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DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF CINACALCET IN TABLET DOSAGE FORM

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ABSTRACT

In the present work a simple, selective, accurate, precise and rapid RP-HPLC method was developed for quantitative estimation of cinacalcet in tablet dosage form. This method was developed by using a C_{18} column with mobile phase a mixture of methanol and phosphate buffer (pH 6.8) in the ratio 60: 40 v/v at a flow rate 1.0 ml min^{-1}. Detection of the drug was performed at 232nm using PDA detector. The developed method was validated as per ICH guideline for linearity, accuracy, precision, specificity, robustness and selectivity. The developed method was successfully applied for quantitative estimation of cinacalcet in its tablet dosage form (Ceracal) having retention time is 2.82 minute and percentage of drug content was found to be 100.45%. Validation study result revealed that the method is specific, accurate, precise, reliable and reproducible for estimation of cinacalcet in it's dosage form. Concentration with in the range of 5-50 µg/ml was found to be linear. Limit of detection and limit of quantitation was found to be 0.32 and 0.91µg/ml respectively. Recovery concentration was found to be in the range 99.85-100.14%, with coefficient of variance (COV) < 2.0. High percentage of recovery and acceptance limit of statistical data for validation study assure that the developed method was suitable for estimation of cinacalcet in it's bulk and dosage forms.

KEYWORDS: Cinacalcet, RP-HPLC, Validation and Mobile Phase.

INTRODUCTION

Cinacalcet is a naphthalene derivative calcimimetic agent which mimics the action of calcium on different organ tissues. It acts as allosteric activators of the calcium sensing receptor (CaSR) in the parathyroid glands and other tissues.[1-3] It lowers the threshold for CaSR activation by extracellular calcium ions and diminishes parathyroid hormone (PTH) release from parathyroid cells during the treatment of hyperparathyroidism in parathyroid carcinoma. Also used in the treatment of secondary hyperparathyroidism in patients with end-stage renal disease on maintenance dialysis therapy to treat higher calcium level.[4] Chemically it is (*R*)-*N*-[1(naphthalene-1-yl) ethyl]-3-[3(trifluoromethyl) phenyl] propan-1-amine with chemical formula $C_{22}H_{22}F_3N$ having molecular weight 357.4 g/mol.[5] Chemical structure of Cinacalcet is given in Fig.01.

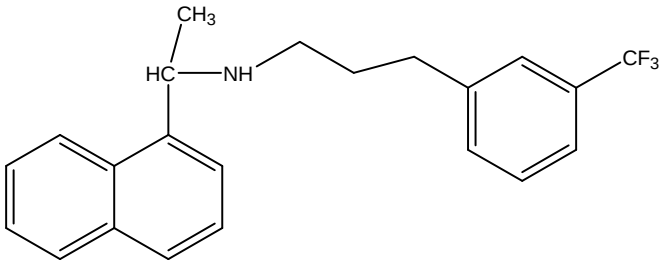

Fig. 01: Chemical Structure of Cinacalcet.

Cinacalcet is available in the market with various brand names such as Sensipar, Mimpara, Ceracal and PTH. Literature survey revealed that very few analytical methods have been reported for the estimation of Cinacalcet in pure drug and pharmaceutical dosage forms using liquid chromatography.[6-8] The aim of the present work is to develop a validated simple, precise and accurate RP-HPLC method with UV detection for the determination of cinacalcet in tablet dosage form.

MATERIAL AND METHODS

Chemical and Reagents

HPLC grade methanol and water were used for this work. AR grade KH_2PO_4 (Potassium Dihydrogen Phosphate) and K_2HPO_4 (Dipotassium Hydrogen Phosphate) were used to prepare phosphate buffer. API of Cinacalcet hydrochloride was obtained as a gift sample from Cheminsol Pharma Solutions Pvt. Ltd, Panvel, Mumbai and the tablets Ceracal (Cinacalcet hydrochloride, 30mg) was procured from local market manufactured by the Jubilant Life Sciences Pvt. Ltd.

Instrument

Study was carried out with a Shimadzu chromatograph equipped with a LC-10 AT VP solvent-delivery system, a universal loop injector (Rheodyne 7725 i) of injection capacity of 20 µL, and an SPD-10 AVP UV–Visible photodiode-array detector set at 232 nm. The equipment was controlled by a PC work station. Separation is carried out on a 25 cm × 4.6 mm, 5-µm particle, Phenomenex Luna C_{18} column under reversed-phase partition conditions. The mobile phase was a 60:40 (*v*/*v*) mixture of methanol and phosphate buffer (pH 6.8). The flow rate was 1.0 mL min^{-1} and the run time was 05 mins. Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through a 0.2-µm filter. Identification of the compound was established by comparing the retention times of compound in the sample solution with it's standard solution. Chromatography was performed in an air-conditioned room maintained at 25 ± 2°C.

Preparation of Mobile Phase

1000ml of Phosphate buffer of pH 6.8 was prepared by dissolving required quantity of K_2HPO_4 and KH_2PO_4 in HPLC grade water. Mixture of methanol and prepared phosphate buffer in ratio 60:40 was used as mobile phase for whole study. The prepared mobile phase was sonicate for degassing and filtered through 0.2µm membrane filter before use.

Preparation of Standard and sample solutions

Cinacalcet (50mg) was weighted accurately and transferred to a 50ml volumetric flask then dissolved with some amount of methanol and further the volume was adjusted upto 50ml with methanol to prepare a stock solution of 1000µg ml^{-1}. The solution was sonicate and filtered by membrane filter and used to prepare different dilutions for study. Twenty tablets of Ceracal were weighted individually and their average weight was determined. Then the tablets were crushed to fine powder and powder equivalent to 50mg of drug content was transfer to a 50ml volumetric flask and dissolved in methanol. The solution was shaken vigorously for 20 minutes and filtered through whatmann filter paper. The residue was washed with methanol and adjusts the volume upto 50ml with methanol. Working concentration solutions were prepared from the above solution and were used for further study.

Linearity Curve

For linearity study different known concentration dilutions were prepared from the standard stock solution of cinacalcet. 20µL of these prepared solutions injected into the instrument one

after another and their peak areas were recorded. Calibration curve was plotted between peak area and concentration. From the curve it was clear that the drug has linearity range between 5-50µg/ml. Unknown assay samples were quantified by reference to the calibration plot.

Assay of Tablet Dosage form

Three replicates of 30µg/ml were prepared from tablet stock solution and sonicate before injecting into the instrument. These solutions (20µL) were injected one by one for analysis, then the drug content in these solutions were calculated by extrapolating peak area from the calibration curve.

Validation

The developed method was further validated as per ICH guidelines for accuracy, Precision, LOD, LOQ, specificity, sensitivity, and robustness.[9,10]

Accuracy

To check the accuracy of the developed method recovery study was performed by stand addition method as per ICH guideline. During this to a pre analysed tablet sample solution known concentration of standard drug solutions were added and % of drug recovery was calculated.

Precision

Precision of the method was verified by repeatability study and intermediate precision study. Three replicates of the tablet formulation were analysed for the repeatability study. The standard deviation, coefficient of variance and standard error were calculated. Intermediate precision of the method was checked by assay the sample solution on same day at an interval of one hour (intraday precision) for three hours and on three different days (interday precision).

LOD & LOQ

Limit of Detection (LOD) is the lowest amount of analyte in a sample that can be detected under the stated experimental conditions. It is calculated by using the formula, LOD= 3.3* SD/Slope.

Limit of Quantitation (LOQ) is the lowest amount of analyte in a sample that can be quantified with acceptable precision and accuracy under the stated experimental conditions. It is calculated by using the formula LOQ= 10 *SD/Slope.

Where SD= Standard deviation, obtained by injecting replicates of the sample and slope is obtained from the calibration curve of the analyte.

Specificity and Robustness

Specificity was assessed by comparing the chromatograms of tablet solution and the drug standard solution. Because the retention time of the drug for standard solution and tablet solution were identical, and no co-eluting peaks from the diluents were observed indicates the method was specific for quantitative estimation of the drug in the commercial formulation. Robustness of the method was investigated under a variety of conditions like change in flow rate (±0.2 ml/min) and change in mobile phase composition. In each variation analysis was made in replicates and %COV of peak areas were determined.

RESULT AND DISCUSSION

Method Development and Optimization

Initial studies revealed that the drug is highly soluble in methanol, acetonitrile, sparingly soluble in tetrahydrofuran. A standard solution (50µg/ml) of cinacalcet was scanned in the range of 200-400 nm and the maximum absorption was found at 232 nm. Hence 232nm was selected as the detection wavelength for the analysis of the drug. Column chemistry, solvent type, solvent strength (volume fraction of organic solvent(s) in the mobile phase and pH of the buffer solution) and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimised so there was no interference from solvent and excipients. Other criteria, for example time required for analysis, appropriate *k* range for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of the drugs from the formulations during drug analysis were also considered.

Mixture of methanol and phosphate buffer having pH 6.8 in ratio 60:40 was selected as mobile phase because the drug gave symmetric peak with good peak shape and optimum retention time. Flow rate was set at 1.0 ml/minute. The above optimized conditions were used for further analysis of the drug. Under the optimised conditions the retention time of cinacalcet was found to be 2.82 min for standard and marketed formulation given in Fig.02 and 03. The information of the sample analysis was given in Table.01.

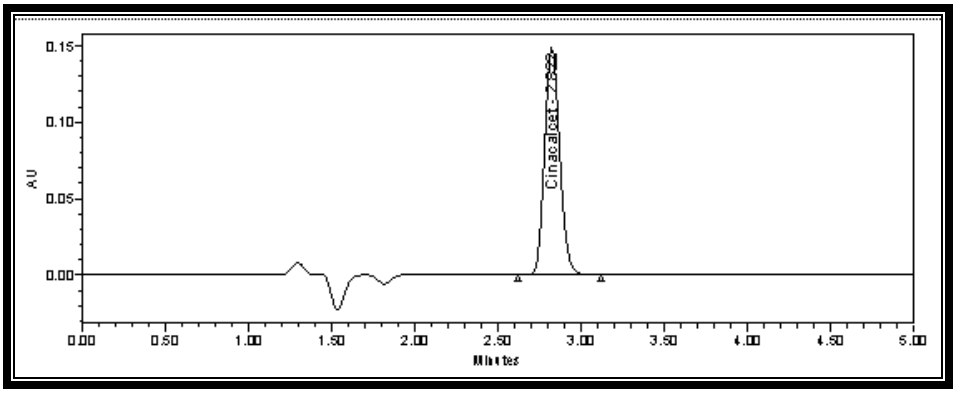

Fig. 02: Chromatogram of Cinacalcet in Standard Solution.

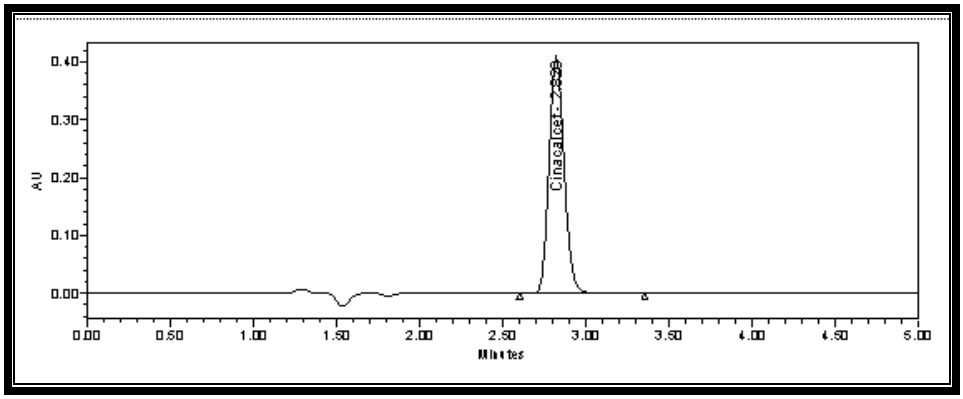

Figure 03: Chromatogram of Cinacalcet in Tablet Solution.

Table 01: Information of Tablet analysis.

	Peak Name	RT	Area	% Area	Int Type	USP Tailing
1	Cinacalcet	2.820	2565243	100.00	BB	1.11

Method Validation

Linearity

Linearity curve was plotted between peak area and concentration by injecting the dilutions made from standard stock solution. From the calibration curve it was revealed that the drug follows linearity in concentration range 5-50 µg/ml. The regression equation of the calibration curve was found to be $y = 82416x + 126472$ with coefficient correlation value R^2 = 0.998. Linearity curve was given in Fig. 04 and values of peak area for each concentration given in Table.02.

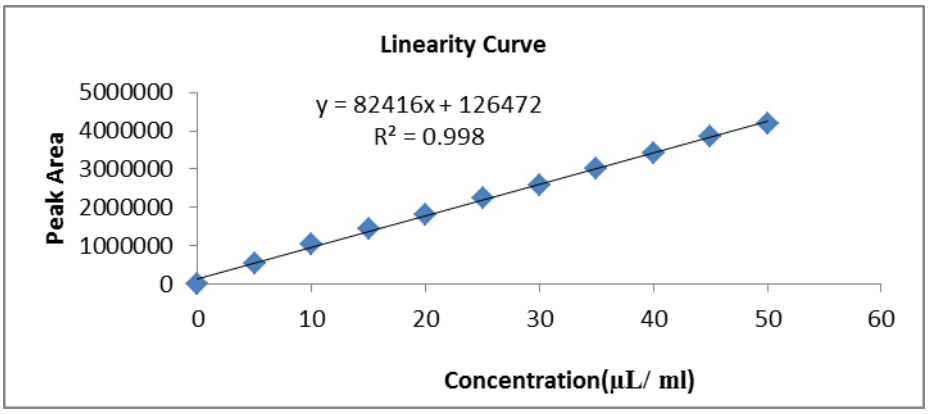

Fig. 04: Linearity Curve of Cinacalcet.

Table 02: Results of Linearity Study.

S. No.	Concentration (µg/ml)	Mean Peak Area	COV %
1	5	515865	0.321
2	10	1028408	0.309
3	15	1434992	0.289
4	20	1818818	0.268
5	25	2230326	0.288
6	30	2565298	0.129
7	35	3013991	0.318
8	40	3406181	0.239
9	45	3854125	0.215
10	50	4187597	0.527

COV- Coefficient of Variance.

Accuracy

Accuracy of the method was confirmed by performing recovery study. During this to a pre analysed tablet solution of 20 µg/ml known amount of standard drug solution (80%,100% and 120% of drug content) were mixed and % of drug recovery were calculated by extrapolating the peak areas from calibration curve equation. Drug recovery was found to be with in concentration range of 99.85-100.14% with COV (%) < 2, indicates the developed method is accurate for quantitative estimation of cinacalcet in it's formulation. The result of recovery study was given in Table.03.

Table 03: Result of Recovery Study.

S. No.	Amount of Drug taken (µg/ml)	Amount of drug added		% of Recovery	COV (%)
		%	µg/ml		
1	20	80	16	99.85	1.524
2		100	20	100.14	1.254
3		120	24	100.04	1.017

COV- Coefficient of Variance

Precision

Intra and Inter day precision study of the tablet solutions were performed and the result of the study was given in Table.04. Precision study clear that this method is précised for quantitative estimation of cinacalcet as the statistical data for the intra and inter study were within the acceptable range i,e standard deviation is < 1, COV (%) is < 2 and lower value of standard error.

Table 04: Result of Precision Study.

Concentration (µg/ml)	Intraday Study			Interday Study		
	Mean±SD	COV (%)	S.E	Mean±SD	COV (%)	S.E
10	10.084±0.124	0.542	0.079	9.857±0.324	1.012	0.379
20	20.175±0.524	0.685	0.125	19.775±0.512	1.201	0.412
30	30.547±0.412	0.287	0.231	29.995±0.811	0.975	0.564
40	40.087±0.025	0.478	0.147	39.781±0.625	1.478	0.847
50	50.023±0.235	0.312	0.301	49.744±0.435	1.302	0.901

SD- Standard Deviation, COV- Coefficient of Variance, S.E- Standard Error

LOD & LOQ

Selectivity of the developed method was confirmed by calculating LOD and LOQ, the values were 0.32µg/ml and 0.91µg/ml respectively.

Specificity and Robustness

Study for specificity and robustness of the develop method were performed. As there is no significant difference found in retention time of cinacalcet after modifying flow rate of mobile phase as well as composition confirms that this method is suitable and robust for quantitative estimation of cinacalcet in tablet formulation. The result was reported in Table.05.

Table 05: Result of Robustness Study.

Parameters	Variation	Retention time	% COV
Flow Rate (ml/min)			
0.8	-0.2	2.92	0.35
1.0	0.0	2.81	0.58
1.2	+0.2	2.74	0.62
Mobile Phase composition (Change in volume of Methanol by ±2ml)			
58	- 2.0	3.12	0.21
60	0.0	2.82	0.32
62	+2.0	2.58	0.52

Assay of Tablet Formulation

Quantitative estimation of cinacalcet was performed in it's tablet dosage form (Ceracal, 30mg) by using this developed method. Replicates of tablet solution having concentration 30 µg/ml were prepared from it's stock solution and injected into the system and concentration of drug content was calculated by extrapolating peak area from the calibration curve. The % of drug content was found to be 100.45 in market formulation and the statistical data revels that the developed method was suitable for quantitative estimation of cinacalcet. The result was given in Table.06.

Tablet 06: Result for Assay of Cerecal.

Drug	Label Claim (mg)	Amount Present (mg)	% of drug content	S.D	% COV	S.E
Cinacalcet. HCl	30	30.135	100.45	0.554	0.447	0.654

SD- Standard Deviation, COV- Coefficient of Variance, S.E- Standard Error

CONCLUSION

In the present work a new RP-HPLC method has been developed for quantitative analysis of cinacalcet in tablet formulation. Validation study result of this developed method revels that the developed method is accurate, precise, linear, repeatable, specific, selective and reliable. The relatively short run time enables rapid quantitation of many samples during analysis. The same solvent was used throughout the experimental work and no interference of any excipients was found. The developed method is economical and therefore can be used as a quality control tool for estimation of cinacalcet in bulk as well as in formulation.

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