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Research Article

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Method Development and Validation by RP-HPLC for the Simultaneous Estimation of Cinacalcet and Maxacalcitol in Tablet Dosage Form

N. Usha*, M. Gobinath, V. Hari Baskar, Ramesh Dhani, A. Soniya

Department of Pharmaceutical Analysis, Ratnam Institute of Pharmacy, Pidathapolur, Muthukur, SPSR Nellore, A.P, India.

ABSTRACT

A Simple, selective, accurate, precise and linear RP-HPLC method was developed subsequently validated for estimation of cinacalcet in bulk & tablet dosage form. Gradient elution at a flow rate of 0.8 ml/min was used for separation of drugs in reversed-phase mode using Waters HPLC 22695 model on an INERTSIL ODS C18 column (150 x 4.6 mm; 5 μ) at a ambient temperature. Mobile phase consisted of water: methanol: acetonitrile (20:60:20). The UV detection wavelength was 235 nm 20 μ l was injected. The retention time for cinacalcet was 3.7 min. 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.

Keywords: Cinacalcet, Maxacalcitol, RP-HPLC, validation

ARTICLE INFO

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*Corresponding Author

N. Usha
Department of Pharmaceutical Analysis,
Ratnam Institute of Pharmacy,
Pidathapolur, SPSR Nellore, A.P, India.
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1. Introduction

Pharmaceutical analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals. Pharmaceutical analysis comprises those procedures necessary to determine the Journal of Pharmaceutical and Biomedical Analysis Letters

“identity, strength, quality and purity” of such articles on the synthesis of new compounds, the analyst is an indispensable team mate of the synthesis. Pharmaceutical analysis includes both qualitative and quantitative analysis

of Drugs and Pharmaceutical substances starts from bulk drugs (starting materials) to form product. In the new current practice of medicine, there are so many Analytical procedures are used in the analysis of chemical constituents found in human body whose altered concentrations during diseases states serve as diagnostic aids and also used to analyze the medicinal agents and their metabolites found in biological system.

High Performance Liquid Chromatography

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the coloured substances by filtration through column, followed by developments with pure solvents. High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids

Structures:

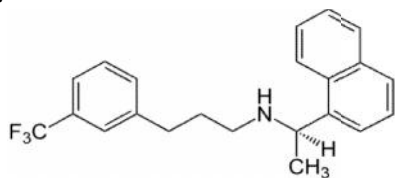


Figure 1: structure of cinacalcet

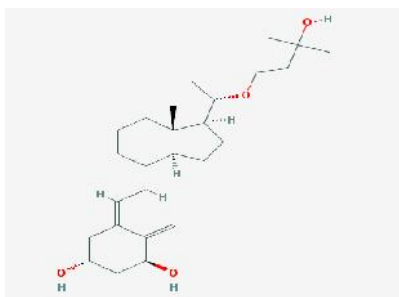


Figure 2: structure of Maxacalcitol

2. Materials and Methods

Materials:

Cinacalcet and maxacalcitol, Combination cinacalcet and maxacalcitol tablets distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetrahydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument: HPLC instrument used was of WATERS HPLC 2695 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer Systronics Instruments and matched quartz was used for measuring absorbance for cinacalcet and maxacalcitol solutions.

Methods:

Standard Preparation:

Accurately Weighed and transferred 250 mg of cinacalcet and 10 mg maxacalcitol of working Standards into a 25 ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1 ml from the above two stock solutions was taken into a 10 ml volumetric flask and made up to 10 ml.

Sample Preparation:

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For analysis of commercial formulation, 20 tablets of cinacalcet and maxacalcitol were weighed the average weight was calculated and powdered. A quantity equivalent to 500 mg of cinacalcet and 2.5 mg of maxacalcitol was weighed and transferred to a 100ml volumetric flask, which contain mobile phase and then shake it for 10 mins and sonicate it for 20 mins. The solution was allowed to stand at a room temperature for 20-30 mins and filtered it through a Whatmann filter paper.

Method Development

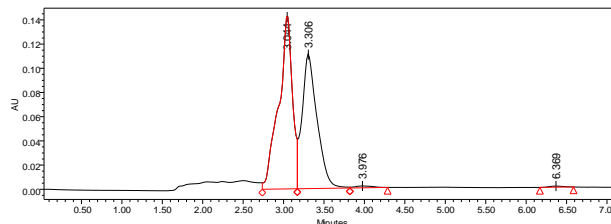


Figure 3: Chromatogram of Trial 1

Inference: Two peaks are not separated, completely merged

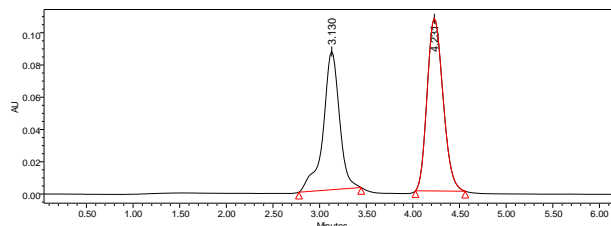


Figure 4: Chromatogram of Trial 2

Inference: Peaks shapes are not good.

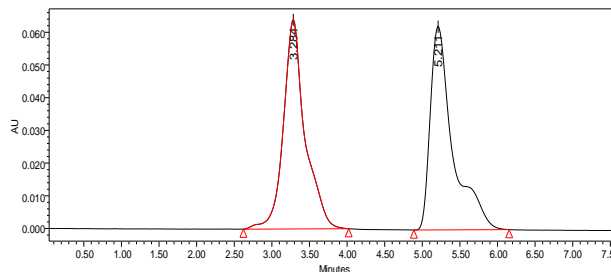


Figure 5: Chromatogram of Trial 3

Inference: peaks are not separated completely.

Table 1: Optimized Method

Parameters	Method
Stationary phase (column)	Inertsil -ODS C ₁₈ (250 x 4.6 mm, 5 μ)
Mobile Phase	Methanol : Water at 90:10V/V
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature	Ambient
Volume of injection loop	20
Detection wavelength	246 nm
Drug RT (min)	2.432 min for Cinacalcet and 3.094 for Maxacalcitol.

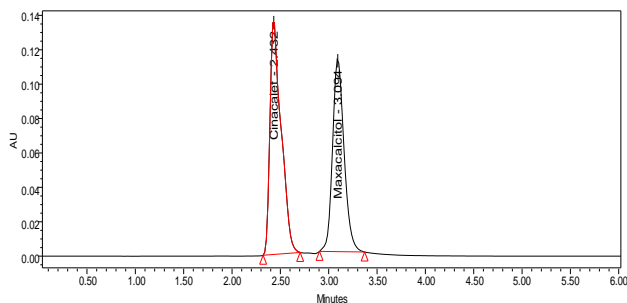


Figure 6: Chromatogram of standard

Inference: Got chromatogram at RT's of 2.9 min to Cinacalcet and 4.1 min to Maxacalcitol

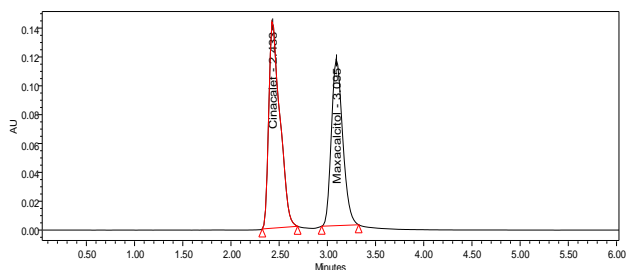


Figure 7: Chromatogram of sample

Inference: Got same chromatogram with same RT values as of standard

3. Results and Discussion

System suitability:

Table 2(a): Data of System Suitability for Cinacalcet

Injectio	RT	Peak	USP Plate	USP
1	2.433	1260823	5890.96406	1.23891
2	2.435	1261454	5915.42362	1.23063
3	2.433	1262001	5934.79698	1.24085
4	2.437	1260790	5976.25374	1.23899
5	2.431	1261698	5953.81415	1.24107
Mean	2.4372	1261353	5934.251	1.23649
SD	0.00083	535.490	-----	-----
% RSD	0.02836	0.04245	-----	-----

Table 2(b): Data of System Suitability for Maxacalcitol

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.095	1058029	8676.113795	1.099100
2	3.098	1056275	8803.641669	1.103929
3	3.095	1055781	8616.937115	1.111477
4	3.097	1057810	8820.182543	1.117660
5	3.098	1054712	8735.115629	1.119004
Mean	3.09754	10565211	8730.398	1.110234
SD	0.002683	1397.872	-----	-----
% RSD	0.064022	0.132309	-----	-----

Linearity:

Six Linear concentrations of Cinacalcet (20-80 ppm) and maxacalcitol (5-20 ppm) are prepared and injected. Regression equation of the cinacalcet and maxacalcitol are found to be $y = 132226 x - 44792$, and $y = 13948 x + 95766$. And regression co-efficient was 0.999.

Table 3(a): Data of Linearity (cinacalcet)

S.NO.	Concentration (µg/ml)	Response
1	0	0
2	20	598203
3	30	921576
4	40	1260823
5	50	1571434
6	60	1904090
7	70	2222634
8	80	2526577

Table 3(b): Data of Linearity (maxacalcitol)

S.NO	Concentration (µg/ml)	Response
1	0	0
2	20	551566
3	30	807869
4	40	1058029
5	50	1301310
6	60	1559184
7	70	1828268
8	80	2110397

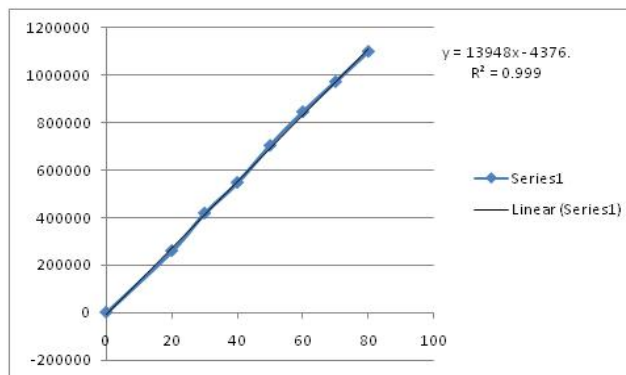


Figure 8: Calibration curve of cinacalcet

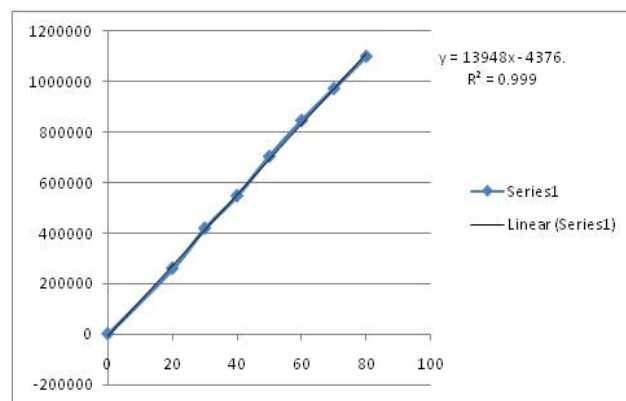


Figure 9: Calibration curve of maxacalcitol

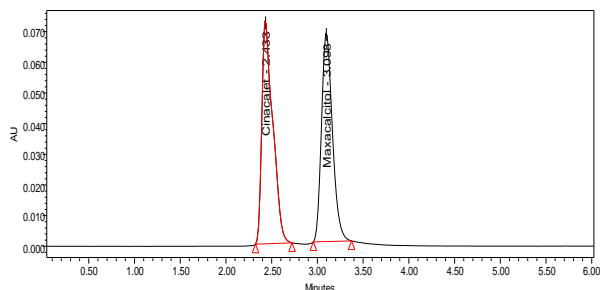


Figure 10: Linearity 20% Chromatogram of Cinacalcet and Maxacalcitol

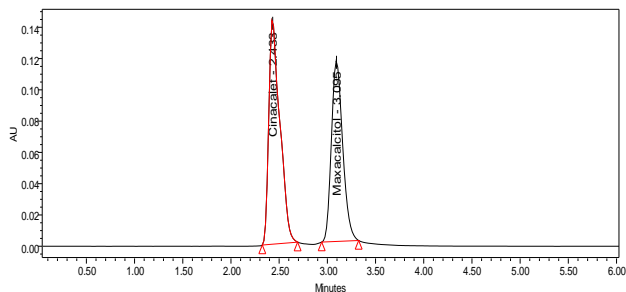


Figure 11: Linearity 80% Chromatogram of Cinacalcet and maxacalcitol

Precision:

Table 4: Data of Repeatability (Method precision) for Maxacalcitol

Concentration 40ppm	Injection	Peak Areas of Maxacalcitol	%Assay
	1	1057731	100.31
	2	1058192	100.35
	3	1053924	99.94
	4	1058321	100.37
	5	1054480	99.99
Statistical Analysis	6	1059937	100.52
	Mean	1057098	100.2467
	SD	2369.868	0.230014
	% RSD	0.224186	0.229449

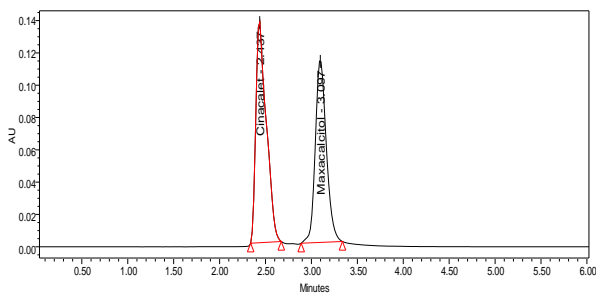


Figure 12: Method precision chromatogram of cinacalcet, maxacalcitol

Intermediate precision:

Table 5: Data of Intermediate precision for Cinacalcet

Conc. 40ppm	Injection	Peak Areas of Cinacalcet	%Assay
	1	1269391	101.05
	2	1262684	100.52
	3	1267859	100.99
	4	1268033	100.94
	5	1267929	100.93
Statistical Analysis	6	1268099	100.95
	Mean	1267333	100.8967
	SD	2347.646	0.189702
	% RSD	0.185243	0.188016

Table 6: Data of Intermediate precision for maxacalcitol

Conc. 40ppm	Injection	Peak Areas of Cinacalcet	%Assay
	1	1057731	100.38
	2	1059144	100.45
	3	1053331	99.89
	4	1052936	99.85
	5	1059901	100.52
Statistical Analysis	6	1058095	100.34
	Mean	1056972	100.2383
	SD	3040.044	0.292124
	% RSD	0.224186	0.29143

Accuracy (Recovery):

Table 7: Data of Accuracy for Cinacalcet

Conc. %mg/ml	Amount added (ppm)	Amount found (ppm)	% Recovery
50%	20	19.85	99.25
100 %	40	39.74	99.35
150%	60	59.04	98.40

Table 8: Data of Accuracy forMaxacalcitol

Concentration %mg/ml	Amount added (ppm)	Amount found (ppm)	% Recovery
50%	20	19.86	99.30
100 %	40	39.54	98.85
150%	60	59.92	99.86

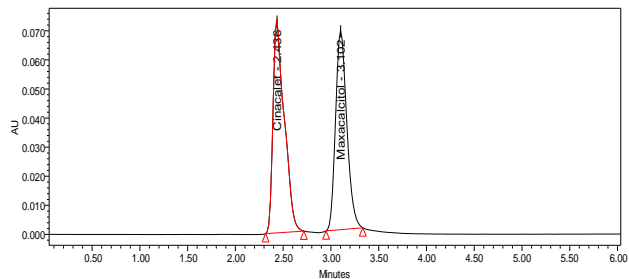


Figure 13: Accuracy 50 % Chromatogram of Cinacalcet and Maxacalcitol

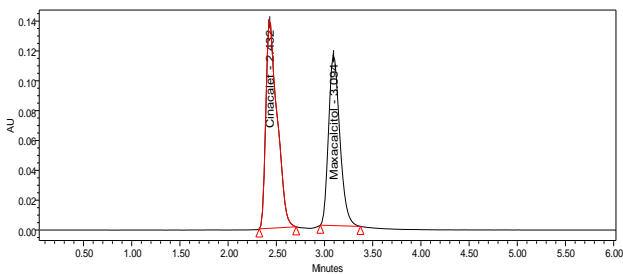


Figure 14: Accuracy 100 % Chromatogram of Cinacalcet and Maxacalcitol

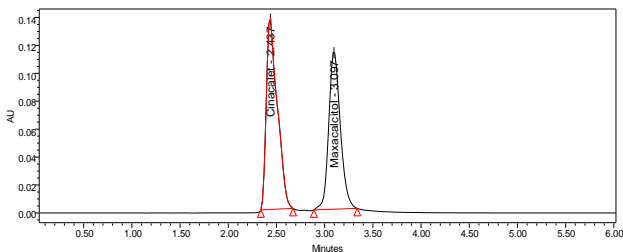


Figure 15: Accuracy 150 % Chromatogram of Cinacalcet and Maxacalcitol

Limit of detection and limit of quantitation (LOD and LOQ):

Cinacalcet:

From the linearity plot, the LOD and LOQ are calculated:

$$\text{LOD} = \frac{3.3}{S} = \frac{3.3 \times 535.4901}{26023} = 0.0554$$

Maxacalcitol

$$\text{LOQ} = \frac{10}{S} = \frac{10 \times 1397.872}{26023} = 0.53716$$

Inference: System suitability Chromatogram for standard-3

Specificity:

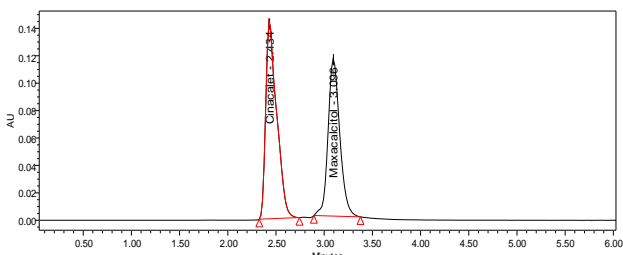


Figure 16: Chromatogram of standard

Inference: Got a peak for standard at an Rt of 2.915 min for Cinacalcet and 4.188 min for Maxacalcitol

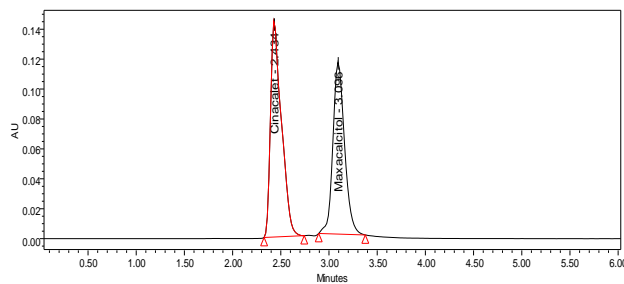


Figure 17: Chromatogram of sample

Inference: Got a peak for sample at an Rt of 2.945min for Cinacalcet and 4.186 min for Maxacalcitol

Precision:

Repeatability: System precision

Table 9: Data of Repeatability (System precision) for Cinacalcet

	Injection	Peak Areas of Cinacalcet	%Assay
Conc. 40ppm	1	1263521	100.58
	2	1260854	100.38
	3	1267091	100.87
	4	1270854	101.16
	5	1268321	100.96
Statistical Analysis	Mean	1266128	100.79
	SD	3958.637	0.31
	% RSD	0.312657	0.30257

Table 10: Data of Repeatability (System precision) for Maxacalcitol

	Injection	Peak Areas of Maxacalcitol	%Assay
Concentration 40ppm	1	1057326	100.27
	2	1050981	99.66
	3	1049333	99.50
	4	1053876	99.94
	5	1058430	100.38
Statistical Analysis	Mean	1053989	99.95
	SD	3924.195	0.378814
	% RSD	0.372318	0.379003

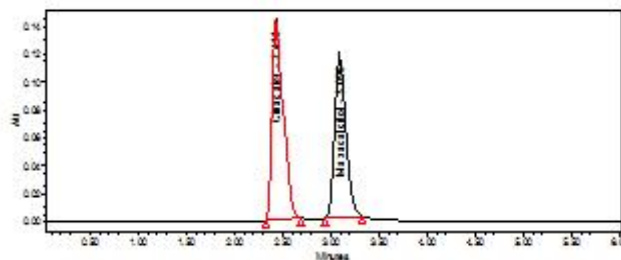


Figure 17

Inference: Chromatogram for system precision (standard- 1)

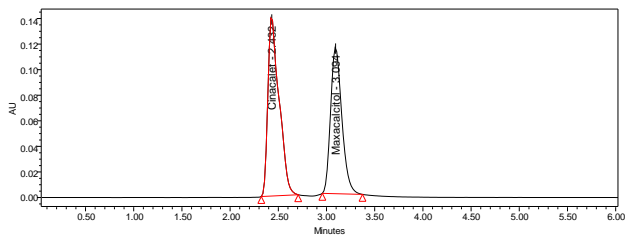


Figure 18

Inference: Chromatogram for system precision (standard-2)

Method precision:

Table 11: Data of Repeatability (Method precision) for Cinacalcet

	Injection	Peak Areas of Cinacalcet	%Assay
Concentration 40ppm	1	1268670	100.99
	2	1260339	100.33
	3	1259781	100.29
	4	1258844	100.22
	5	1261734	100.45
	6	1268327	100.96
Statistical Analysis	Mean	1262949	100.54
	SD	4400.643	0.345254
	% RSD	0.348442	0.343399

Table 12: Data of Repeatability (Method precision) for Maxacalcitol

	Injection	Peak Areas of Maxacalcitol	%Assay
Concentration 40ppm	1	1057731	100.31
	2	1058192	100.35
	3	1053924	99.94
	4	1058321	100.37
	5	1054480	99.99
	6	1059937	100.52
Statistical Analysis	Mean	1057098	100.2467
	SD	2369.868	0.230014
	% RSD	0.224186	0.229449

Chromatograms of Repeatability

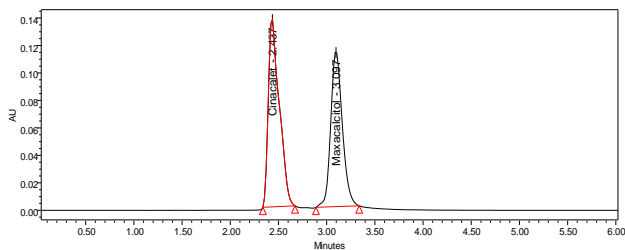


Figure 19

Inference: Chromatogram for Repeatability (standard-1)

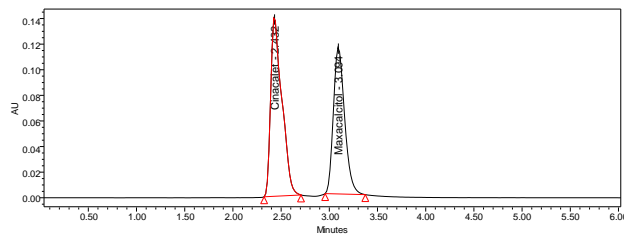


Figure 20

Inference: Chromatogram for Repeatability (standard - 2)

Intermediate precision.

Table 13: Data of Intermediate precision (Analyst 2) for Cinacalcet

	Injection	Peak Areas of Cinacalcet	%Assay
Conc. 40ppm	1	1269391	101.05
	2	1262684	100.52
	3	1267859	100.99
	4	1268033	100.94
	5	1267929	100.93
	6	1268099	100.95
Statistical Analysis	Mean	1267333	100.8967
	SD	2347.646	0.189702
	% RSD	0.185243	0.188016

Table 14: Data of Intermediate precision (Analyst 2) for Cinacalcet

	Injection	Peak Areas of Cinacalcet	%Assay
Conc. 40ppm	1	1057731	100.38
	2	1059144	100.45
	3	1053331	99.89
	4	1052936	99.85
	5	1059901	100.52
	6	1058095	100.34
Statistical Analysis	Mean	1056972	100.2383
	SD	3040.044	0.292124
	% RSD	0.224186	0.29143

Chromatograms of Intermediate precision

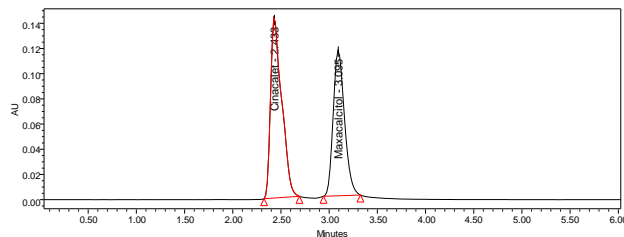


Figure 20

Inference: Chromatogram for Intermediate Precision

Chromatograms for accuracy (100%)

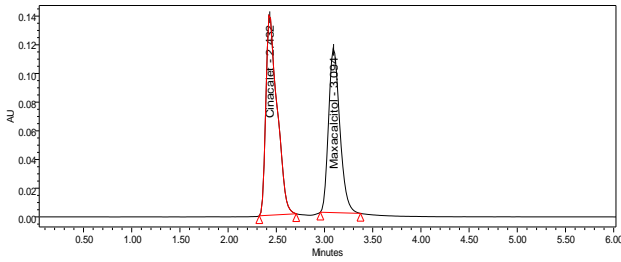


Figure 21

Inference: Chromatogram for standard 1

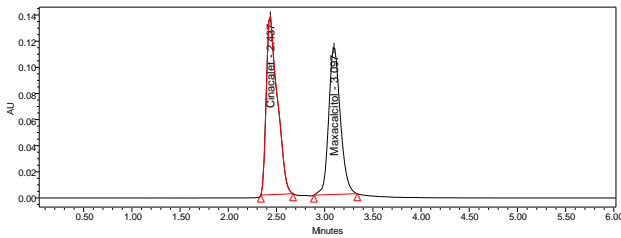


Figure 22

Inference: Chromatogram for standard 2

Linearity

Chromatograms for 20 ppm:

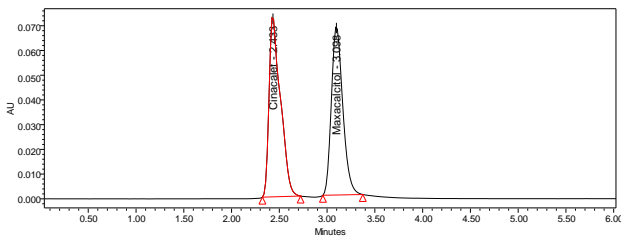


Figure 23

Inference: Chromatogram for 20 ppm standard 1

Chromatogram for 40 ppm standard 1

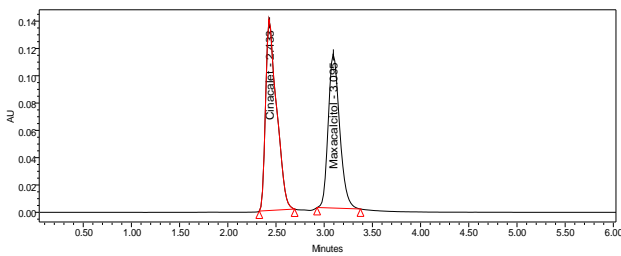


Figure 24

Inference: Chromatogram for 40 ppm standard

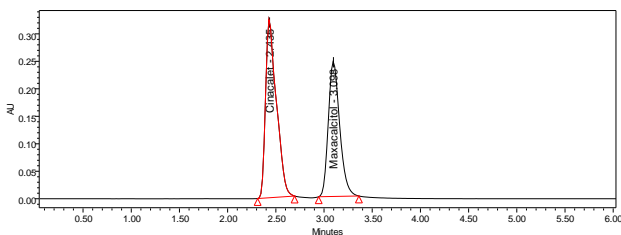


Figure 25

Inference: Chromatogram for 80 ppm standard 1

Ruggedness:

a) System to System variability:

Table15: Data of system to system variability (Cinacalcet)

S.No	Peak area	Assay % of Cinacalcet
1	1261844	100.46
2	1263783	100.60
3	1269021	101.02
4	1264684	100.68
5	1268093	100.95
6	1267704	100.91
Mean	1265855	100.77
%RSD	0.224008	0.222531

Table 16: Data of system to system variability (Maxacalcitol)

S.No	Peak area	Assay % of Maxacalcitol
1	1058621	100.39
2	1054933	100.04
3	1053781	99.93
4	1058136	100.35
5	1049722	99.54
6	1050936	99.66
Mean	1054355	99.985
%RSD	0.345403	0.348463

Chromatograms of system to system variability

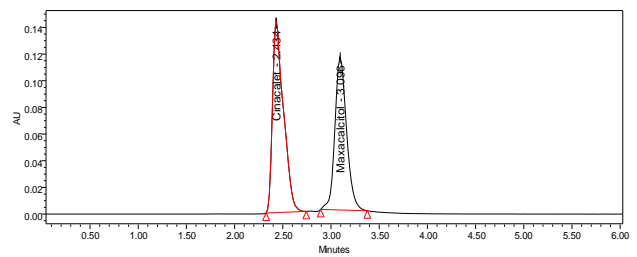


Figure 26

Inference: Chromatogram of system to system variability std- 1

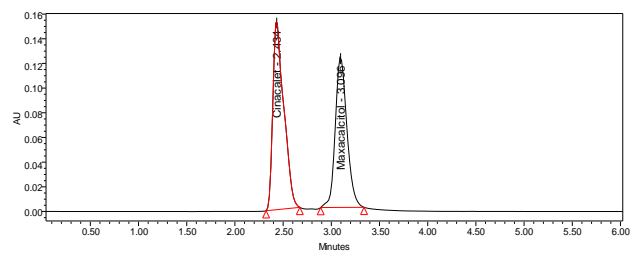


Figure 27

Inference: Chromatogram of system to system variability std- 2

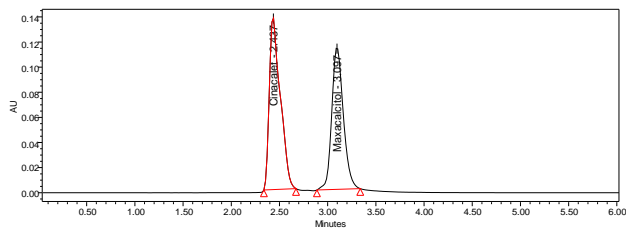


Figure 28

Inference: Chromatogram of system to system variability std- 3

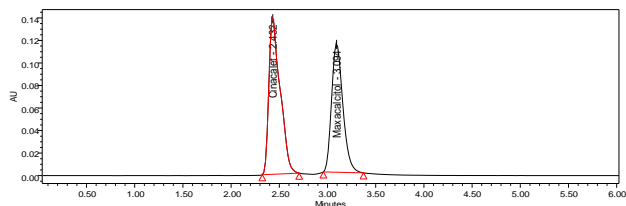


Figure 29

Inference: Chromatogram of system to system variability std- 4

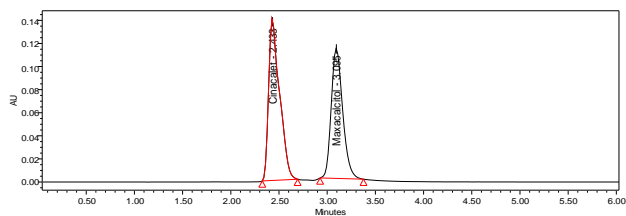


Figure 30

Inference: Chromatogram of system to system variability std- 5

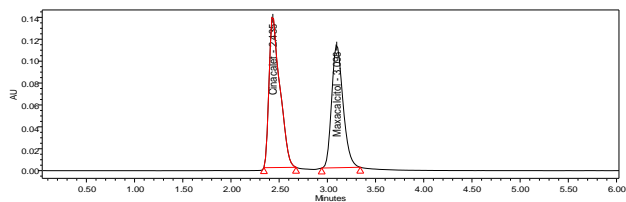
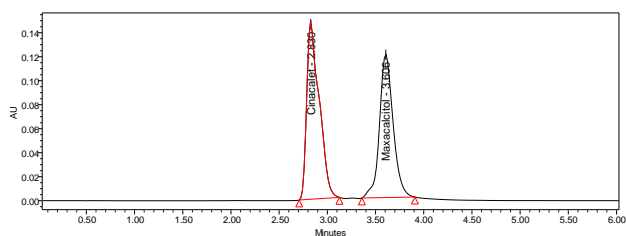


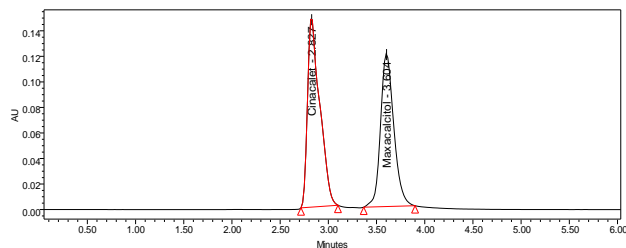
Figure 31

Inference: Chromatogram of system to system variability std- 6

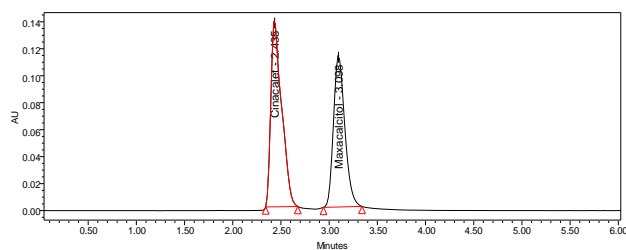
Fig 32-33 Chromatograms of robustness
a) Effect of variation of flow rate (for 0.8 ml/min flow)



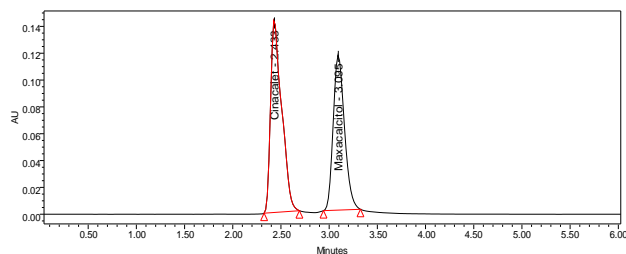
Inference: Chromatogram for robustness standard - 1



Inference: Chromatogram for robustness standard - 2
Fig 34-35: Chromatograms for 1ml/min

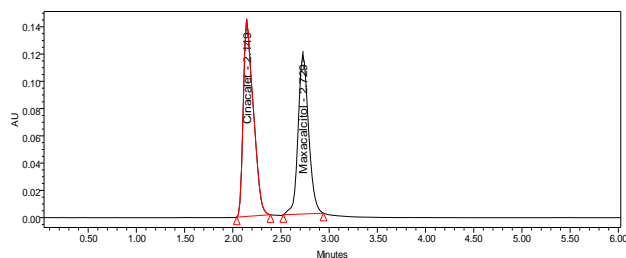


Inference: Chromatogram for robustness standard - 1

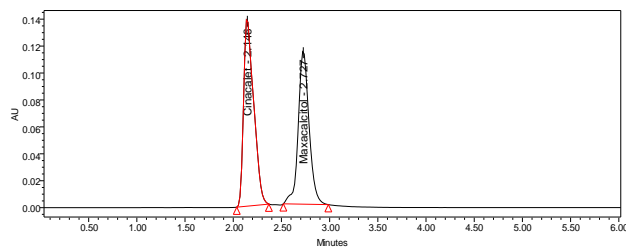


Inference: Chromatogram for robustness standard - 2

Fig 36-37: Chromatograms for 1.2ml/min



Inference: Chromatogram for robustness standard - 1



Inference: Chromatogram for robustness standard - 2

Limit of Detection and Limit of Quantitation (LOD and LOQ): Cinacalcet:

From the linearity plot, the LOD and LOQ are

$$\text{LOD} = \frac{3.3}{S}$$

$$= \frac{3.3 \times 535.4901}{31897} = 0.0554$$

$$\text{LOQ} = \frac{10}{S}$$

$$= \frac{10 \times 535.4901}{31897} = 0.1678$$

Maxacalcitol:

$$\text{LOD} = \frac{3.3}{S}$$

$$= \frac{3.3 \times 1397.872}{26023} = 0.17727$$

$$\text{LOQ} = \frac{10}{S}$$

$$= \frac{10 \times 1397.872}{26023} = 0.53716$$

Table 17 (i): Data of Accuracy for Cinacalcet

Concentration % mg/ml	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.85	99.25	MEAN	99.88
50% Injection 2	20	19.96	99.80		
50% Injection 3	20	20.12	100.6		
100 % Injection 1	40	39.74	99.35	MEAN	99.81
100 % Injection 2	40	40.08	100.2		
100% Injection 3	40	40.24	100.6	%RSD	0.399
150% Injection 1	60	59.04	98.40	MEAN	99.19
150% Injection 2	60	59.62	99.36		
150% Injection 3	60	59.89	99.81	%RSD	0.72

Table 17(ii): Data of Accuracy for Maxacalcitol

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50% Injection 1	20	19.86	99.30	MEAN	99.46
50% Injection 2	20	19.98	99.90		
50% Injection 3	20	19.84	99.20		
100 % Injection 1	40	39.54	98.85	MEAN	99.76
100 % Injection 2	40	39.82	99.55		
100% Injection 3	40	39.96	99.9	%RSD	0.189
150% Injection 1	60	59.92	99.86	MEAN	100.0067
150% Injection 2	60	60.08	100.13		
150% Injection 3	60	60.02	100.03	%RSD	0.136

Table 18(i): Data for Effect of variation in flow rate (Cinacalcet):

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1290454	1.238915		1268345	1.251658		1230845	1.262464
	1297807	1.230637		1260951	1.245435		1238451	1.251658
	1298120	1.240858		1267320	1.262464		1238412	1.237018
	1290875	1.238995		1268057	1.237018		1238741	1.239010
	1297354	1.241073		1259102	1.239010		1234510	1.238915
Avg	1294922	1.236496	Avg	1264795	1.247117	Avg	1236192	1.245813
SD	3898.919	0.005254	SD	4420.129	0.010328	SD	3462.24	0.010984
%RSD	0.301093	0.424907	%RSD	0.349474	0.008282	%RSD	0.280073	0.00881712

Table 18(ii): Data for Effect of variation in flow rate (Maxacalcitol)

Flow 0.8 ml	Std Area	Tailing factor	Flow 1.0 ml	Std Area	Tailing factor	Flow 1.2 ml	Std Area	Tailing factor
	1075442	1.099100		1056610	1.122813		1023622	1.121321
	1073854	1.103929		1058041	1.112181		1028241	1.122813
	1078048	1.111477		1058761	1.121321		1025025	1.124805
	1073452	1.117660		1056241	1.124805		1027849	1.123373
	1073855	1.119004		1057760	1.123373		1027405	1.099100
Avg	1074930	1.110234	Avg	1057483	1.120899	Avg	1026428	1.118282
SD	1902.758	0.008622	SD	1039.96	0.00503	SD	2006.414	0.047969
%RSD	0.177012	0.77655	%RSD	0.098343	0.004488	%RSD	0.195475	0.965376

4. Conclusion

The proposed RP-HPLC method is suitable technique for determination of cinacalcet and Maxacalcitol. All the parameters for this drug met the criteria of ICH guidelines for method validation. Percentage of recovery shows that the proposed method is free from interferences of excipients used in the formulation. The developed method is recommended for routine and QC analysis of the investigated drugs to provide simple and accurate quantitative analysis for the determination of cinacalcet and Maxacalcitol.

5. Acknowledgement

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6. References

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