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Development and Validation of Stability Indicative Analysis for Cinacalcet Hydrochloride and Stress Study

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Abstract: **The present research deals with development and validation of simple, economical, for Cinacalcet Hydrochloride along with its process impurities. The mobile phase consists of phosphate buffer (pH 3.0) and Acetonitrile. The YMC pack butyl column was used for separation. The LOD value for Cinacalcet and known impurities were about 0.17 ppm and 0.20 ppm respectively, whereas LOQ values were about 0.50 and 0.60 ppm. The regression coefficient was more than 0.999. The % accuracy for each impurity was within the range of 90 to 110. The method is capable to quantify the degradant impurities generated during stress study, thus proving stability indicative nature of method.**

Index Terms: **Cinacalcet Hydrochloride, Dehydro impurity, Ethanamine impurity, Mesylate impurity, Stress Study, Validation.**

I. INTRODUCTION

Cinacalcet Hydrochloride (Cinacalcet HCl) is used to control excessive level of calcium in the blood of patients. It usually occurs due to primary and secondary hyperparathyroidism seen in kidney disease or cancer of parathyroid gland. (Drüeke, 2018; Miller et al., 2012) Cinacalcet is a first calcimimetic drug which controls the secretion of parathyroid hormone. It mimics the action of calcium on tissues by allosteric activation of the receptor which sense extracellular levels of calcium ions. (Jensen & Bräuner, 2007, Torres, 2006).

The International Union of Pure and Applied Chemistry (IUPAC) Name of Cinacalcet is (*R*)-*N*-[1-(1-naphthyl) ethyl]-3-[3-(trifluoromethyl) phenyl] propan-1-amine.

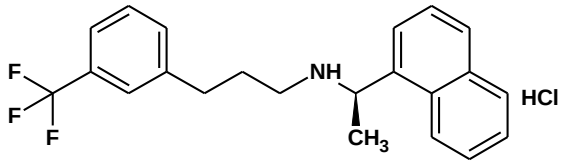

Fig. 1. Structure of Cinacalcet HCl

Cinacalcet HCl may potentially contain various impurities, like intermediate steps used in synthetic process or impurity that may be formed as by-product during synthesis. Three such impurities are described below.

1-(naphthalen-1-yl) ethan-1-amine is key raw material for used synthesis of Cinacalcet drug substance.

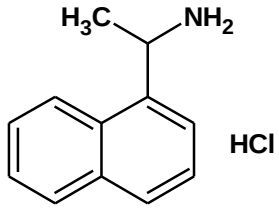

Fig. 2. Structure of Cinacalcet Ethanamine (Impurity A)

Cinacalcet mesylate is an intermediate step. It has IUPAC name 3-(3-(trifluoromethyl)phenyl) propyl methanesulfonate.

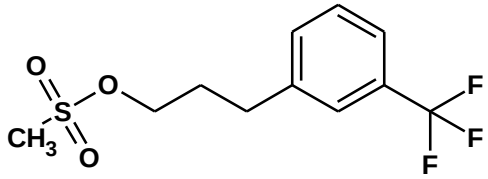

Fig. 3. Structure of Cinacalcet mesylate (Impurity B)

* Corresponding Author

Dehydro cinacalcet or (E)-N-(1-(naphthalen-1-yl) ethyl)-3-(3-(trifluoromethyl) phenyl) prop-2-en-1-amine is also a process impurity of Cinacalcet.

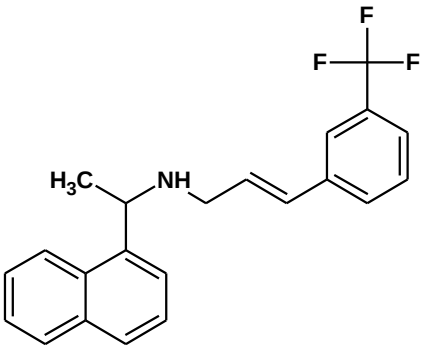

Fig. 4. Structure of Dehydro Cinacalcet (Impurity C)

The impurity profile analysis of Drug substance is very critical regulatory expectations. (ICH Q3AR2, 2006). The prime requirements of regulatory analysis are method should be stability indicative, which means method should be capable to quantify impurities those may be generated on aging. (Ruben et al., 2013; Sengupta et al., 2018). There are several spectroscopic methods reported for estimation of Cinacalcet. (Darwish et. Al., 2012; Loni et. Al., 2012; Rao & Gowrisankar, 2016) However, being non stability indicative, these spectroscopic methods have limitation to adopt in regulatory environment.

There are some quantitative estimation methods are reported for analysis of Cinacalcet Active pharmaceutical Ingredient (API) or formulation (Panigrahi et. Al., 2018; Rao & Gowrisankar, 2020). However, these methods are not suitable for impurity profiling. Some impurity profiling methods are also reported for Cinacalcet. However, these methods have very high analytical run time. (Sigala et. Al.,2009) or requires high-cost Ultra pressure liquid chromatography (Reddy et. al., 2015). Certain HPLC method are also described (Karikalan et. al., 2016; Krishnan et al,2015). However, none of the available literature had covered suitability of method for quantification of process impurities viz. ethanamine, cinacalcet mesylate and dehydro cinacalcet along with API.

The research exercise was strategized to develop a simple, economical, precise, stability indicating Related Substances method for Cinacalcet HCl, which will be suitable to identify and quantify the process impurities mentioned above.

To generate potential degradants which may appear on aging, Cinacalcet API was exposed to harsh physicochemical degrading agents like temperature, light, humidity, oxidation, acidic and basic hydrolysis. (Blessy et. al., 2014; ICH Q1B 1996). These force-degraded samples were analyzed to ensure all process and degradation impurities are well resolved. To adopt this method in regulated quality control environment, it was comprehensively validated as per requirements of major international Guidelines. (ICH Q2 R1, 2005; USFDA, 2015; WHO 2018)

II. MATERIALS AND METHODS

A. Materials

Cinacalcet HCl API was received from Mehta API Private Limited, Maharashtra. The API standard and impurity standards were received from Daicel technologies Hyderabad. Potassium Dihydrogen Phosphate, Acetonitrile, orthophosphoric acid were procured from Merck. The analysis was done on Dionex Ultimate 3000 with PDA detector controlled by Chromelon software version 6. Newtronic photostability Chamber (model: NLPS4SI) was used for photostability study.

B. Methods

1) Chromatographic Method Development

Various trials were made to develop the Chromatographic condition for analysis. Ultra-violate (UV) detector was set to 215 nm based on UV spectra of all four analytes. The chromatographic trails and retention time (RT) are mentioned in Table 1.

Table 1. Chromatographic Trials

Trial	Mobile Phase A	Mobile Phase B	Column	RT
1	Water	Methanol	Inertsil cyano	4.3
2	phosphate Buffer pH 3.0	Methanol	Inertsil cyano	7.9
3	phosphate Buffer pH 3.0	Acetonitrile	YMC C18	13.3
4	phosphate Buffer pH 3.0	Acetonitrile	YMC C4	7.4

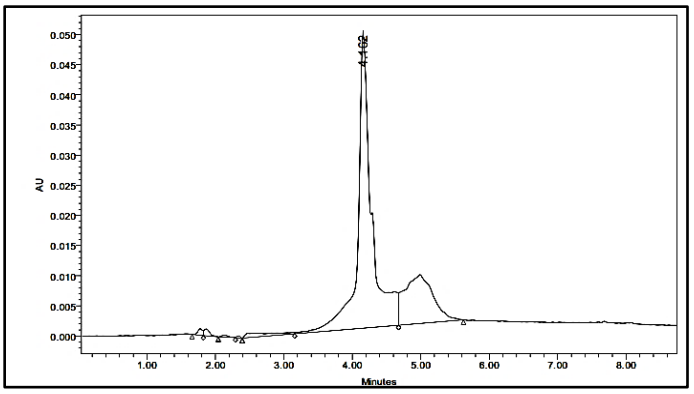

Fig. 5. Chromatogram of Cinacalcet (Trail 1)

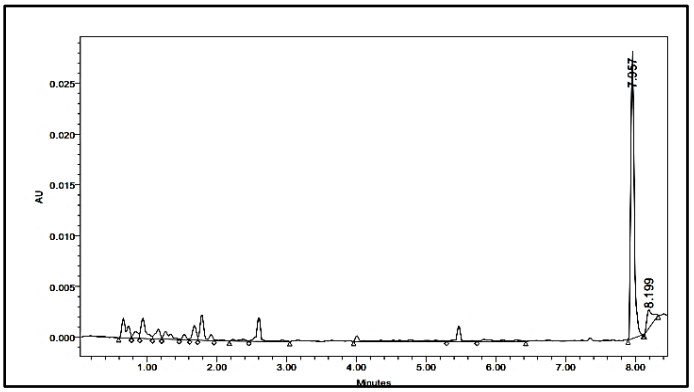

Fig. 6. Chromatogram of Cinacalcet (Trail 2)

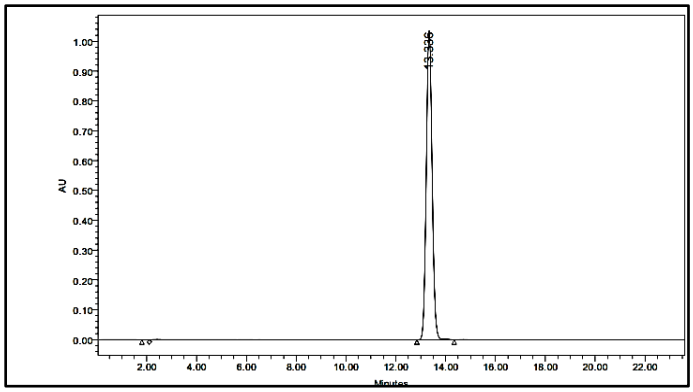

Fig. 7. Chromatogram of Cinacalcet (Trial 3)

Peak shape was good however, method with shorter run time was preferred.

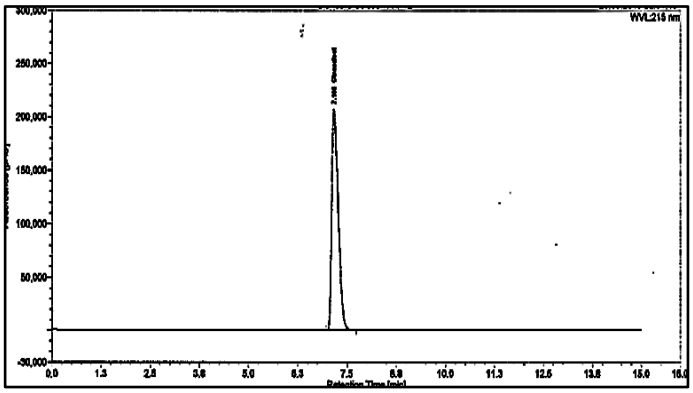

Fig. 8. Chromatogram of Cinacalcet (Trial 4)

2) Analytical Method Validation

Preparation of Standard solution: A separate stock solution for Cinacalcet Hydrochloride (10 ppm), ethanamine impurity (15 ppm), mesylate impurity (15 ppm) and dehydro impurity (15 ppm) was prepared by accurate weighing and appropriate dilution. From each flask, 1 ml of solution was pipetted out and volumetrically diluted to 10 ml in a standard flask. This standard solution contained 1 ppm of cinacalcet and 1.5 ppm of each impurity.

Preparation of Sample solution: In a 10 ml volumetric flask, about 10 mg sample was quantitively transferred. It was dissolved in about 2 ml of diluting solution with aid of gentle sonication. It was then made up to the volume using diluting solution. Sample concentration is 1000 ppm.

a) Specificity

Specificity was checked for by injecting Cinacalcet, Impurity A, Impurity B, and Impurity C. (Imp A, Imp B, and Imp C)

b) LOD

A concentration of individual analyst giving signal to noise ratio (S/N) greater than 3 was selected as LOD.

c) LOQ

A concentration of individual analyst giving signal to noise ratio (S/N) greater than 9 was selected as LOQ.

d) Linearity and Range

The linearity of Cinacalcet HCl was established in range of 0.5 ppm (i.e., LOQ) to 1.5 ppm (i.e., 50 % to 150% of working concentration). Linearity for each impurity was established in range of 0.6 ppm to 2.4 ppm (i.e., 40 % to 160 %)

e) Precision

As a repeatability study, all three impurities were spiked at working level concentration (1.5 ppm) in sample. Six samples were prepared.

Analysis was repeated with fresh preparation on other day for intermediate precision.

f) Solution stability

Solution stability of Standard and sample preparation was monitored till 24 hours.

g) Accuracy

For accuracy study, three levels of known concentrations (0.06%, 0.15% and 0.24%) of each were spiked in sample solution. Each level was prepared in triplicate.

h) Robustness

For Robustness, sequential changes were made in flow rate (±0.1 ml/ min) and then buffer concentration (±10 %). For each condition, three sample preparations were done.

3) Stress Study

Degradation study done to check impact of physical stress parameters like UV and Visible light, higher temperature, elevated humidity and chemical stress conditions like oxidation, hydrolysis at acidic and basic pH.

a) Physical Stress Conditions

(1) Higher Temperature: Cinacalcet API was exposed to a temperature 80° C in drying oven for two days.

(2) UV- Visible light: Solid Sample was exposed to uv-visible light (as per ICH Q1B option 1) in photostability chamber with controlled humidity.

(3) High Temperature and humidity: Solid sample was kept in humidity chamber set at temperature 40° C and 80 % RH (Relative humidity) for 5 days.

b) Chemical stress Condition.

(1) Oxidative Degradation. 10% H_2O_2 was prepared by diluting H_2O_2 solution in diluting solution. Sample was dissolved in 10% H_2O_2 and refluxed for about 2 hours on heated water bath.

(2) Acid hydrolysis**.** 1 M HCl was prepared in diluting solution. Sample was dissolved in 1 M HCl and refluxed on heated water bath for 2 hours. Before analysis, the content was neutralized with NaOH.

(3) Base hydrolysis**.** 1 M NaOH was prepared in diluting solution. Sample was refluxed on water bath for 2 hours in 1 M NaOH. As higher degradation was observed in this condition, sample was refluxed in 0.5 M NaOH for two hours. In this condition also, higher degradation was observed, hence exposure time was reduced to half an hour. Before analysis, the content was neutralized with dilute HCl.

III. RESULTS AND DISCUSSION

Finalized chromatographic conditions are described below.

Column: YMC Pack C4

Column dimension: 4.6 mm x 100 mm, i.d. 3.0 µ

Column oven Temperature: 25^0 C

Flow rate: 1.0 ml/min

Wavelength: 215 nm

Injection volume: 10 µl

Autosampler Temperature: ambient

Mobile phase A: 0.05 M KH_2PO_4 buffer, pH adjusted to 3.0 ± 0.05 with orthophosphoric acid.

Mobile phase B: Acetonitrile

Diluting Solution: 1:1 (v/v) mixture of Mobile phase A: Mobile phase B

Table 2. Gradient Program

Time (min)	% Mobile phase A	% Mobile phase B
0.0	50	50
4.0	50	50
15.0	30	70
16	50	50
21	50	50

Fig. 9 to Fig. 11 shows specificity of individual analyte i.e., Cinacalcet Hydrochloride, Imp. A, Imp. B and Imp. C

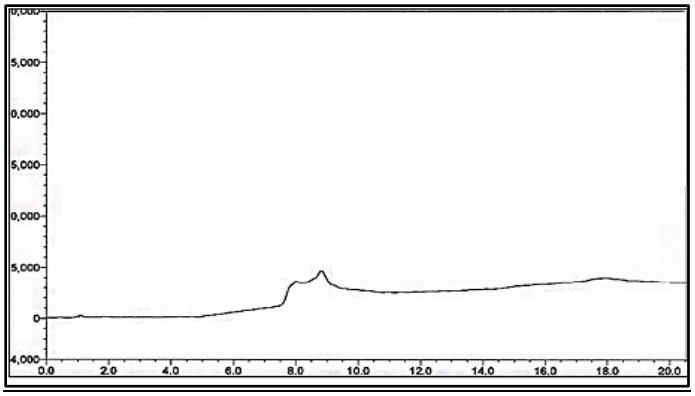

Fig. 9. Specificity Blank

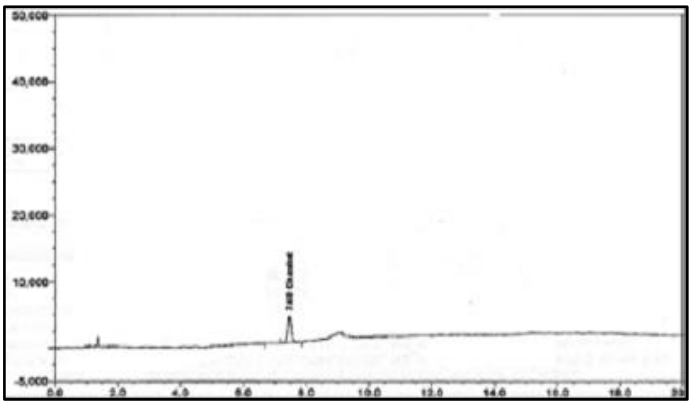

Fig. 10. Specificity - Cinacalcet HCl

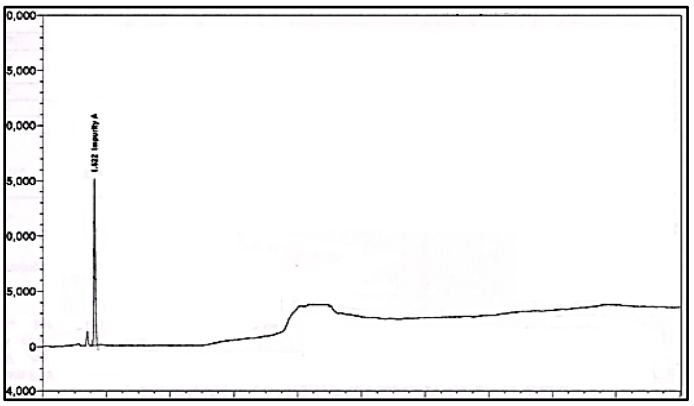

Fig. 11. Specificity- Ethanamine impurity (Imp A)

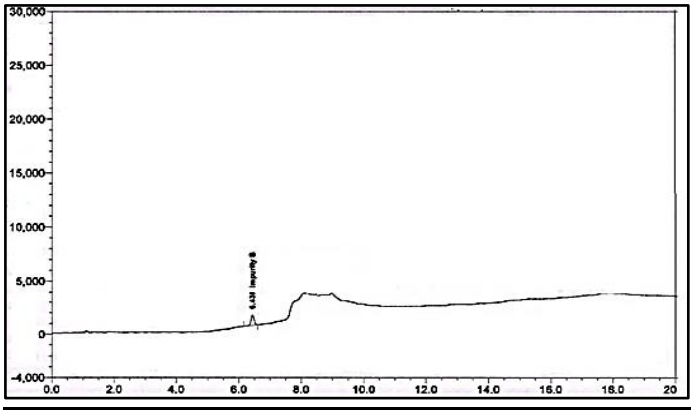

Fig. 12. Specificity- Mesylate impurity (Imp B)

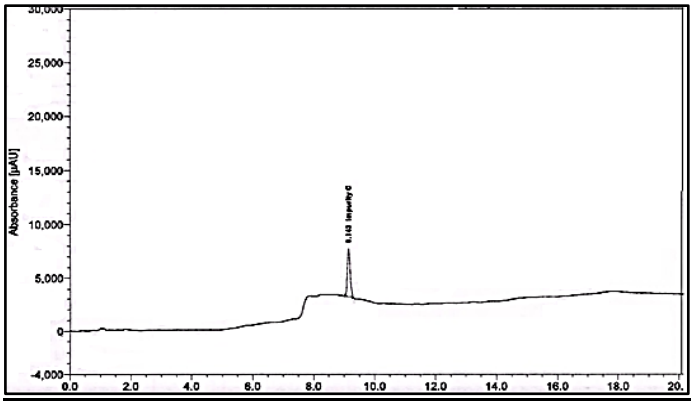

Fig. 13. Specificity Dehydro Impurity (Imp C)

Table 3: S/N ratio for LOD concentration

Peak Name	Concentration (ppm)	Peak Height (H)	Noise Height (h)	S/N= 2H/h
Cinacalcet	0.168	0.7	0.1	14
Impurity A	0.206	1.1	0.1	22
Impurity B	0.200	0.25	0.1	5
Impurity C	0.202	0.45	0.1	9

Table 4. S/N ratio for LOQ concentration

Peak Name	Concentration (ppm)	Peak Height (H)	Noise Height (h)	S/N= 2H/h
Cinacalcet	0.505	23	0.1	46
Impurity A	0.618	36	0.1	72
Impurity B	0.600	9	0.1	18
Impurity C	0.606	13	0.1	26

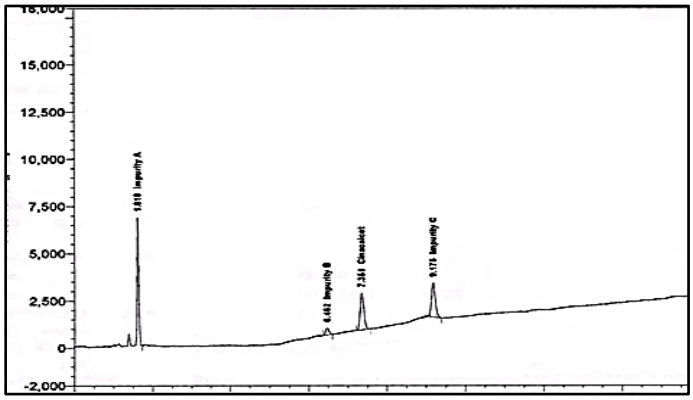

Fig. 14. Linearity level- I (LOQ)

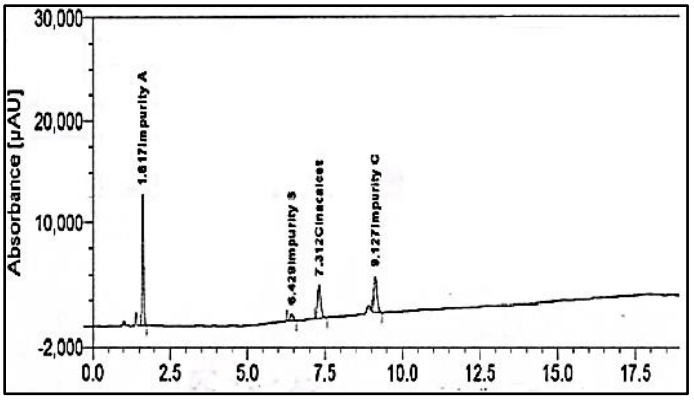

Fig. 15. Linearity level- II

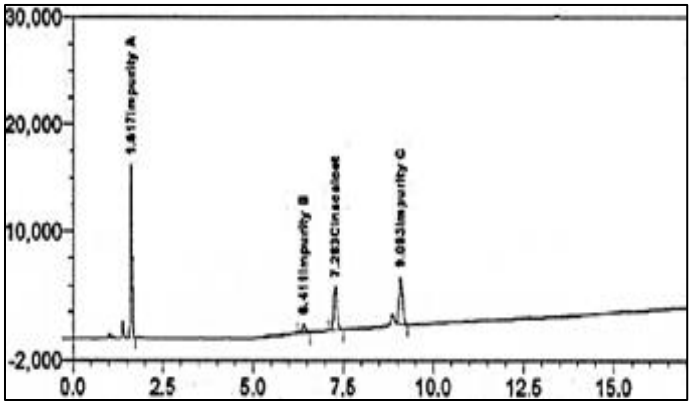

Fig. 16. Linearity Level III

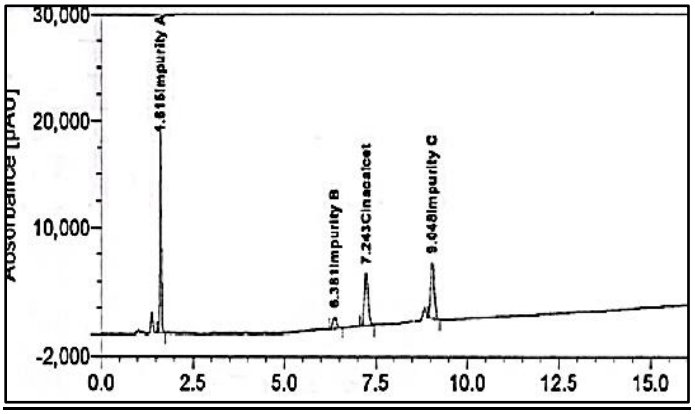

Fig. 17. Linearity Level IV

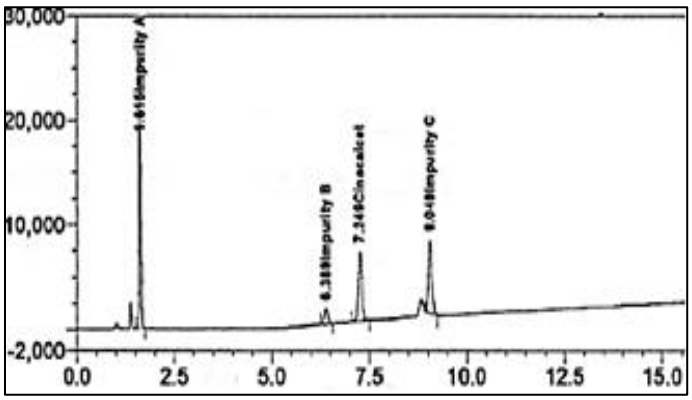

Fig. 18. Linearity Level V

The graphs of peak response of respective analyte verses concentration were plotted for Cinacalcet HCl and all three impurities. It was also processed for statistical assessment.

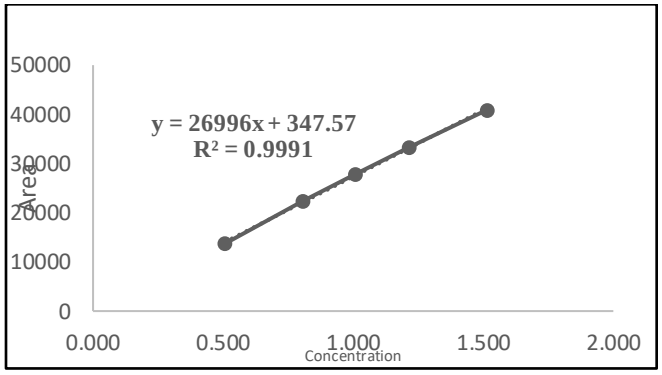

Fig. 19. Linearity of Cinacalcet HCl

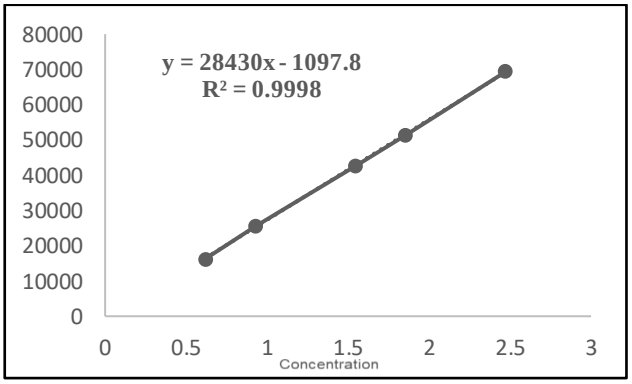

Fig. 20. Linearity of Impurity A

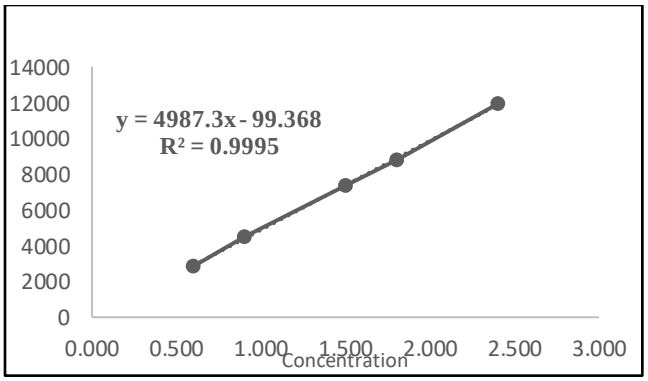

Fig. 21. Linearity of Impurity B

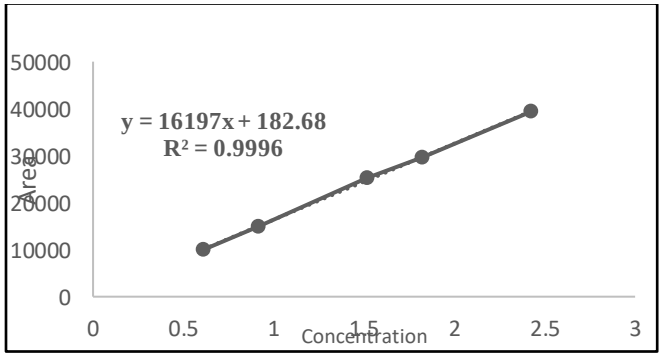

Fig. 22. Linearity of Impurity C

The system suitability is calculated on six replicate injections of Standards solution, results are tabulated in Table 5. Resolution between adjacent pair of peaks was calculated.

Table 5. System suitability

	Imp A	**Imp B**	**Cinacalcet**	**Imp C**
% RSD	1.02	2.25	1.92	1.92
Plate	8513	19283	22146	32908
Tailing	1.1	1.1	1.1	1.1
Resolution		40	1.6	5.9

Table 6. Precision- % RSD for Impurity Content

	% of Impurity A	**% of Impurity B**	**% of Impurity C**
Repeatability			
% RSD	1.67	2.71	3.64
Intermediate Precision			
% RSD	1.20	1.60	3.64
% Variation between Repeatability and Intermediate Precision			
% Variation	0.24	0.12	0.13

Solution Stability of Standard and Sample was monitored up to 24 hours and found to be stable. % RSD for content of each impurity at all time intervals was found to be less than 5.0%.

Table 7. % Accuracy for Impurity A, B and C

	Impurity A	**Impurity B**	**Impurity C**
Level 1	99.64	90.15	103.71
	104.19	92.02	109.08
	102.88	94.14	111.09
Level 2	106.83	96.78	108.25

	101.96	100.01	100.36
	102.30	98.19	101.67
Level 3	108.48	99.35	107.89
	104.84	102.52	108.68
	107.00	101.77	110.42

% Mean Accuracy for Impurity A was found to be 104%

% Mean Accuracy for Impurity B was found to be 97%

% Mean Accuracy for Impurity was found to be 107%

Table 8. % Variation for impurity Content (Robustness and Precision Study)

	Imp A	**Imp B**	**Imp C**
Rob1	1.74	0.02	0.38
Rob1	3.73	1.18	1.78
Rob1	0.68	1.91	0.86
Rob 2	0.65	2.49	2.19
Rob 2	2.61	1.79	0.51
Rob 2	0.59	1.18	3.78
Rob 3	7.86	1.22	6.09
Rob 3	3.36	2.05	7.86
Rob 3	1.39	2.84	0.02
Rob 4	3.86	2.66	0.23
Rob 4	2.17	0.56	6.09
Rob 4	3.95	1.77	1.71

(Rob = Robustness)

Stress Study.

No degradation was observed in any of the physical stress conditions (higher temperature, uv-visible light, and higher humidity. Cinacalcet was more susceptible for base hydrolysis. Various secondary degradations were formed in initial base hydrolysis study, hence final conditions were made moderate. In acidic condition, % degradation was achieved up to 4.6 %; in basic condition degradation was achieved to 6.2 % and for oxidation degradation was up to 8.5%.

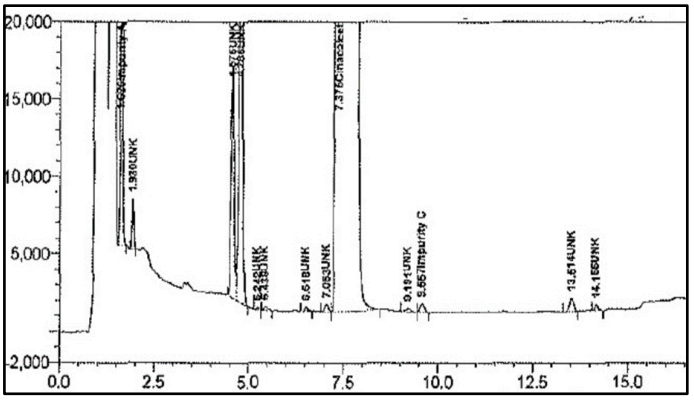

Fig 23. Oxidative Degradation of Cinacalcet HCl

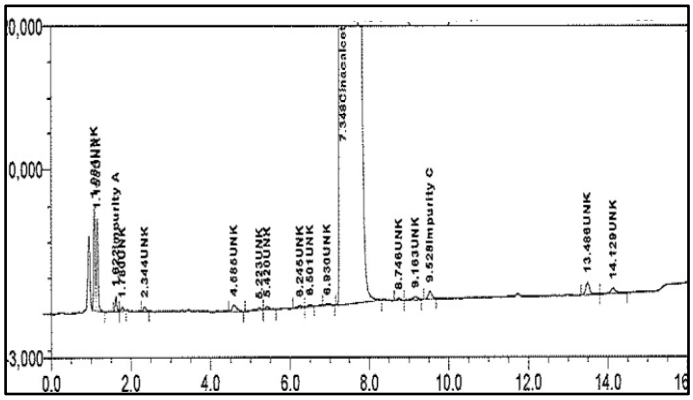

Fig 24. Acidic Hydrolysis of Cinacalcet HCl

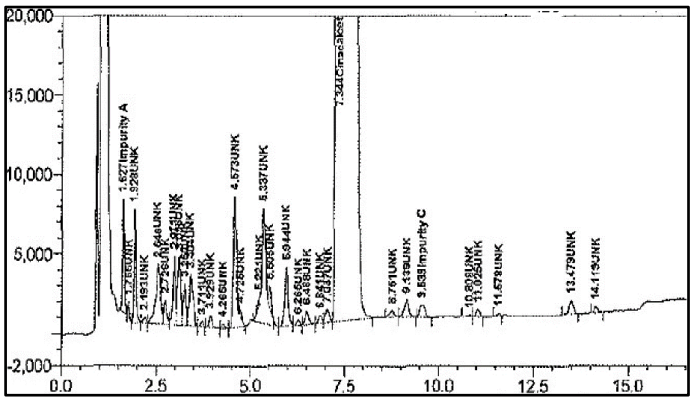

Fig 25. Basic Hydrolysis of Cinacalcet HCl

The peak purity for Cinacalcet and all known impurities was more than 950, confirming homogeneity of peak.

CONCLUSION

The Related substances method was developed for Cinacalcet Hydrochloride along with ethanamine impurity, Cinacalcet mesylate and Cinacalcet dehydro impurity. The shorter method was achieved without using high cost UPLC instrument. The method was duly validated according to current expectation from global regulatory agencies. The statistical assessment of the method, accuracy, precision, robustness meets the requirement. The sample and standards preparations are stable for more than 24 hours. The stress study establishes the method's competency to

resolve all potential degradants which may generate on aging of API or during formulation. Thus, this stability indicating method can be adopted for regular GMP environment for release and stability.

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