AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation RP-HPLC Method for Estimation of Cinacalcet in Bulk and Tablet Dosage Form

N. Siva Ganesh,[1] M. Ratanlal[1], A. Venkat Narsaiah[1], K. V. L. D. Spandana,[2] Sridhar Thota[2], Sagar Narala[2]*

1. Organic and Biomolecular Chemistry Division, Indian Institute of Chemical Technology, Hyderabad-500007, Telangana, India.

2. Department of Pharmaceutical Analysis, St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda-506001, Telangana, India.

ABSTRACT

The objective of the method was to develop a simple, rapid, efficient, cost effective and reproducible, stability indicating reverse phase high performance liquid chromatography method (RP-HPLC) for the quantification of cinacalcet in bulk and pharmaceutical dosage form. As very few analytical methods have been reported for the analysis of cinacalcet by chromatography, we aimed to develop a method which would be simple with optimum retention time by the use of simple mobile phase, which results in the economic method that can be used for routine analysis of the drug. The RP-HPLC analysis was carried out on Inertsil ODS C18 with a mobile phase of methanol, acetonitrile and water in the ratio of 70:15:15 v/v/v. Detection was carried out at 280 nm using a PDA detector. The method was validated for linearity, accuracy, precision, LOD, LOQ and robustness as per ICH guidelines. The method was found to be linear in the range of 10-50 µg/ml. Limit of detection and limit of quantitation was found to be 0.22 and 0.74 µg/ml respectively. Recovery was found to be in the range 99.7-100.02% and precision less than 1%. The developed method was successfully applied for the estimation of cinacalcet in marketed tablet formulation (PTH 30) and percentage assay was found to be 100.8 %. The developed RP-HPLC method was simple, rapid, accurate, precise and stability indicating for the quantification of cinacalcet in bulk and tablet dosage form.

Keywords: Cinacalcet, RP-HPLC, ODS C18, Validation, mobile phase.

*Corresponding Author Email: nsagar980@gmail.com

Received 05 January 2015, Accepted 13 January 2015

Please cite this article as: Sagar N *et al*., Development and Validation RP-HPLC Method for Estimation of Cinacalcet in Bulk and Tablet Dosage Form. American Journal of PharmTech Research 2015.

INTRODUCTION

Cinacalcet is a calcimimetic agent and acts on calcium sensing receptor of the parathyroid. It is approved for the treatment of secondary hyperthyroidism in patients with chronic kidney disease (CKD) placed on dialysis and for the treatment of elevated calcium levels in the patients with parathyroid carcinoma [1].Chemically it is (*R*)-*N*-[1(naphthalene-1-yl) ethyl]-3-[3(trifluoromethyl) phenyl] propan-1-amine with a molecular weight, 357.4 g/mol and molecular formula, $C_{22}H_{22}F_3N$ respectively.The calcium sensing receptor on the surface of the chief cell of the parathyroid gland is the principal regulator of parathyroid hormone secretion (PTH). It acts by directly lowering 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].

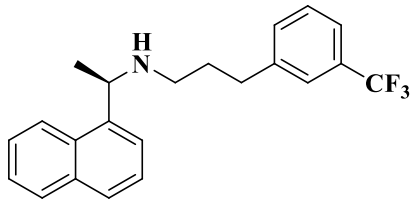

Figure 1: Structure of cinacalcet

Cinacalcet is available in the market with various brand names such as Sensipar, Mimpara and PTH. It is available as oral dosage forms in multiples of 30 mg. 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 which is shown below in **Table 1**. The aim of our present work is to develop and validate a simple and reliable isocratic RP-HPLC method with UV detection for the determination of cinacalcet in bulk and tablet dosage forms.

Table 1: Comparison of reported methods for the estimation of cinacalcet in pure and pharmaceutical dosage forms with the proposed method

S.No	Title	Mobile phase	Stationary phase	Flow rate	Range	Rt	r^2
1.	Stability indicating HPLC method for the estimation ofCinacalcet hydrochloride API	Methanol: Water (70:30v/v) pH 3.6 with dilute Orthophosphoric Acid	Phenomenex C18 column	1.3 ml/min	50-300 mcg/ml	5.52 min	0.999
2.	Method Development and Validation and Degradation Studies for Cinacalcet HCl Drug by RP-HPLC	Buffer solution and methanol ratio(30:70)	AgilentZorbax C18 column	1.3 ml/min	50-150 mcg/ml	3.81 min	0.99

	Method						
3.	Validated RP-HPLC Method for the Estimation of Cinacalcet in Bulk and Tablet Dosage form	Water: methanol: acetonitrile (20:60:20).	Inertsil ODS C18 column	0.8ml/min	20-80 mcg/ml	3.7min.	0.999
4.	A Validated Chiral LC Method for the Enantiomeric Separation of Cinacalcet Hydrochloride	n-hexane, Ethanol and TFA (95:5:0.1, v/v).	(Chiralpak AD-H)	1.0 ml/min	0.16-1.2 mcg/ml	5.95 & 9.65 min	>0.99
5.	Present work	Methanol: acetonitrile: water	Inertsil ODS C18 column	1.0 ml/min	10-50 mcg/ml	3.0 min	0.996

In the reported methods, estimation of cinacalcet in API and pharmaceutical dosage forms was carried out using different mobile phases which include buffers and solvents of acidic and basic nature such as triethylamine, triflouroacetic acid etc. which makes the process tedious and expensive. The retention time in the reported methods also was found to be in the range of 3.7-5.5 minutes in the racemic form. In the estimation of enantiomers of cinacalcet the retention time of *R* and *S* enanatiomers was found to be 5.95 and 9.65 minutes respectively. In the present method, our aim is to develop a method using simple mobile phase system with decreased retention time compared to other methods. Decrease in retention time provides advantages such as consumption of less quantity of solvents and decreases the analysis time which results in fast analysis and economical method with increased column life.

MATERIALS AND METHOD

Chemicals and Reagents

HPLC grade solvents such as methanol, acetonitrile were obtained from sigma Aldrich and Millipore water was prepared by distillation and vacuum filtration through 0.45µ membrane filter. Cinacalcet bulk sample was obtained as a gift sample from IICT, Hyderabad and cinacalcet formulation (PTH 30) manufactured by Intas pharmaceuticals Ltd. were purchased.

Instruments

Schimadzu HPLC system model 2020 equipped with Lab solutions software was used for the analysis. Detection was carried out using a PDA detector and samples were injected through an autosampler. Other instruments used include ultrasonicator of model S410 by spectra lab instruments, Hyderabad and electronic balance Afcoset (ER-200A), Hyderabad. The chromatographic separation was carried out on Inertsil ODS C18 column (150×4.6 mm,5 µ).

Elution was performed with a mobile phase containing methanol, acetonitrile and water in the ratio of 70:15:15 v/v/v. Mobile phase was freshly prepared and filtered through 0.45 µm membrane filter and degassed prior to the analysis.

Preparation of mobile phase

A mixture of 700 volumes of methanol,150 volumes of acetonitrile and 150 volumes of Millipore water (150 mL) were sonicated and filtered through 0.45µ, membrane filter and is used as mobile phase.

Preparation of standard solution

Accurately (25 mg) weighed Cinacalcet standard (API) is transferred into volumetric flask (25 mL) to which small amounts of HPLC grade methanol is added. It was allowed to dissolve and the volume is made up to 25 mL with the same solvent. The solution is sonicated and filtered through 0.45µ, membrane filter. This is called primary stock solution from which desired working concentration solutions are prepared.

Preparation of sample solution

Twenty tablets were weighed and powdered. Tablet powder equivalent to 25 mg of cinacalcet was weighed and transferred into 25 ml volumetric flask to which diluent is added and the volume was made up to the mark with the same solvent, sonicated for 5 minutes and filtered through 0.45 µm membrane filter. Working concentration solutions are prepared from the above solution and are used for further analysis.

Validation of the method

The developed method was validated as per ICH guidelines in terms of linearity and range, accuracy, Precision, LOD, LOQ, specificity, sensitivity, and robustness.

Linearity and range

The linearity of an analytical procedure is its ability to obtain test results that are directly proportional to concentration of analyte in samples. The range of an analytical is the intervals between the upper and lower concentration of analyte in the sample for which it has been demonstrated. Linearity was established in the range of 10-50 µg/ml.

Accuracy

Accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value of the analyte. Accuracy of the method was studied by % recovery of the samples.

Precision

Precision is the analytical method describes the closeness of individual measures of the analyte

when the procedure is repeatedly to multiple aliquots of a single homogenous sample. Intraday and interday precisions were carried by injecting the samples in triplicates. For intraday precision samples are injected at regular intervals on the same day and analysed whereas for inter-day precision analysis is being done by injecting the sample on three consecutive days.

Limit of detection (LOD)

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value, under the stated experimental conditions. It is calculated by using the formula

LOD= 3.3*SD/Slope

Where SD=standard deviation, obtained by replicate injections of the sample and slope is obtained from the calibration curve of the analyte.

Limit of quantification (LOQ)

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. It is calculated by using the formula

LOD= 10 *SD/Slope

Where SD=standard deviation, obtained by replicate injections of the sample and slope is obtained from the calibration curve of the analyte.

Specificity

Specificity was established by complete separation of analyte in the presence of tablet excipients and without interferences at the retention time of cinacalcet.

Robustness

Robustness of the method was investigated under a variety of conditions like change in flow rate (±0.2 ml/min) and wavelength (±2 nm), change in mobile phase composition (±2^0 C). In each variation analysis was made in three replicates and %RSD of peak areas were determined.

RESULTS AND DISCUSSION

Solubility studies and selection of detection wavelength

Preliminary studies revealed that the drug is freely soluble in methanol, acetonitrile, sparingly soluble in tetrahydrofuran and insoluble in water. A standard solution (100µg/ml) of cinacalcet was scanned in the range of 200-400 nm and the maximum absorption was found at 280 nm. Hence 280nm was selected as the detection wavelength for the analysis of the drug. Chromatographic method was selected on the basis of physico chemical characteristics of the drug like the nature of the drug, molecular weight and solubility. Since the selected drug is polar in nature, a reversed phase chromatographic method has been selected for the analysis. During the

course of method development, various mobile phase systems in different compositions were tried which include mixtures of polar solvents consisting of methanol, acetonitrile and water in various compositions. Mixture of methanol, acetonitrile and water in the ratio of 70:15:15 (V/V/V) gave symmetric peak with good peak shape and optimum retention time. Hence mobile phase consisting of methanol, acetonitrile and water in the ratio of 70:15:15 (V/V/V) was considered as the optimum mobile phase concentration. Flow rate was set at 1 ml/minute. The above optimized conditions were used for further analysis of the drug.

METHOD VALIDATION

Linearity and range

The calibration curve was constructed between peak area and respective concentrations. The calibration curve was linear over the range of 10-50 µg/ml. Correlation coefficient was found to be 0.997. The regression equation for calibration curve was found to be y= 49290x + 65407. Results of linearity are shown in Table 2 and Figure. 4

Precision

Precision of the method was determined in terms of intraday and interday precision and is expressed in terms of % RSD. The % RSD obtained for intraday and interday precision was less than 2%, which is within the levels of acceptance criteria. The results of precision were shown in Table 3.

Accuracy

Accuracy was calculated by recovery studies in three concentrations i.e., 10,30 and 50 µg/ml by standard addition method. The results of accuracy was shown in Table 4.

Limit of detection

Limit of detection was calculated by the above mentioned equation and was found to be 0.24 µg/ml.

Limit of quantification

Limit of quantification was calculated by the above mentioned equation and was found to be 0.81 µg/ml.

Robustness

Robustness of the method was determined by deliberately changing parameters like flow rate, composition of mobile phase and wavelength. Samples were analysed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in Table 5.

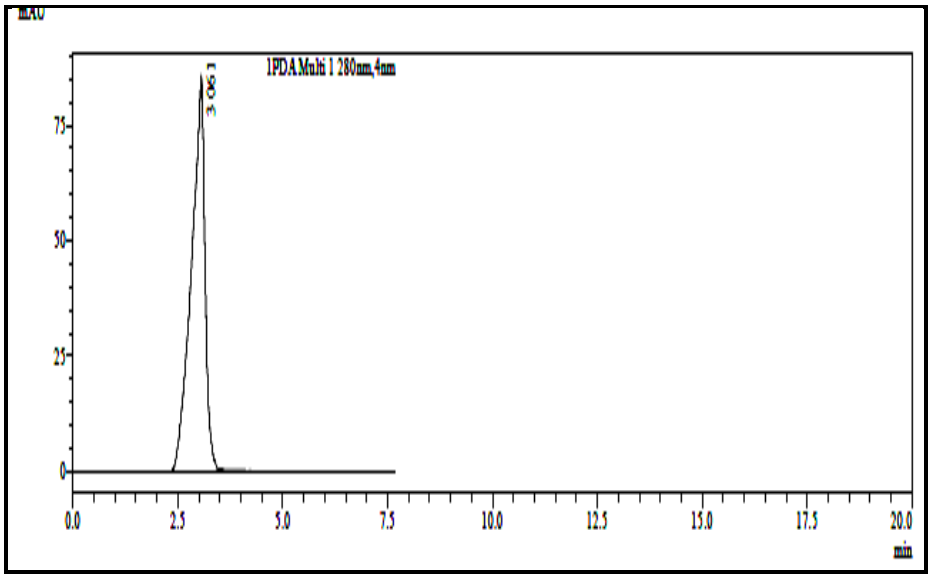

Figure 2: chromatogram of test preparation (marketed formulation)

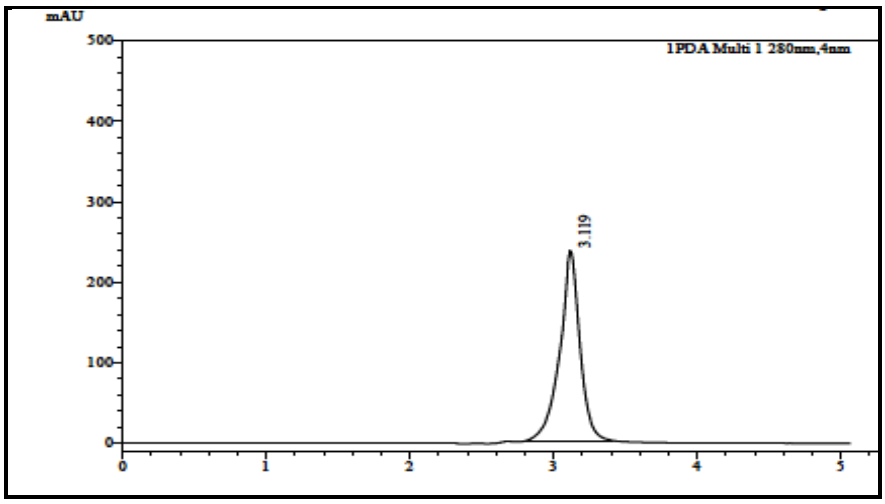

Figure 3: chromatogram of standard preparation (API)

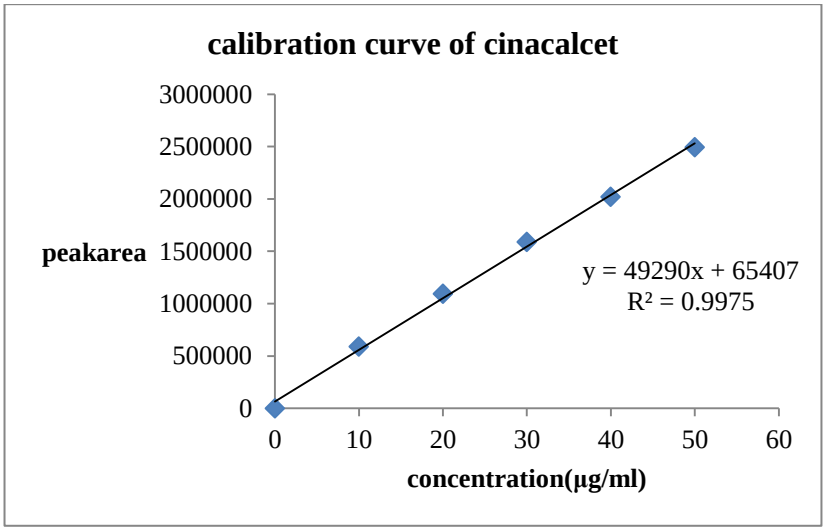

Figure 4: Calibration curve of cinacalcet in the linear range

Table 2: Analytical parameters for cinacalcet

Parameters	Analyte
Range	10-50µg/ml
Slope	49290
Correlation coefficient(r^2)	0.997

Table 3: Precision results of cinacalcet

Concentration (µg/ml)	% RSD	
	Intra day	Inter-day
10	0.63	0.61
20	0.31	0.33
30	0.26	0.084
40	0.24	0.03
50	0.27	0.23

Table 4: Results for accuracy and % recovery of cinaclacet

S.No	Amount present	Amount recovered	% recovery
1	10	9.32 µg/ml	99.32
2	30	30.33 µg/ml	100.11
3	50	50.37 µg/ml	100.47

Table 5: Results for robustness of the proposed method

S.No	Parameter	variation	Results	% RSD
1.	Flow rate	0.8 ml	3.2	0.15
		1.0 ml	3.1	0.25
		1.2 ml	2.9	0.19
2.	Mobile phase Composition	62	2.8	0.25
		60	3.1	0.38
		58	3.2	0.24

Table 6: Review of system suitability parameters for the proposed method

Parameters	Cinacalcet
Theoretical plates	9589
Tailing factor	0.88
LOD (µg/ml)	0.24
LOQ (µg/ml)	0.81

Assay of cinaclacet formulation by the proposed method

The validated method was used for the estimation of cinacalcet in marketed formulation of cinacalcet tablets(PTH 30). The parameters such as retention time and tailing factor comply with that of standard and were within the limits of acceptance. The chromatogram was shown in Figure.5.The percentage assay was found to be 100.8%, within the acceptance criteria. Hence the proposed method can be termed as simple, economic, sensitive and robust method for the analysis of the formulation.

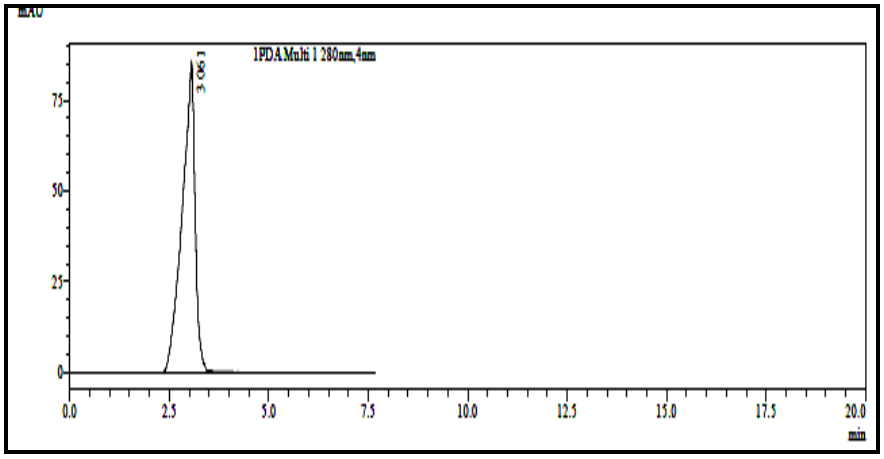

Figure 5: Chromatogram showing assay of PTH 30 tablets.

CONCLUSION

As there are very few methods for the estimation of cinacalcet through chromatographic methods, there is a need to establish a simple method for the analysis of cinacalcet. In the present study, a simple, sensitive, specific, accurate and precise RP-HPLC method was developed and validated for the routine analysis of bulk and tablet dosage form of cinacalcet. The method is sensitive enough for the detection of analyte in pharmaceutical formulation when compared to the research works found in the literature. The method can be termed as simple due to the use of simple mobile phase systems which proves the method economical and the results obtained in precision and accuracy indicate that the method is precise, accurate and therefore can be used for the routine analysis of the analyte in marketed formulations.

REFERENCES

1. Padhi D, Harris R. Clinical pharmacokinetic and pharmacodynamic profile of cinacalcet hydrochloride. Clinical Pharmacokinetics. 2009;48(5):303-11
2. Manjula A *et al.*, Spectrophotometric determination of cinacalcet hydrochloride in Bulk, Int J Pharm Review Res 2012, 2(2), 111-118.
3. Amruta B. Loni*et al.*, Spectrophotometric estimation of cinacalcet hydrochloride in Bulk and tablet dosage form**,** Int J Pharm Pharma Sci 2012, 4(3), 513-515.
4. Nirogi R *et al.*, Quantification of cinacalcet by LC–MS/MS using liquid–liquid extraction from 50 µL of plasma. J Pharma Biomedical Analysis 2011,56,373-381.
5. Shaik.Saida*et al.*,Liquid chromatographic separation, determination and ESI-MS/MS, FTIR and NMR characterization of the forced degradation products of cinacalcet. Analytical Methods, 2014,6(14), 5076-5087.

6. Manikandan K *et al.*, Stability indicating HPLC method for the estimation of Cinacalcet HCl API,. Indian J Res Pharma Biotechnology, 2013, 1(3):346-50.
7. Radhika T *et al.*, validated RP-HPLC method for the estimation of Cinacalcet in Bulk and Tablet dosage Form, American J Pharmtech Res 2013, 3(3), 409-414.
8. Manoj S. Charde, A. S. Welankiwar, Jitendra Kumar Method development by liquid chromatography with validation Int J Pharma Chemistry,2014,4(2), 57-61.
9. ICH, Validation of analytical procedures: text and methodology Q2 (R1), in: proceedings of the International Conference on Harmonization, IFPMA, Geneva, 2005.

\