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Development and Validation of Analytical Method for the Estimation of Related Substances in Cinacalcet Tablets by RP – HPLC

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ABSTRACT

A simple yet suffice, accurate and precise method was developed for the estimation of impurities in cinacalcet tablets (30/60/90 mg). The samples were injected and flows through Hypersil BDS 100 mm×4.6mm, 3µm column (stationary phase), The mobile phase being a combination of MP-A/Solution A: Prepared a degassed mixture of Buffer and Methanol(HPLC Grade) in the ratio of 70:30, v/v. Solution-B: Prepared a degassed mixture of Acetonitrile: Methanol: Water (HPLC Grade) in the ratio of 90:5:5, v/v and the temperature was maintained at 30°C. The wavelength was optimised to 210 nm. The retention time of Cinacalcet and its impurities (Amine impurity,Alcohol impurity,Mesylated impurity,Regio impurity and Dimer impurity) are ,6.70, 1.49, 1.57, 1.89, 6.38, 19.75. The %RSD of Cinacalcet is 0.6. This method has eluted Cinacalcet and all its impurities with high resolution so this makes the method easy and economical, and this method is validated for parameters such as System Sitability, specificity, Linearity, Accuracy, LOD and LOQ. The developed method with the specified chromatographic parameters was acceptable and found to be linear and accurate and highly suitable to for routine RS analysis

Key words: Cinacalcet, Amine impurity, Alcohol impurity, Mesylated impurity, Regio impurity and Dimer impurity, RP-HPLC.

INTRODUCTION:

Cinacalcet hydrochloride is an oral calcimimetic drug that works by binding to the calcium-sensing receptor (CaR) on the surface of parathyroid gland cells. This binding causes a structural change in the CaR that makes it more sensitive to calcium in the blood. This increased sensitivity reduces the amount of parathyroid hormone (PTH) secreted by the parathyroid gland. Lower PTH levels can lead to: Lower serum calcium levels, Inhibited osteoclast activity, Decreased bone turnover and fibrosis, and Lower PSA levels.

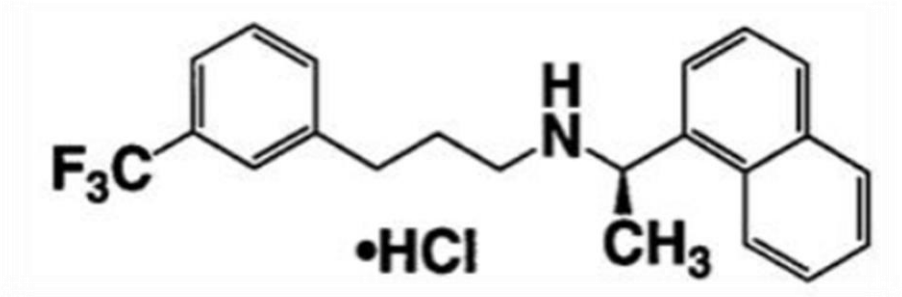


Fig:1-Structure of Cinacalcet

The Analytical method developed through Reverse Phase High Performance Liquid Chromatography for the estimation of impurities in Cinacalcet Hcl Tablets. Impurities can pose health risks, cause adverse reactions, or be toxic, so it's vital to detect and control them to protect consumer health. Impurities can affect the efficacy, stability, and overall quality of a product. In pharmaceuticals, for instance, impurities can impact the drug's effectiveness or cause unintended side effects. Analytical Method development helps ensure that products maintain consistent quality by monitoring and controlling impurity levels.

METHODS AND MATERIALS:

The Cinacalcet working standard and tablets were provided by laboratory. Materials and their Grades used are mentioned in the below table

Table:1 Chemicals used

S.NO	Chemicals/Reagents	Make	Grade
1.	Acetonitrile	Supelco	HPLC Grade
2.	Methanol	Supelco	HPLC Grade
3.	Orthophosphoric acid	Supelco	Lab reagent Grade
4.	Potassium dihydrogen ortho Phosphate	Supelco	Reagent Grade
5.	1-Octane sulphonic acid sodium salt	Supelco	Reagent Grade
6.	Water	Millipore	Milli-Q

INSTRUMENTATION:

The analysis was carried out by using WATERS2695 Alliance HPLC system, equipped with PDA detector and Auto sampling mechanism incorporated with Empower 3 software. And column used Hypersil BDS 100 mmX4.6mm, 3µm or its equivalent

Semi micro balance (Sartorius), pH meter (Hanna), centrifuge (Remi), rotary shaker (Remi), Sonicator (Samarth), Vacuum/ Pressure pump (Borosil)), were used.

Chromatographic conditions:

Optimised Liquid chromatographic conditions equipped with PDA detector

Table-2 Chromatographic conditions

Column	**Hypersil BDS 100 mmX4.6mm, 3µm**
Wave length	**210 nm**
Column temperature	**30°C**
Flow rate	**0.8 ml/min**
Run time	**60 minutes**
Injection volume	**10µl**

Table-3 Gradient program

Time (in minutes)	% Mobile Phase- A (%)	% Mobile phase- (%)
0.01	55	45
15	40	60
30	5	95
45	5	95
50	55	45
60	55	45
60.01	STOP	

Optimised Mobile phase preparation:

Preparation of buffer solution: Accurately weigh and transfer 1.36 gm of Potassium dihydrogen ortho Phosphate into 1000 mL of Milli-Q water and add 1 gm of 1-Octane sulphonic acid sodium salt, mix well. Adjust the pH of the solution to 3.0±0.05 with diluted ortho phosphoric acid and filter the solution through 0.45 µ membrane filter

Preparation of Mobile phase:

Solution-A: Prepare and mix pH 3.0 Buffer: Methanol in the ratio of 70:30 v/v and degas**.**

Solution-B: Prepare and mix Acetonitrile: Methanol: Water in the ratio of 90:5:5 v/v and degas.

Diluent: Prepare and mix Acetonitrile and Milli-Q water in the ratio of 90:10 v/v.

Preparation of Blank solution: Diluent is used as blank.

Preparation of Standard Stock Solution: (53.6 ppm)

Weigh accurately and transfer about 67 mg of Cinacalcet HCl Working Standard to a 50 mL volumetric flask. Add about 30 mL of diluent, sonicate to dissolve and dilute to volume with diluent (D1). Further dilute 2 mL from the standard stock solution to 50 mL volumetric flask, dilute to volume with diluent (D2).

Preparation of Standard Solution: (2.144 ppm)

Further dilute 2 mL from the standard stock solution (D2) to 50 mL volumetric flask, dilute to volume with diluent (D3).

Preparation of Placebo Solution: Weighed and transferred accurately 271.38 mg of powdered placebo sample in to a 50 mL volumetric flask. Added 30 ml of diluent and Sonicated with intermittent shaking for 20 minutes. Diluted to volume with diluent. Centrifuged the solution for 5-10 minutes at 2000 RPM.

Preparation of Sample Stock Solution :(30 / 6 0 / 90 mg) Weigh accurately about not less than 10 tablets of respective strength, crush in to fine powder. Weigh and transfer an accurately weighed powdered equivalent to 50 mg Cinacalcet into 50 mL volumetric flask. Add 30 mL of diluent, sonicate for 20 minutes. Cool the flask to room temperature. Dilute to volume with diluent. Centrifuge the solution at 2000 RPM for 10 minutes.

Method development:

After Stabilisation of system which is done through continueous flow of mobile phase through column and then injecting the solution to determine the necessary parameters of the system, a single injection of blank and single injection of Cinacalcet standard solution was runed.

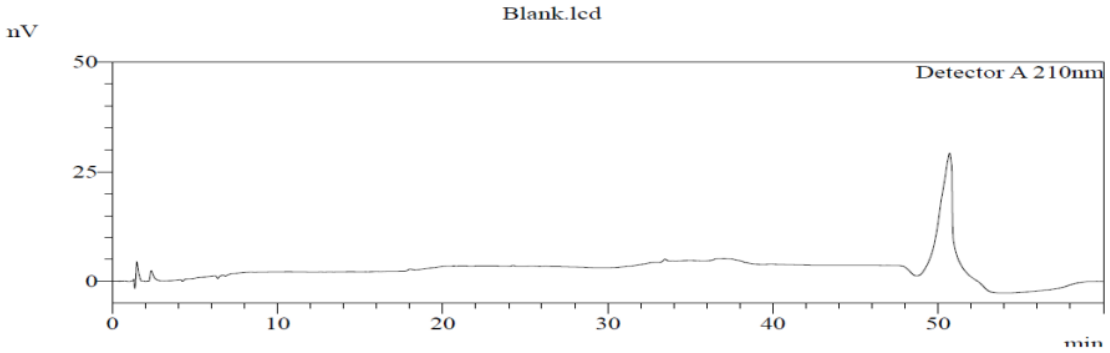


Fig:2 **CHROMATOGRAM OF BLANK**

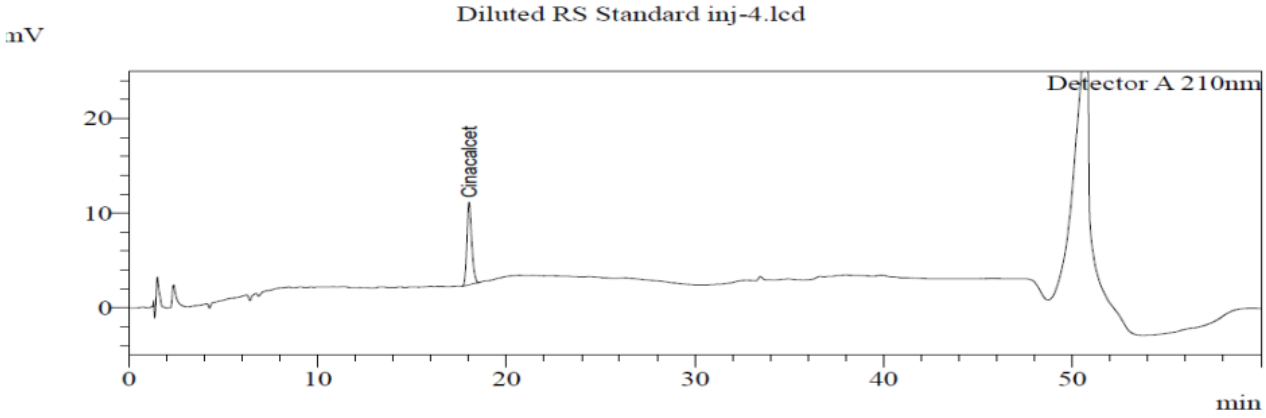


Fig:3 **CHROMATOGRAM OF STANDARD**

METHOD VALIDATION:

SYSTEM SUITABILITY TEST:

System Suitability Test Solution: The Standard Solution was used to determine the system suitability criteria. It is estimated by separately injecting 10µL of Blank, Placebo and Six replicate injections of Standard solution, System suitability solution and individual Impurity solutions into the chromatographic system. Record the chromatograms and measure the peak responses.

For system suitability lowest plate count and highest tailing factor was considered.

The system suitability criteria were observed well within the acceptance limits. Any Impurity interference, Blank and Placebo interference was also not observed.

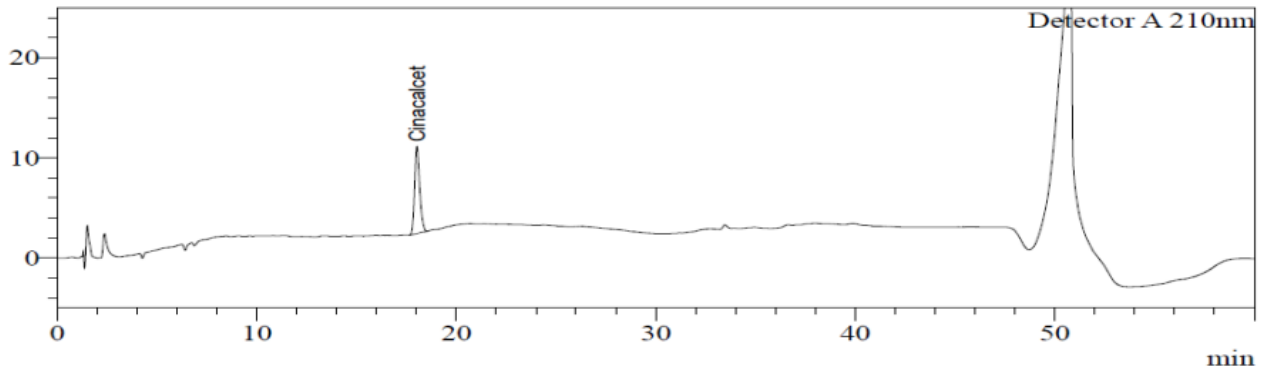


Fig:4 **CHROMATOGRAM OF SYSTEM SUITABILITY**

SPECIFICITY:

Specificity is a test in which at the retention time of the main peak (active ingredient) the blank and the placebo and impurity do not interfere with the retention time of the main peak i.e., the blank placebo and impurities should not show any peaks at the retention time of the Active ingredient.

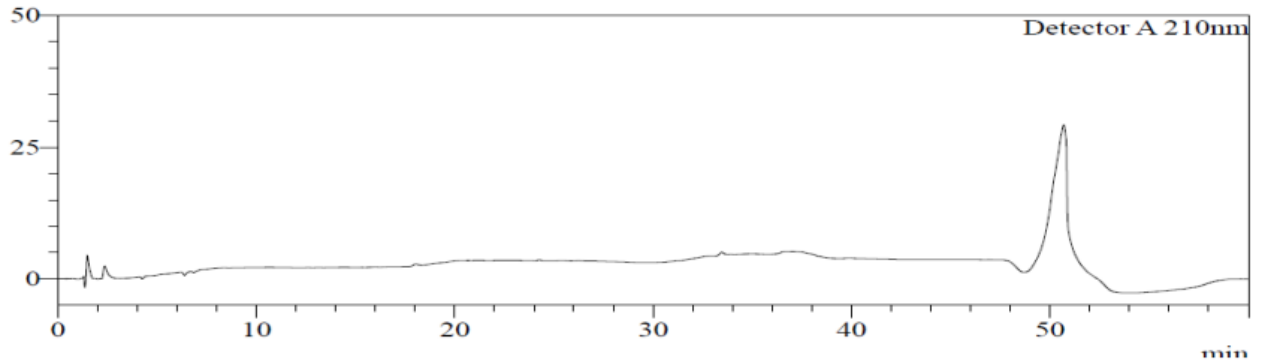


Fig:5 **CHROMATOGRAM OF BLANK**

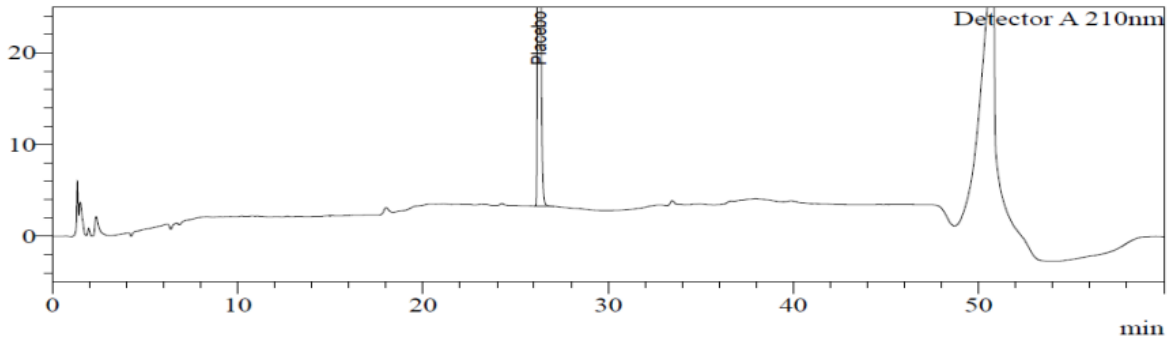


Fig:6 **CHROMATOGRAM OF PLACEBO**

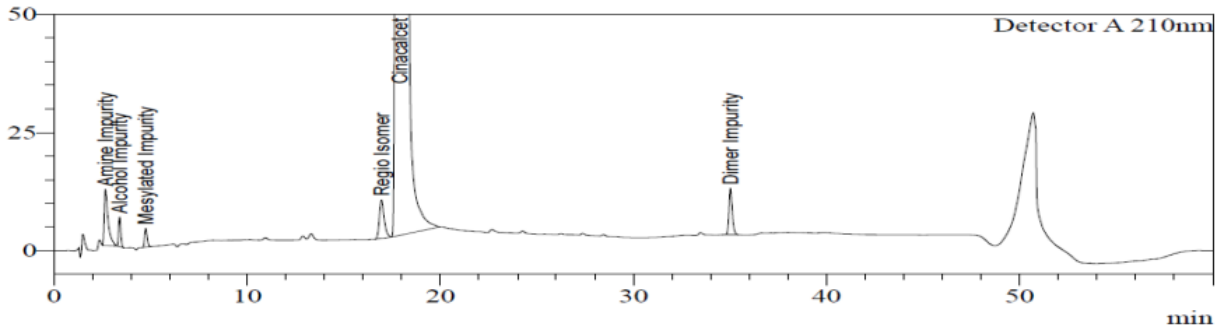


Fig:7 **CHROMATOGRAM OF SPIKED SAMPLE**

Linearity:

Linearity of detector was established by plotting a graph of concentration and responses of Cinacalcet. All the Known impurities and Cinacalcet were found Linear

Results were tabulated in table

Table:4 **Linearity Table**

S.No.	Description	RT	RRT	Slope	r^2 Value	RRF
1	Cinacalcet	17.778	1.00	3828	0.999	1.00
2	Amine Impurity	2.718	0.15	4793	0.999	1.25
3	Alcohol Impurity	3.315	0.19	1274	0.999	0.33
4	Mesylated Impurity	4.611	0.26	977	0.998	0.26
5	Regio Isomer	16.828	0.95	3844	0.999	1.00
6	Dimer Impurity	34.867	1.96	2790	0.999	0.73

Conc Level	**Area**
25%	**93092**
50%	**193531**
100%	**387327**
150%	**576614**
200%	**763782**

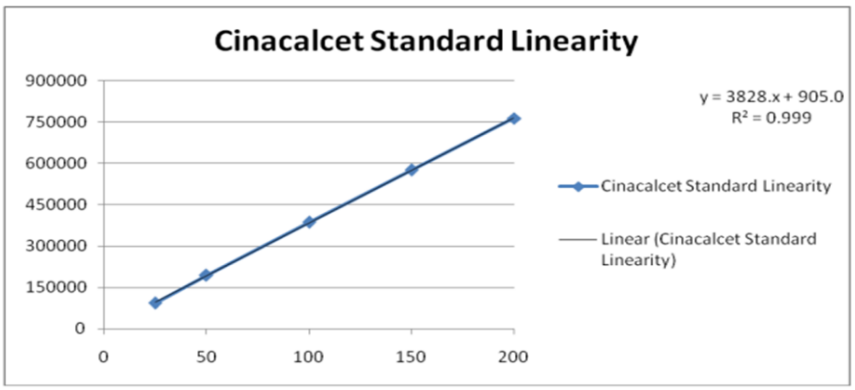


Fig:8 **Calibration Curve of Cinacalcet**

Conc Level	Area
25%	**98309**
50%	**211136**
100%	**444930**
150%	**696211**
200%	**932898**

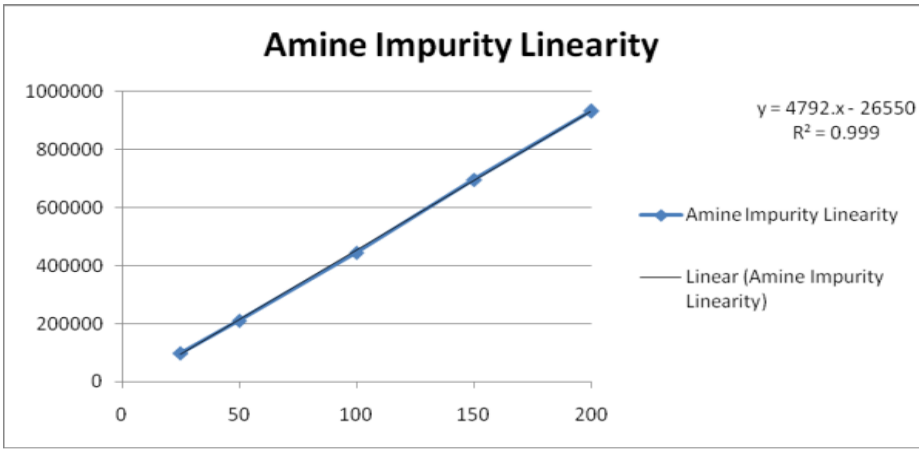


Fig:9 **Calibration Curve of Amine Impurity**

Conc Level	Area
25%	**30337**
50%	**61587**
100%	**125899**
150%	**191051**
200%	**252257**

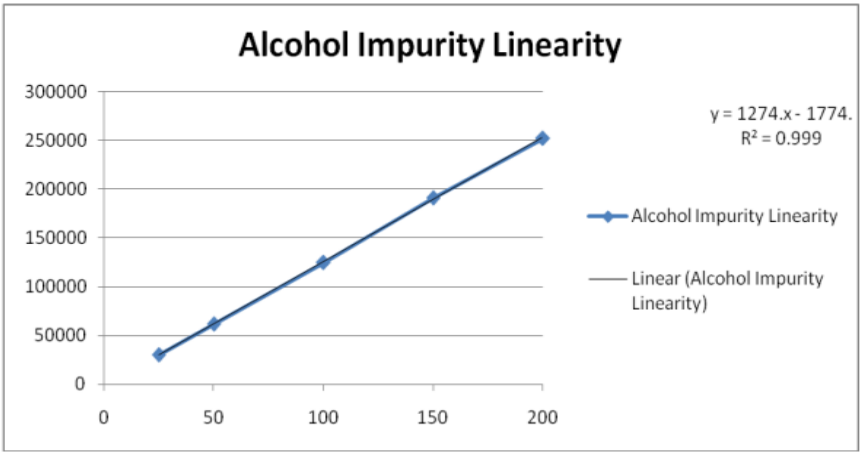


Fig:10 **Calibration Curve of Alcohol Impurity**

Conc Level	**Area**
25%	**22709**
50%	**47598**
100%	**93700**
150%	**149904**
200%	**191340**

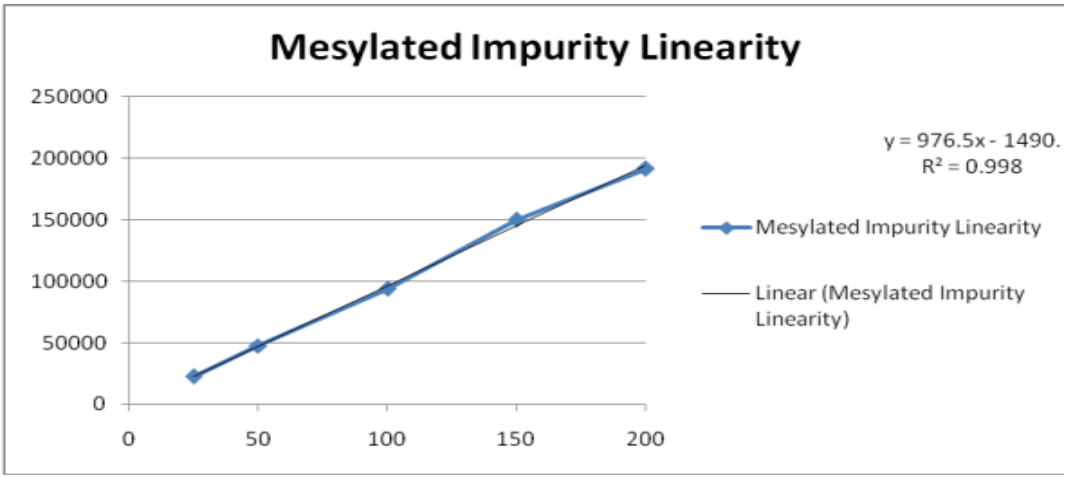


Fig:11 **Calibration Curve of Mesylated Impurity**

Conc Level	Area
25%	93520
50%	190584
100%	386035
150%	587463
200%	760663

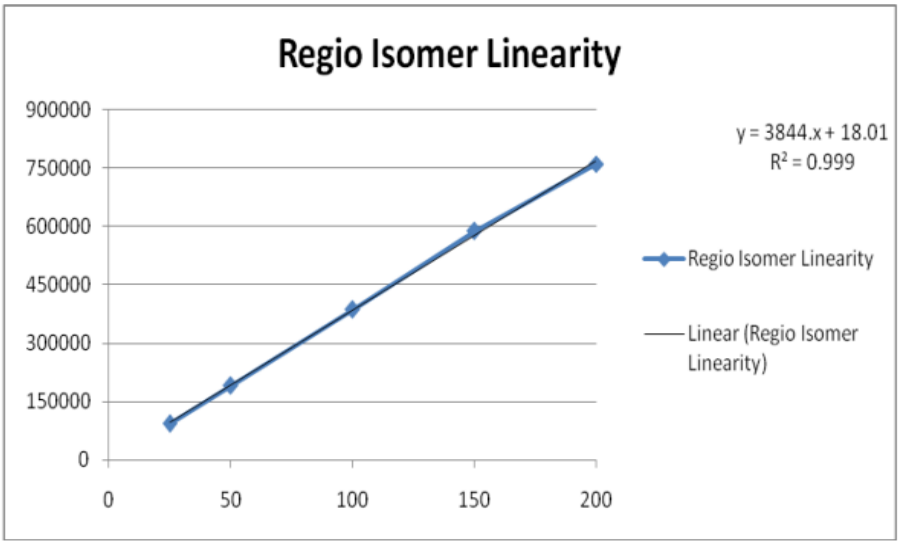


Fig:12 **Calibration Curve of Regio Impurity**

Conc Level	**Area**
25%	**70757**
50%	**135730**
100%	**272123**
150%	**426781**
200%	**552359**

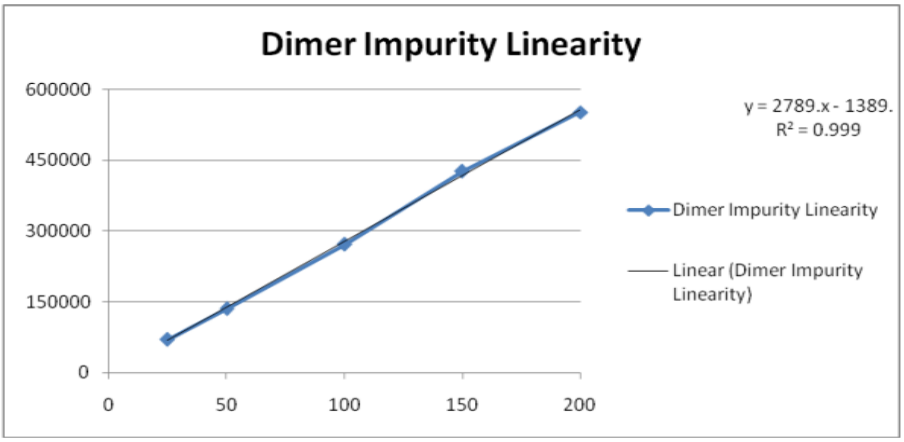


Fig:13 **Calibration Curve of Dimer Impurity**

Result: All the Known impurities and Cinacalcet were found Linear

ACCURACY:

The accuracy study was performed to check the closeness of the obtained/test value to the true value.

Table:5

% Conc	Dilution scheme from each stock solution in mL						Final Volume (mL)	Level (%)
	Amine Imp	Alcohol Imp	Mesylated Imp	Regio Isomer	Cinacalcet (Std)	Dimer Imp		
25%	1.465	0.270	0.165	2.520	2.05	0.880	200	0.125
50%	1.470	0.268	0.165	2.520	2.05	0.880	100	0.25
100%	2.940	0.540	0.330	5.100	4.10	1.760	100	0.50
150%	2.200	0.400	0.247	3.780	3.08	1.316	50	0.75
200%	2.940	0.540	0.330	5.100	4.12	1.760	50	1.00

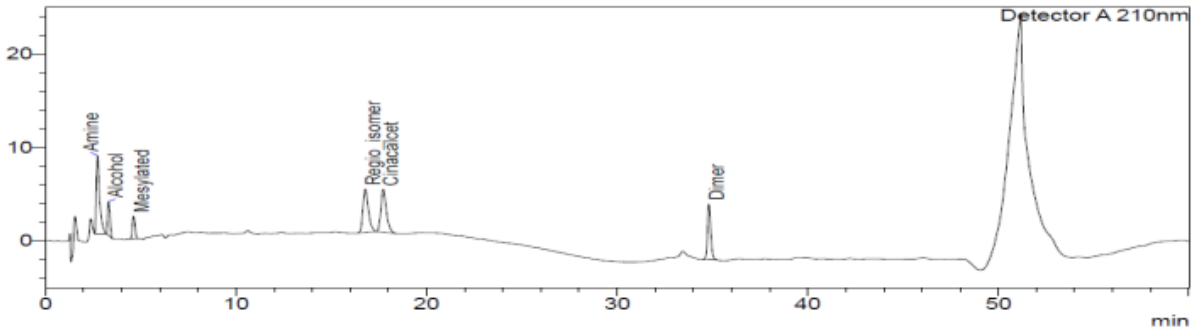


Fig:14 **25% specification level solution**

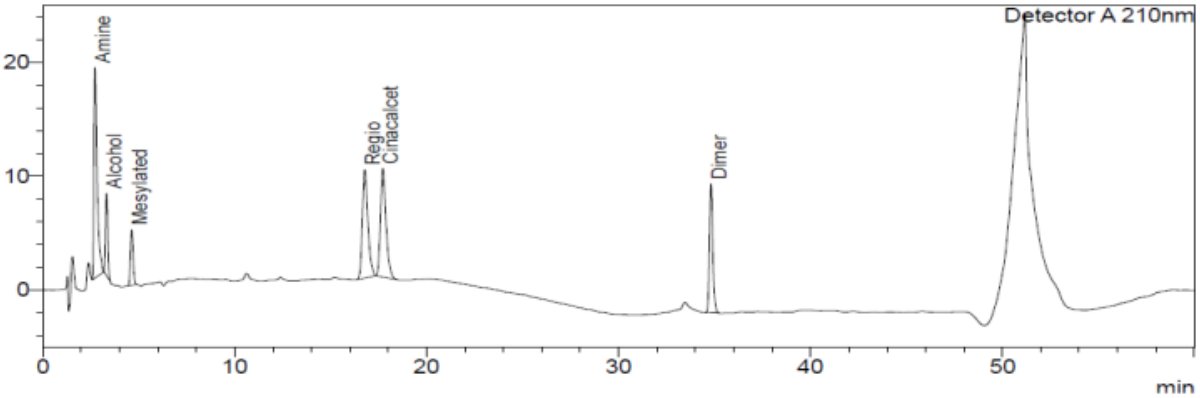


Fig:15 **50% specification level solution**

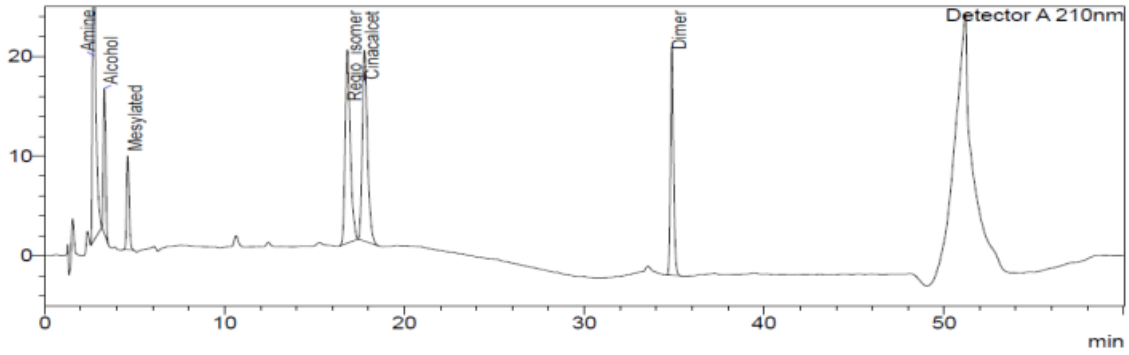


Fig:16 **100% specification level solution**

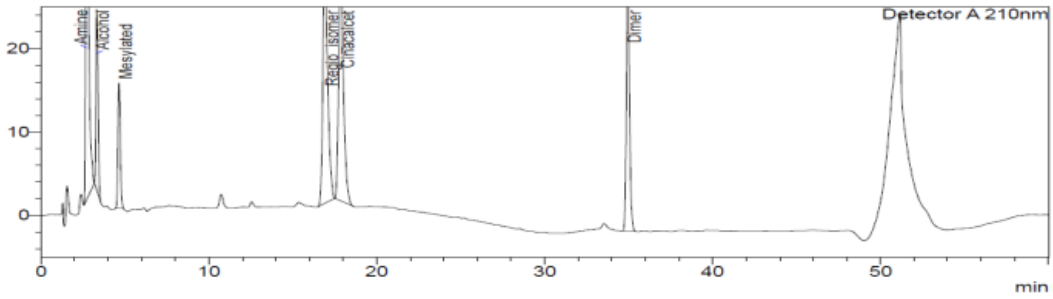


Fig:17 **150% specification level solution**

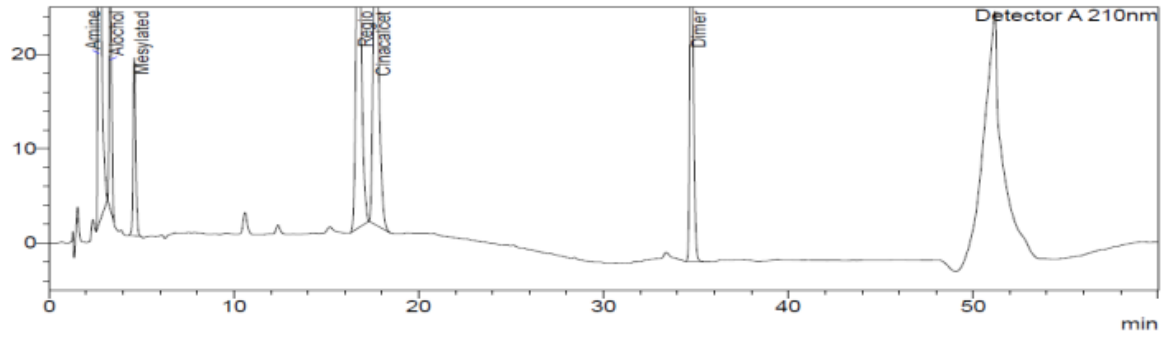


Fig:18 **200% specification level solution**

Result: The method was found to be accurate

LOD AND LOQ:

Preparation of 0.1% Reference solution for LOD & LOQ establishment:

To establish the LOD and LOQ concentrations, a solution of 0.1% Linearity solution was prepared as specified in the below table and used as Reference solution for determine the S/N ratio.

Table:6

Conc	Level (%)	Dilution scheme from stock solution						Final volume (ml)
		Amine Imp	Alcohol Imp	Mesylated Imp	Regio Isomer	Cinacalcet (Std)	Dimer Imp	
10%	0.1	0.300	0.055	0.035	0.550	0.45	0.190	50

LOQ Solution Establishment:

Based on the S/N ratio obtained from the reference solution of 0.1% prepared as above, the LOQ Solution was prepared as specified the in the below table.

Table:7

Level %	Dilution scheme from stock solution for LOQ solution in mL						Final volume (ml)
	Amine Imp	Alcohol Imp	Mesylated Imp	Regio Isomer	Cinacalcet (Std)	Dimer Imp	
LOQ	0.5	0.5	0.4	1.1	0.2	0.7	100
Conc in µg/mL	0.068	0.094	0.121	0.083	0.108	0.075	

LOD Solution Establishment:

Based on the S/N ratio obtained from the LOQ level solution prepared as above, the LOD Solution was prepared as specified the in the below table.

Table:8

Level %	Dilution scheme from stock solution for LOD solution in mL						Final volume (ml)
	Amine Imp	Alcohol Imp	Mesylated Imp	Regio Isomer	Cinacalcet (Std)	Dimer Imp	
LOD	0.8	0.5	0.6	1.2	0.06	0.5	250
Conc in µg/mL	0.044	0.037	0.073	0.036	0.013	0.021	

Results:

The S/N Ratio were tabulated as below:

Table:9

Level of Conc.	S/N Ratio details						Acceptance criteria
	Amine Imp	Alcohol Imp	Mesylated Imp	Regio Isomer	Cinacalcet (Std)	Dimer Imp	
0.1% (ref)	259.3	116.0	81.7	108.5	121.3	143.7	NA
LOQ Solution	15.6	11.4	9.5	10.6	15.3	18.2	8.0-20.0
LOD Solution	3.4	6.1	5.2	5.6	5.9	3.5	3.0-7.0

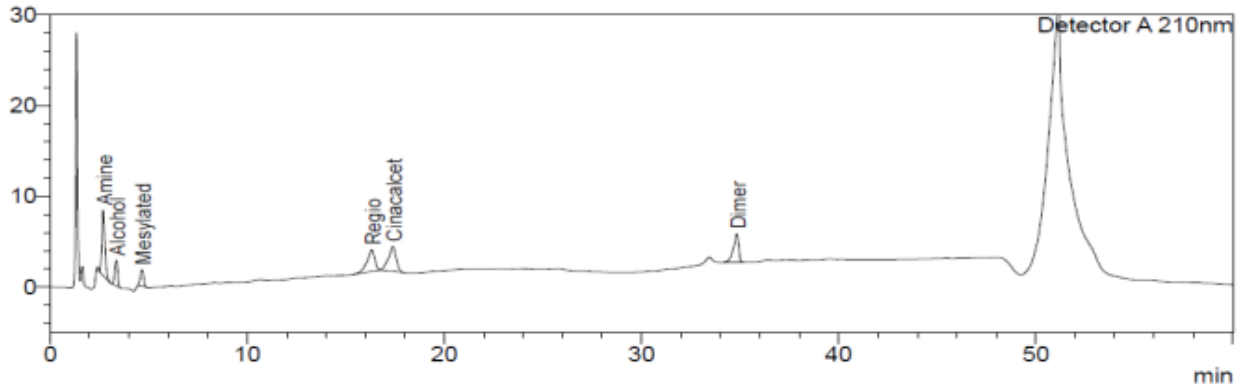


Fig:19 **Chromatogram of 0.1% Spec level solution (Reference)**

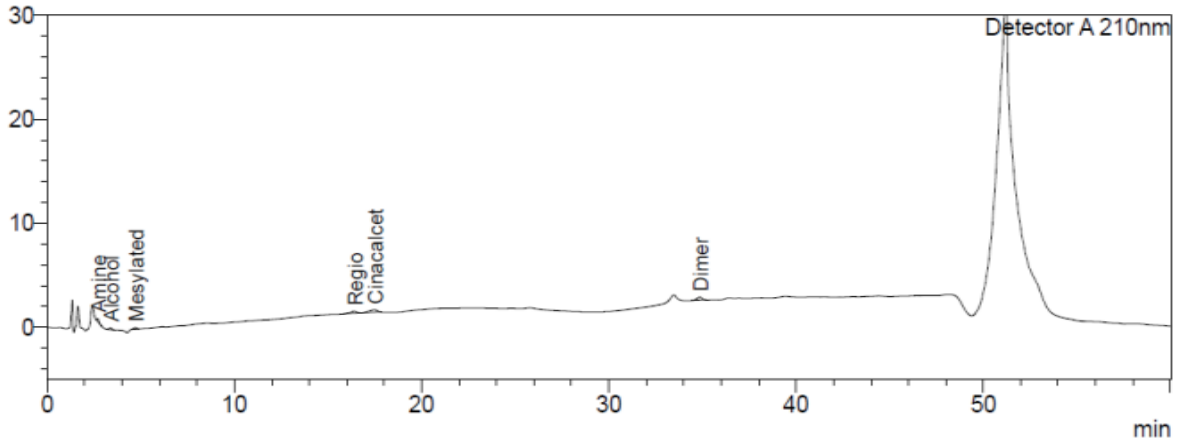


Fig:20 **Chromatogram of LOQ level solution**

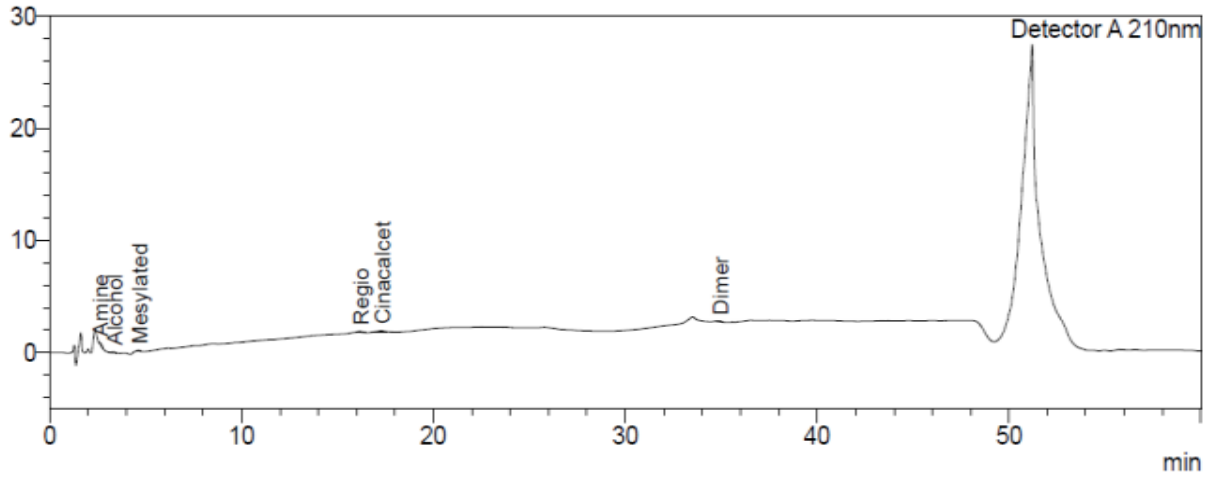


Fig:21 **Chromatogram of LOD level solution**

FORCED DEGRADATION:

The forced degradation is the important test in the method development, where the stability of the drug can be identified. The forced degradation is conducted by treating drug to artificially elevated conditions the forced degradation also helps to find the degradation product which may be drug or its excipients and also their degradation pathways can be known.

Cinacalcet drug product were subjected to following stress conditions:

Heat Treatment : at 105°C for 24 hrs

Humidity : 90% RH for 24hrs

Acid hydrolysis : 1.0N HCL/50°C/1Hour

Base hydrolysis : 1.0N NaOH/50°C/1Hour

Oxidation : 3.0% Hydrogen peroxide/50°C/1Hour

Water : 5ml de-mineralized water/50°C/1Hour

Table:10

Forced Degradation & Mass Balance data of Related Substances									**Peak Purity**	
S. No.	**Condition**	**% Assay (a)**	**Amine (b)**	**Alcohol (c)**	**Highest Unk (d)**	**% Total Imp (e)**	***Total (a+b+c+d+e)**	**Mass Balance**	**Retention time**	**Single Point Purity Threshold**
1	**Sample As Such**	**99.5**	**0.000**	**0.000**	**0.046**	**0.087**	-	-	**18.141**	**0.999998**
2	**Stress-Acid**	**97.3**	**0.000**	**0.000**	**0.027**	**0.059**	**97.4**	**96.9**	**18.107**	**0.999964**
3	**Stress-Base**	**101.5**	**0.000**	**0.020**	**0.035**	**0.079**	**101.6**	**101.1**	**18.047**	**0.999992**
4	**Stress-Oxidation**	**96.7**	**0.003**	**0.000**	**0.174**	**0.419**	**97.1**	**96.7**	**18.105**	**0.999980**
5	**Stress-Heat**	**102.8**	**0.000**	**0.000**	**0.044**	**0.110**	**102.9**	**102.4**	**18.148**	**0.999911**

6	**Stress-Hydrolysis**	**98.2**	**0.002**	**0.000**	**0.053**	**0.141**	**98.3**	**97.9**	**18.122**	**0.999836**
7	**Stress-Humidity**	**100.9**	**0.000**	**0.000**	**0.067**	**0.115**	**101.0**	**100.5**	**18.095**	**0.999943**

* Mesylated Impurity, Regio Isomer and Dimer Impurity Presence was not detected in any Stress condition, hence not reported in the table.

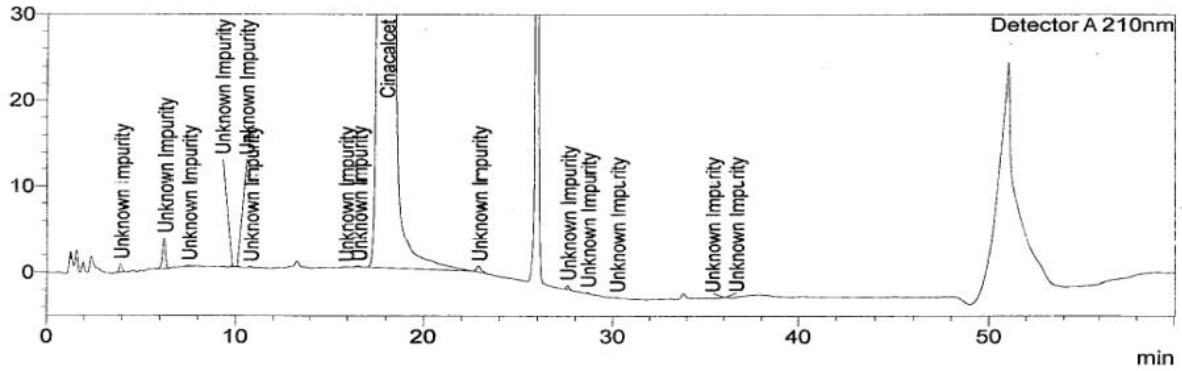
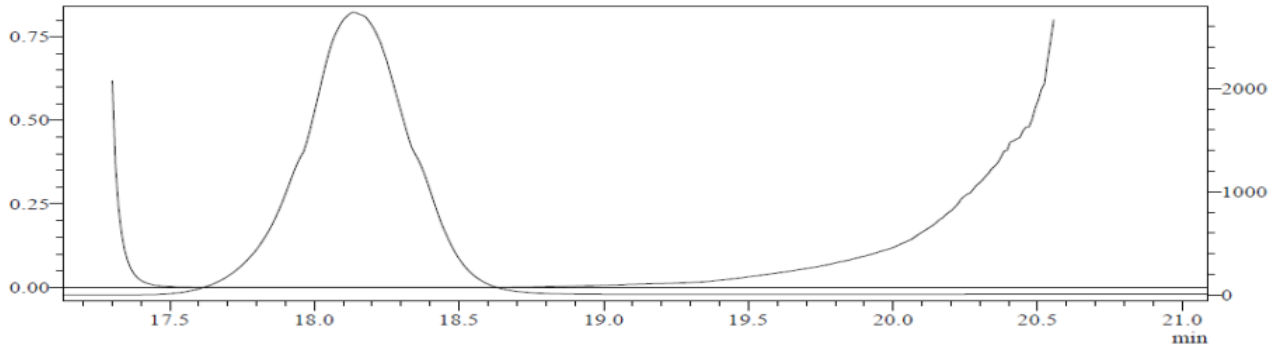


Fig:22 **Chromatogram of As such Sample**



Peak Purity Index:0.999998

Fig:23**Peak Purity Plot of Sample as Such**

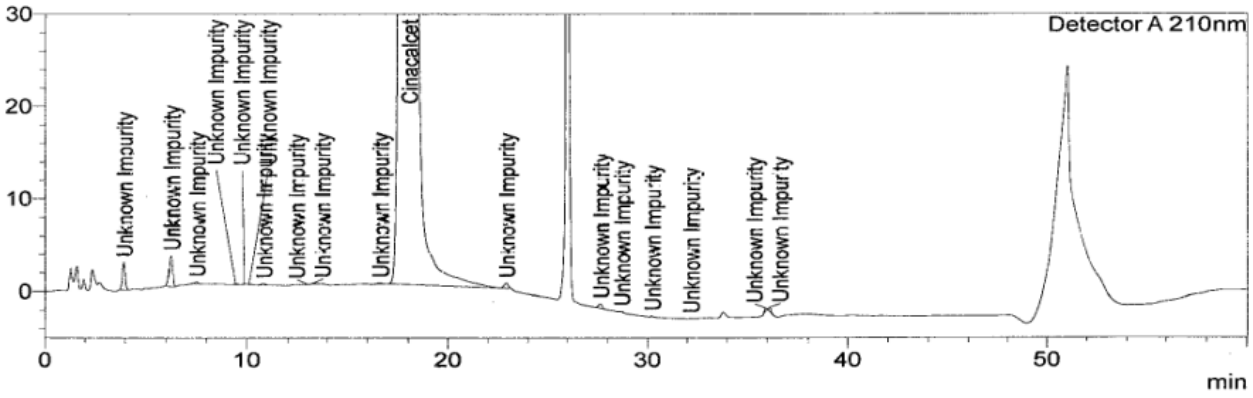
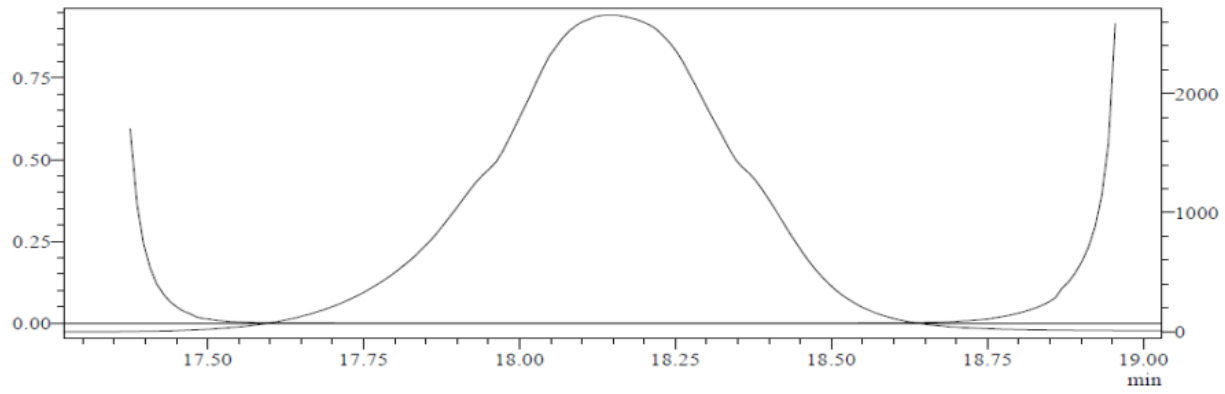


Fig:24 **Chromatogram of Sample - Heat Treated**



Peak Purity Index:0.999997

Fig:25 **Peak Purity plot of Sample - Heat Treated**

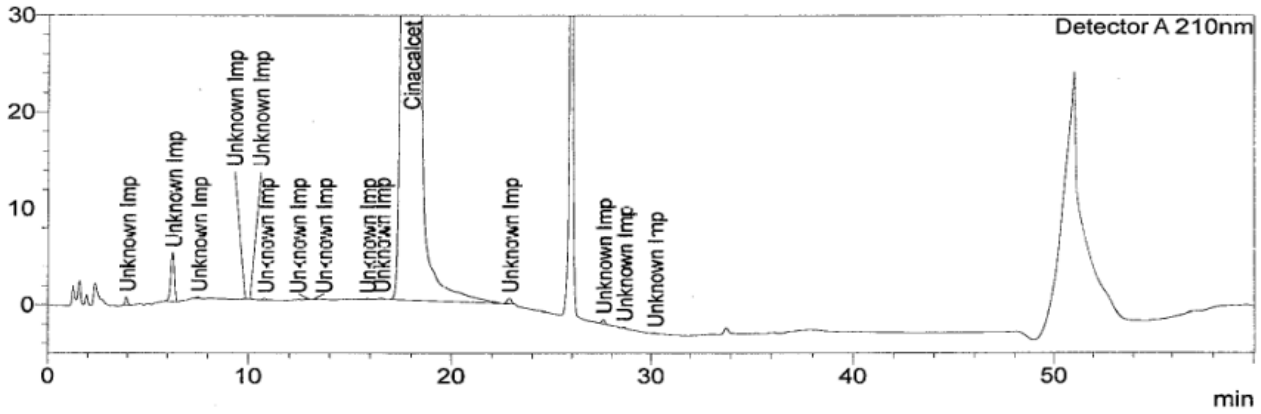
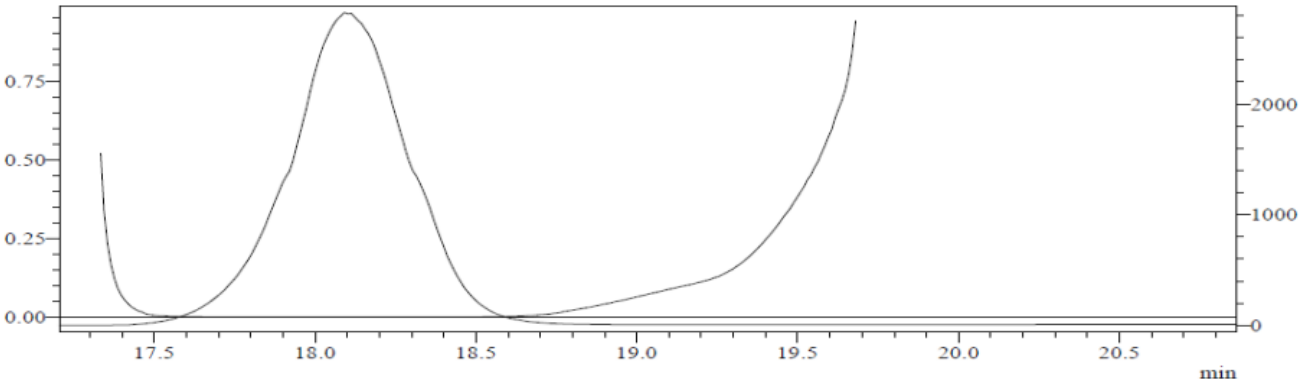


Fig:26 **Chromatogram of Sample - Humidity Treated**



Peak Purity Index:0.999996

Fig:27 **Peak Purity plot of Sample - Humidity Treated**

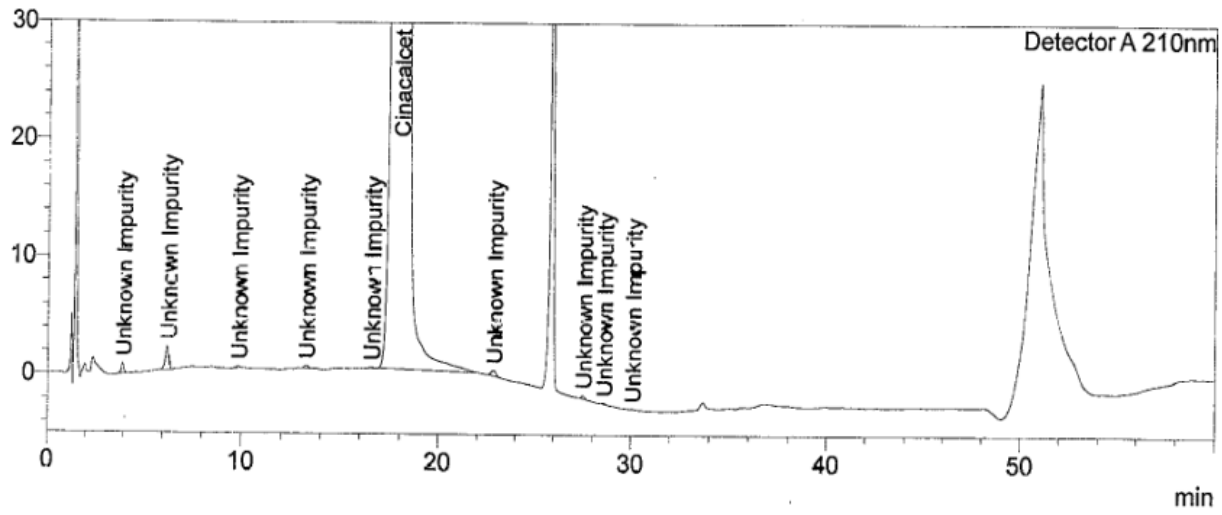
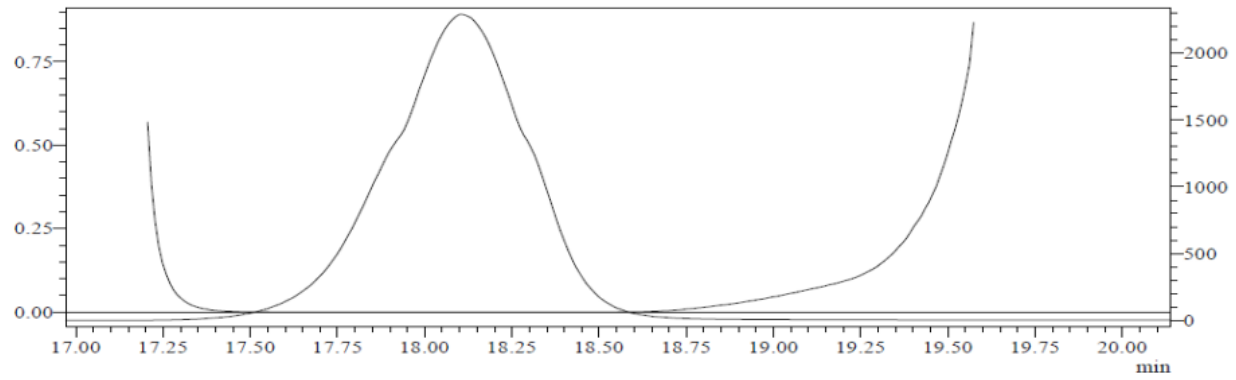


Fig:28 **Chromatogram of Sample-Acid degradation**



Peak Purity Index:1.000000

Fig:29 **Peak Purity Plot of Sample - Acid degradation**

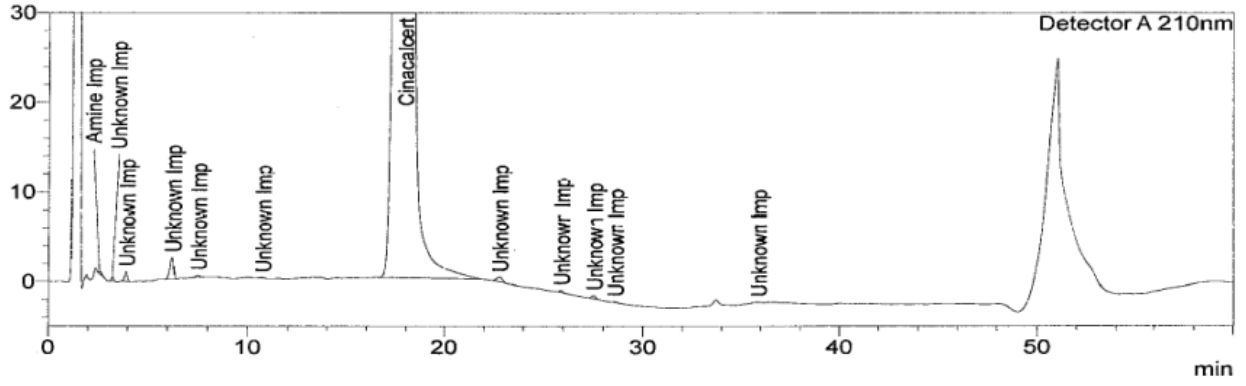
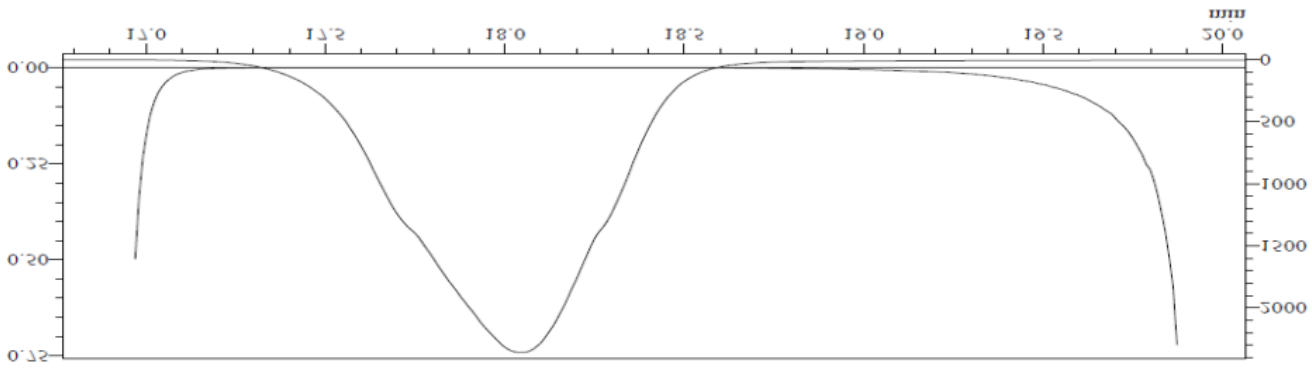


Fig:30 **Chromatogram of Sample - Base degradation**



Peak Purity Index:0.999999

Fig:31 **Peak Purity Plot of Sample - Base degradation**

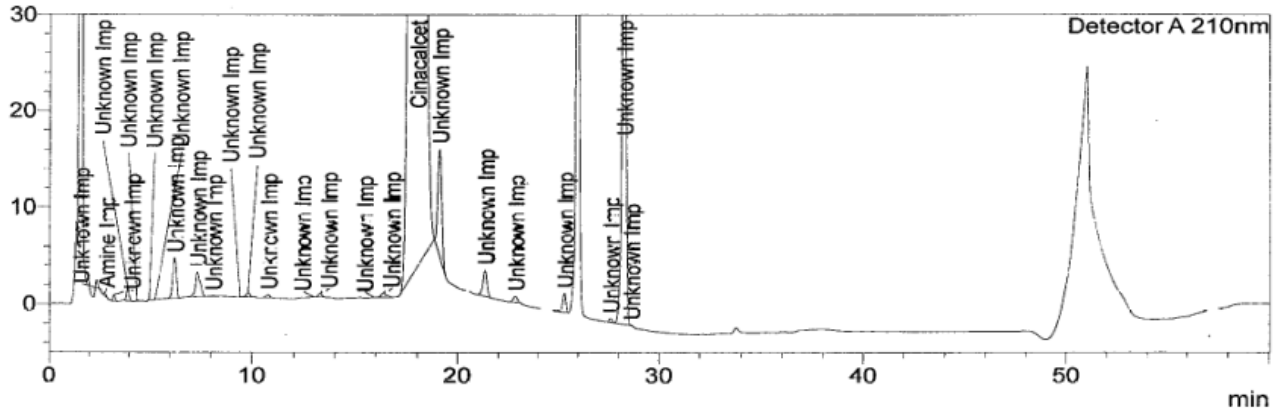
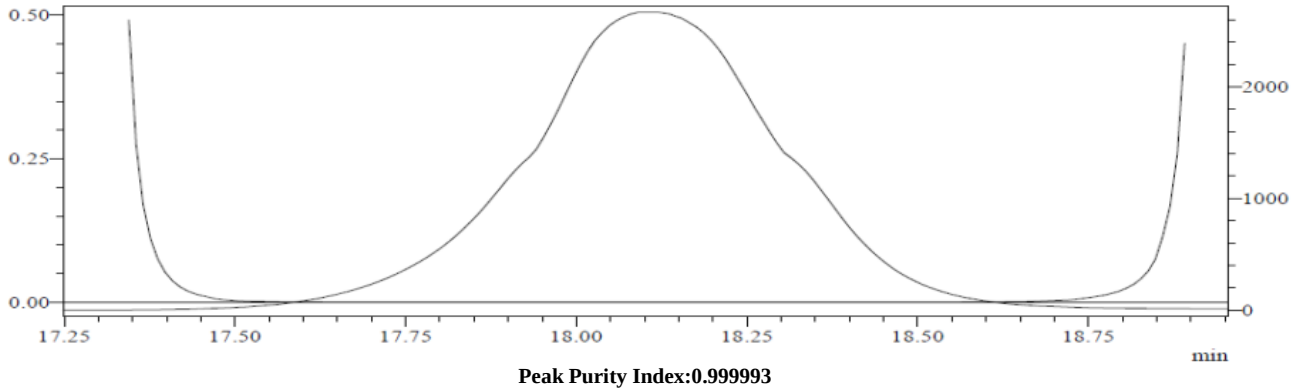


Fig:32 **Chromatogram of Sample – Oxidation**



Peak Purity Index:0.999993

Fig:33 **Peak Purity Plot of Sample – oxidation**

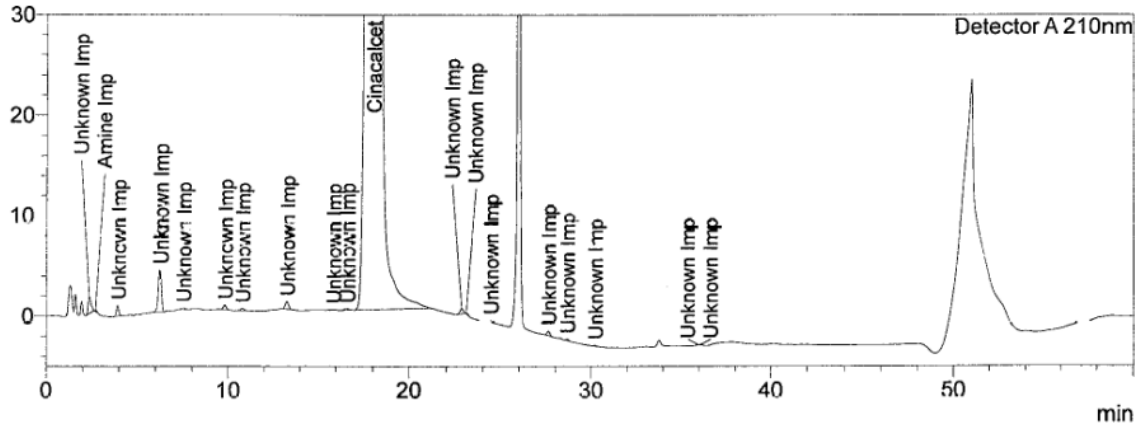
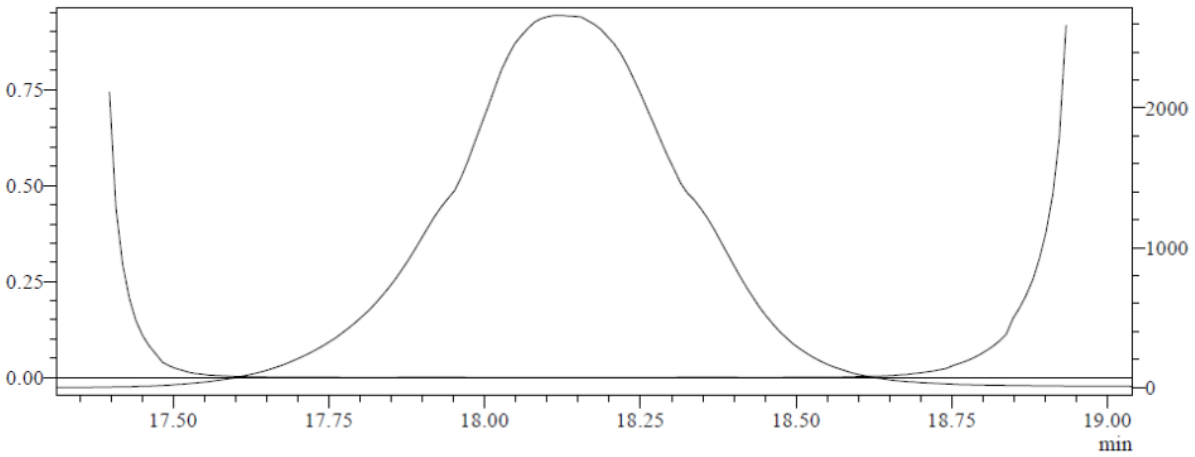


Fig:34 **Chromatogram of Water Treated Sample**



Peak Purity Index:0.999982

Fig:35 **Peak Purity Plot of Water treated Sample**

Result:

Peak purity of Cinacalcet Peak was found passed for all the stress conditions. Any interference due to placebo and blank was not observed at the Cinacalcet RT.

Conclusion:

A simple, accurate and precise method was developed for the estimation of impurities in cinacalcet tablets (30/60/90 mg). The retention time of Cinacalcet and its impurities (Amine impurity,Alcohol impurity,Mesylated impurity,Regio impurity and Dimer impurity) are ,6.70, 1.49, 1.57, 1.89, 6.38, 19.75.And developed method is validated for parameters such as System Sitability, specificity, Linearity, Accuracy, LOD and LOQ. The developed method with the specified chromatographic parameters was acceptable and found to be linear and accurate and Peak purity of Cinacalcet Peak was found passed for all the stress conditions. the developed method was found to be suitable for stability indicating for Cinacalcet Tablets Related substances and hence this can be used for routine analysis of related substances of Cinacalcet Tablets.

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