8

Discussion:

LOD for cinacalcet was 2.913µg/ml respectively, while LOQ was 9.709µg/ml.

Robustness:

flow-1

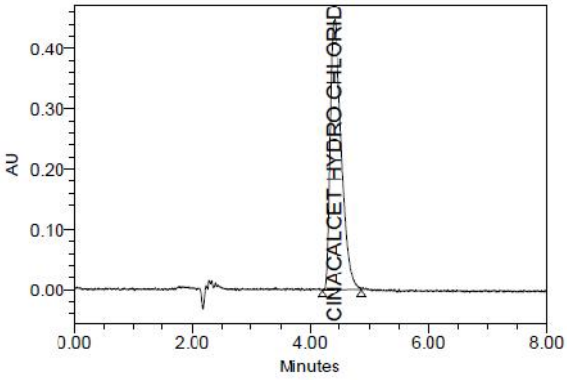

Figure. 9

flow-2

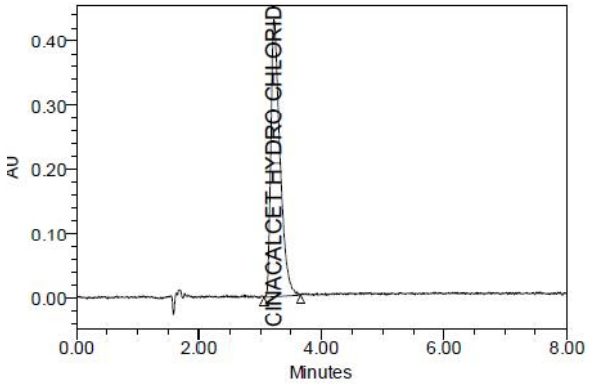

Figure. 10

Temp-1

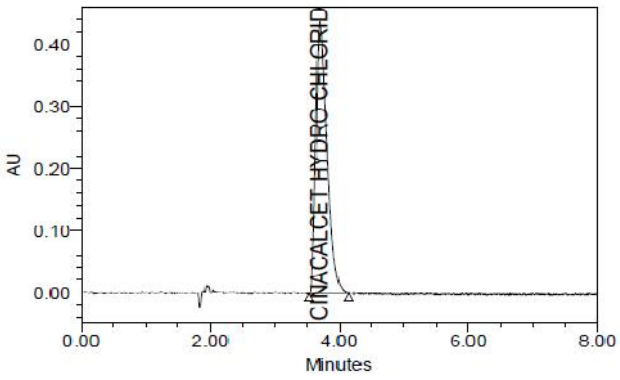

Figure. 11

Temp-2

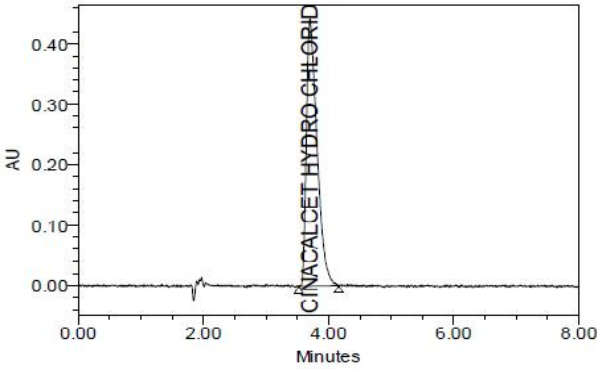

Figure. 12

Table. 7

Factor	RT	%USP Tailing
Flow rate	4.410	1.56
	3.222	1.68
Temp	3.690	1.57
	3.702	1.50

Degradation studies:

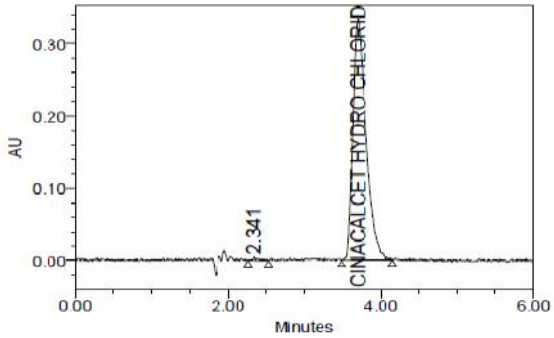

Figure. 13

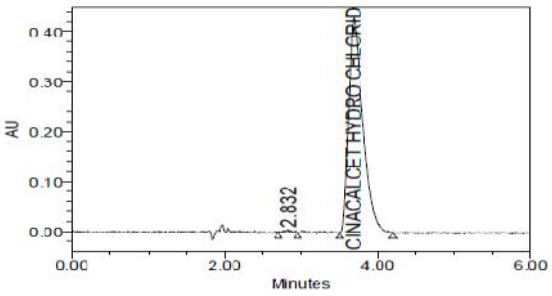

Figure. 14

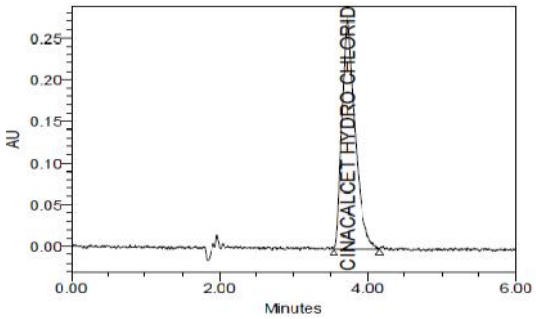

Figure. 15

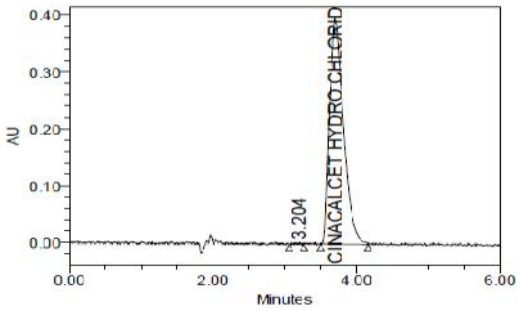

Figure. 16

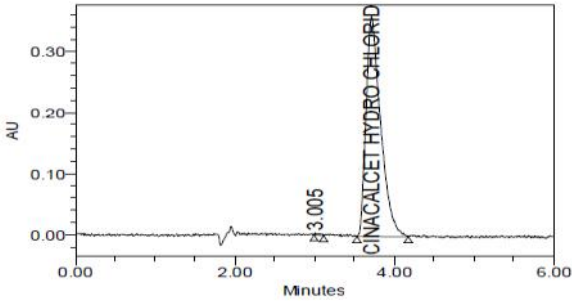

Figure. 17

Table. 8

Sample name	Peak name	RT	Area
Acid	Cinacalcet Hcl	3.705	3183248
Base	Cinacalcet Hcl	3.688	3924866
Hydrogen peroxide	Cinacalcet Hcl	3.731	4526402
Heat	Cinacalcet Hcl	3.703	4835827
sunlight	Cinacalcet Hcl	3.716	4926787

Table. 9

S.No	Sample Weight	Intraday	Interday	% Assay
1	25	5137202	4026509	100
2	25	5132050	4022254	100
3	25	5132184	4074053	100
4	25	5137864	4087765	100
5	25	5130610	4026995	100
6	25	5131991	4046100	100
Avarage Assay:		5133650.2	4047279	100
STD		3068.5	27664	0.06
%RSD		0.1	1	0.06

4. Conclusion

The HPLC method described in this study was proved to be determinations and degradation studies in cinacalcet Hcl 25mg. the proposed method is simple and also cost-effective with moderate analysis time. The specificity and robustness capability of the method was demonstrated through forced degradation studies as per ICH guidelines. LOD was found to be 2.913µg/ml , LOQ was 9.709µg/ml, intraday %RSD is 0.1 and interday %RSD 1 is found. found to be simple, sensitive, accurate and precise. the developed methods may be recommended for routine and QC analysis of the investigated drugs to provide simple, accurate and reproducible quantitative analysis for the determination.

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