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Development and validation of a chiral liquid chromatographic method for determination of S-isomer in cinacalcet API

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ABSTRACT

A reversed phase enantioselective high performance liquid chromatographic method was developed and validated for determination ofS-enantiomer in Cinacalcet drug substance. The enantiomers of Cinacalcet were resolved on a Chiralcel-OJH (250mmx4.6mm, 5microns) column using a mobile phase system containing n-Hexane, ethanol and n-butyl amine (90:10:1). The resolution between enantiomers was found to be more than 2.0. The retention times of S-enantiomer and Cinacalcet were found to be 7.50 and 9.63 min, respectively. The limit of detection and limit of quantification of S-enantiomer were found to be 0.014 and 0.042 respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of S-isomer was found to be 99.9%.Correlation coefficient between detector response and linearity concentration in the range of LOQ to 250% was found to be 0.99. The sample solution and mobile phase were found to be stable for at least 48 hours. The final optimized method was successfully applied for separation of S-enantiomer from Cinacalcet drug substance and proven to be reproducible and accurate for quantitative determination of S-enantiomer in bulk drugs.

Keywords: Cinacalcet, Enantiselective Liquid chromatography, S-isomer and Method validation

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INTRODUCTION

The hydrochloride salt of cinacalcet is described chemically as N-[1-(R)-(-)-(1-naphthyl)ethyl]-3-[3(trifluoromethyl)phenyl]-1-aminopropane hydrochloride and structural formula is depicted in Figure 1. The hydrochloride salt of cinacalcet is a white to off-white, crystalline solid that is soluble in methanol or 95% ethanol and slightly soluble in water.

Its empirical formula is $C_{22}H_{22}F_3N$•HCl with a molecular weight of 393.9 g/mol (hydrochloride salt) and 357.4 g/mol (free base) [1-2]. It has one chiral center having an R-absolute configuration. The R-enantiomer is the more potent enantiomer and has been shown to be responsible for pharmacodynamic activity.

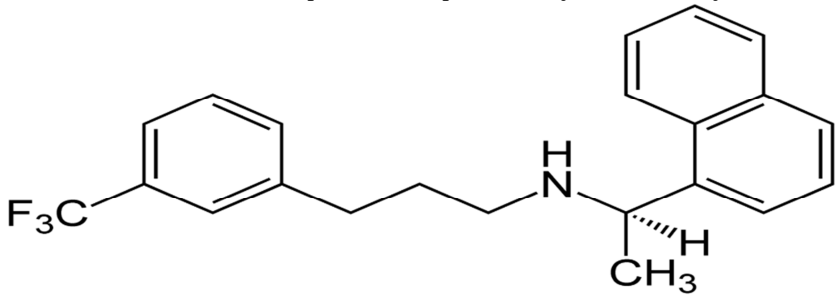

Figure 1: Structure of Cinacalcet

Cinacalcet is a drug that acts as a calcimimetic (i.e. it mimics the action of calcium on tissues) by allosteric activation of the calcium-sensing receptor that is expressed in various human organ tissues. Cinacalcet is used to treat secondary hyperparathyroidism (elevated parathyroid hormone levels), a consequence of end-stage renal disease. It is also indicated for the treatment of hypercalcemia in patients with parathyroid carcinoma. The calcium-sensing receptors on the surface of the chief cell of the parathyroid gland are the principal regulator of parathyroid hormone secretion (PTH). Cinacalcet increases the sensitivity of calcium receptors on parathyroid cells to reduce parathyroid hormone (PTH) levels and thus decrease serum calcium levels. As receptors are already active from the calcimimetic (Cinacalcet) the native rise and fall of Ca levels now interact with the remaining receptors, effectively lowering the threshold for activation of feedback on the parathyroid chief cells [3].

Enantiomers of racemic drugs often show different behaviors in pharmacological action and metabolic process. It is not uncommon for one enantiomer to be active while other isomer is toxic in biological systems. The pharmaceutical industry has raised its emphasis on the generation of enantiomerically pure compounds before under taking phamarmacokinetic, metabolic, physiological, and toxicological evaluation in the search for drugs with grater therapeutic benefits and low toxicity. Nowadays, chiral separations are playing more and more important role for the analysis of single enantiomers in the field of pharmaceutical industry. However, the development of the methods for the quantitative analysis of Chiral compounds and for the assessment of enantiomeric purity is extremely challenging, because the same physical and chemical properties of the two enantiomers make discriminating and separating them very difficulty [14].

A literature survey revealed few liquid chromatography (LC) methods that have been reported for the determination of S-enantiomer in bulk dug and pharmaceutical dosage [4-10].The aim of the present work is to focus on the development of an efficient enantioselective liquid chromatographic method for estimation of S-enantiomer in Cinacalcet API in a short chromatographic run.

The developed Liquid Chromatographic method was validated with respect to specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, precision, accuracy and robustness in accordance with ICH guidelines.

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Cinacalcet and its enantiomer standards were supplied by MSN Laboratories Ltd., Hyderabad as gift samples. n-Hexane, ethanol and n-butylamine chemicals of HPLC grade were procured from SD finechem chemicals, New Delhi.

2.2 Chromatographic Conditions

Enantioselective separation was carried out by using Waters alliance 2695 HPLC system (Waters Corporation, Milford, MA) equipped with a with binary HPLC pump, Waters 2998 PDA detector and Waters Empower2 software. The separation was achieved on a Chiralcel OJH (250mmx4.6mm, 5microns) column. The mobile phase consisting of n-Hexane, ethanol and n-butylamine (HPLC grade) were filtered through 0.45 µm membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 90:10:1 v/v into the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 35°C. The detection was monitored at 220 nm and the run time was 20 minutes. The volume of injection was 20 µl.

2.3 Standard and Sample solution Preparation:

Stock solutions of Cinacalcet (1mg/ml) and its S-enantiomer (1mg/ml) were prepared by dissolving appropriate amount of the substance in diluent, n-Hexane and Ethanol in the ratio (90:10).

The analyte concentration of Cinacalcet was fixed as 1000 µg/mL. Cinacalcet solutions spiked with low levels of (S)-enantiomer were prepared by transferring a calculated amount of S-enantiomer stock solution with pipette into the calculated amount of Cinacalcet stock solution, and then the solution was added to volume with diluent and mixed well.

The chromatographic parameters such as retention factor (k), the separation factor (α), and the resolution (Rs) were selected to evaluate the separation of S-enantiomer and Cinacalcet (R-enantiomer)

RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions:

To develop a rugged and suitable reversed phase HPLC method for the separation of the two enantiomers, different stationary phases and mobile phases were employed. Chiralpak-IA (250mm*4.6*5mic) and Chiralcel-OJH

(250mm*4.6*5mic) columns were used to provide an efficient separation but appropriate chromatographic separation was achieved on Chiralcel-OJH (4.6*250mm*5 mic). Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase containingn-hexane, ethanol and n-butyl amine in the ratio of 90:10:1 (v/v) gave a better resolution. Eluents were monitored at a wavelength of 220 nm by using PDA detector. Amongst the several flow rates tested, the flow rate of 1 ml/min was best suited for resolution of enantiomer peaks. The retention time of Cinacalcet and its enantiomer was found to be 9.63 and 7.50 minrespectively. The chromatograms of blank, standard, spiked and sample solution S-enantiomer were shown in **Figure 2.**The asymmetry factor of S-enantiomer and Cinacalcet was found to be 1.16 and 2.44respectively, which indicates symmetrical nature of the peak. The USP resolution of 4.91 was achieved between S-enantiomer and Cinacalcet. The USP plate count of S-enantiomer and Cinacalcet was found to be 9760 and 4925respectively, which indicates column efficiency for separation. System suitability parameters such as Peak asymmetry, Resolution and Number of theoretical plates are meeting ICH requirements [11-19].

Table 1.System suitability results of Cinacalcet and its S-enantiomer

S. No	Name	R_t	Area	% Area	Asymmetry	Theretical plates	USP Resolution
1	S-Isomer	7.54	68047	0.12	1.16	9760	--
2	Cinacalcet	9.64	57300264	99.88	2.44	4925	4.91

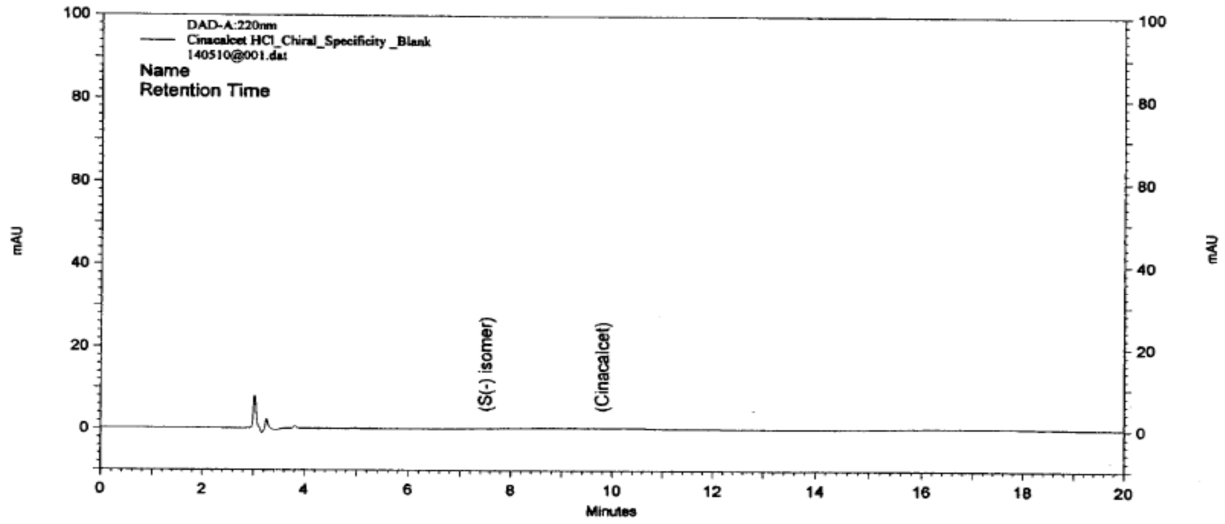

Figure 2: Chromatogram of Blank solution

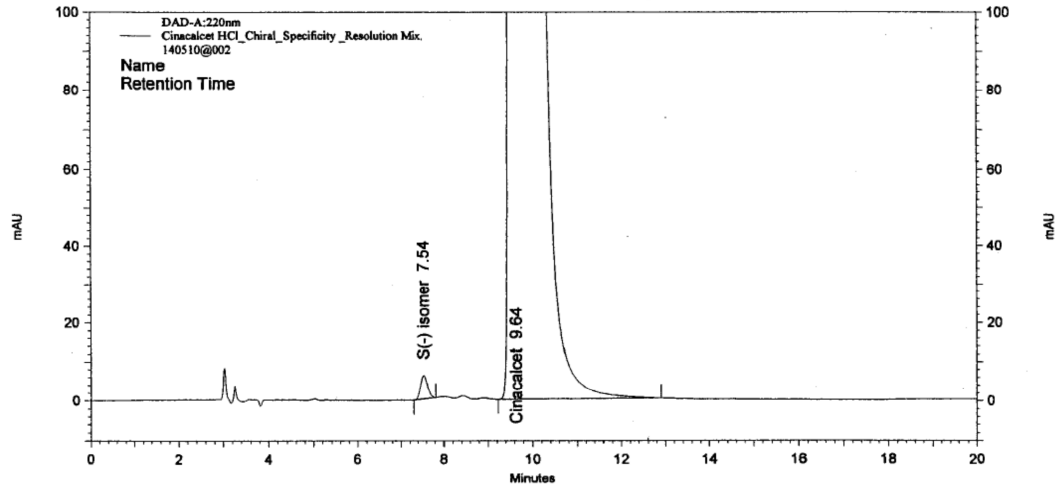

Figure 3: Typical Chromatogram of System suitability

The above result indicates that the system is suitable to carry out the Cinacalcet Enantiomeric Purity by HPLC analysis.

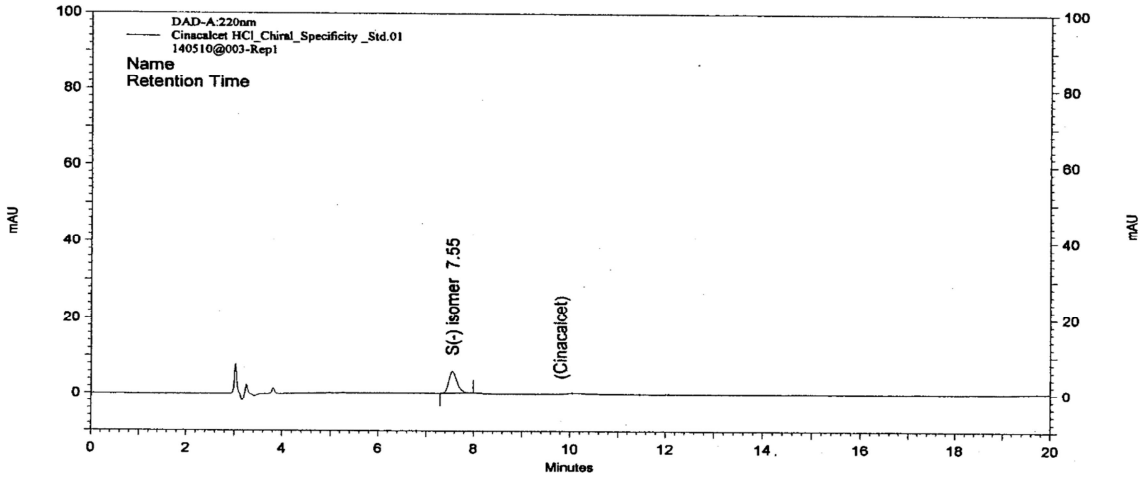

Figure 4: Typical Chromatogram-S-isomer standard

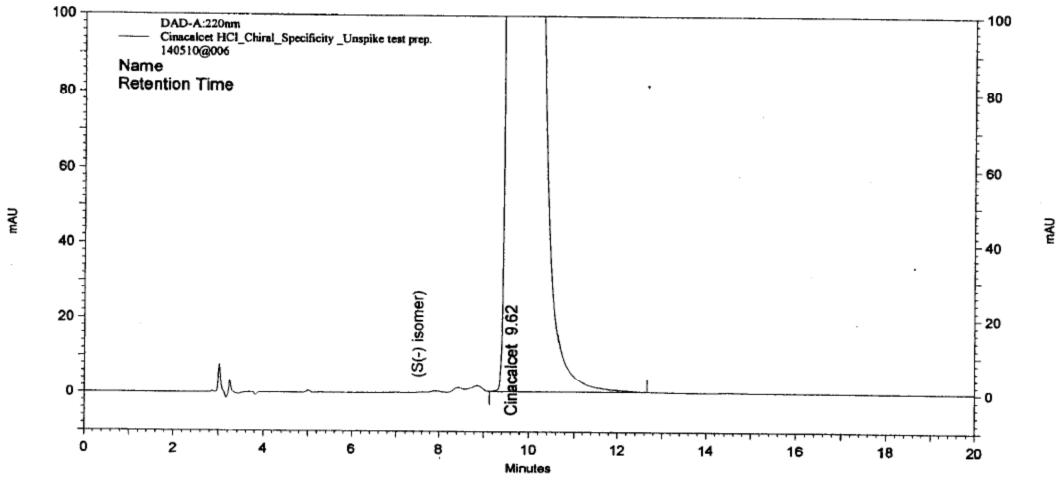

Figure 5: Typical Chromatogram-Test sample

3.2 Validation Results of the Method:

3.2.1.Specificity and Selectivity:

Specificity of analytical method was performed by injecting blank, individual impurities, Cinacalcet standard and spiked solution (S-isomer spiked with Cinacalcet at specification level) to confirm the retention times of the impurities. The peak purity is established by using PDA detector.

Table 2: Purity Angle and Purity thresholds of Cinacalcet and Its S-isomer

Nature of components	Purity Angle	Purity Threshold	Retention time	Relative retention time
S-isomer	08562	2.0245	7.54	0.78
Cinacalcet	0.247	0.320	9.64	1.0

No interference is observed at the retention time of known impurities and Cinacalcet due to blank.The retention time of the individual known impurities and Cinacalcet are comparable with that of the spiked solution. Purity angle is less than purity threshold for S-isomer and Cinacalcet API in spiked solution.

Table 3: System Precision results of S-isomer

Injection	S-Isomer
Preparation 1	71668
Preparation-2	71417
Preparation-3	72079
Preparation-4	71902
Preparation-5	71225
Preparation-6	72070
Mean	71727
SD	353.203
%RSD	0.49
Acceptance criteria	NMT 5.0%

3.2.2.Precision
The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement. System precision is performed by injecting six replicates of S-isomer and Cinacalcet standard solution (0.1%) and summarized results are given in table 3.

Above results indicate that the system is expressing the repeatability under the same operating condition over a short interval of time.

3.2.3. Method precision:
In method precision, a homogenous sample of a single batch should be analysed six times. This indicates whether a method is giving consistent results for a single batch.

Method precision is performed by injecting six preparations of spiked sample and summarized results are given below.

Table 4: Method Precision Results of S-isomer

Sample No	% S-Isomer
Injection-1	0.146
Injection-2	0.148
Injection-3	0.148
Injection-4	0.151
Injection-5	0.151
Injection-6	0.148
Mean	0.149
SD	0.002
%RSD	1.34
Acceptance criteria	NMT 10.05

Above results indicate that the method has expressed the closeness of the results between multiple preparations of same homogeneous sample, under stated condition.

3.2.4. Accuracy:
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy study was conducted by spiking the known amount of active ingredients into the placebo at three different levels (LOQ, 50%, 100% and 150% of target concentration). The samples were analysed as per the proposed test procedure and the % recovery for each spiked level was calculated.

The % recovery at each spike level should be NLT 80.0 and NMT 120.0 of the added amount.

Table 5: Accuracy of S-isomer at 50%, 100% and 150%

Spiked Level	Amount Added (%)	Amount Recovered (%)	% Recovery	Mean
LOQ	0.008	0.008	100.0	100.0
LOQ	0.008	0.008	100.0	
LOQ	0.008	0.008	100.0	
50%	0.075	0.072	96.0	96.65
50%	0.075	0.072	96.0	
50%	0.075	0.073	97.3	
100%	0.150	0.146	97.3	97.06
100%	0.150	0.146	97.3	
100%	0.150	0.145	96.6	
150%	0.225	0.220	97.7	97.7
150%	0.224	0.220	97.7	
150%	0.225	0.220	97.7	

Above results indicate that the method has been expressed the closeness of agreement between observed value and true value. Hence, Accuracy has been established across the specified range (LOQ, 50%, 100% and 150%) of analytical method.

3.2.5. Linearity and Range:
The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.Linearity

for S-isomer is performed from LOQ level to 250% level with respect to the specification level. The linearity of S-isomer is determined by plotting correlation of area response vs concentration and the correlation coefficient. The summarised results are given below.

Table 6: Linearity results of S-isomer:

S-Enantiomer				
% Con.	**Conc of S-isomer**	**Run-1**	**Run-2**	**Mean Area**
LOQ	0.042	4068	4067	4068
50	0.375	35553	35676	35615
100	0.751	72181	72556	72369
150	1.126	109314	109404	109359
200	1.501	145450	144124	144787
250	1.876	182931	180457	181694
Correlation Coefficient				0.99998
Slope				96936.03
Intercept				-305.37

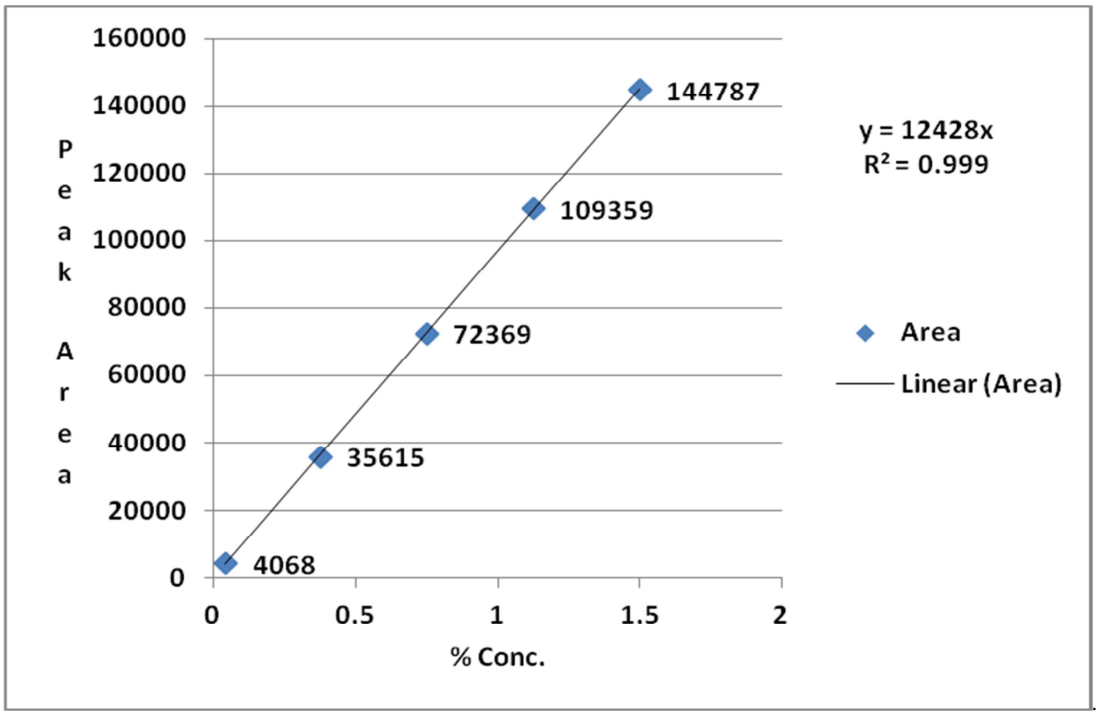

Figure 6: Liearity curve for S-isomer

Above results indicate that the method has ability to generate responses which are directly proportional to the concentration of analytes present in the sample. This concluded that the method was linear throughout the range selected.

Table 7: LOD and LOQ

Concentration (%wrt specification level)	**Concentration of S-isomer (µg/ml)**	**Area**
40.0	0.301	24775
30.0	0.226	17864
20.0	0.151	12648
10.0	0.075	6045
5.0	0.038	2989
2.5	0.019	1878
SD of residual		343.59
Slope		81047.36
LOQ(µg/ml)		0.042
LOD(µg/ml)		0.014
LOQ (%wrt test conc.)		0.008
LOD (%wrt test conc)		0.003

3.2.6. Limit of detection and Limit of quantitation:
Limit of detection is determined by injecting 0.01% concentration of all known impurities and drug substances. Limit of quanitation is three times the limit of detection level. Precision and accuracy at LOQ are performed and the results are tabulated in table 7.

3.2.7.Robustness:
Robustness of the proposed analytical method was evaluated by making deliberate changes in the chromatographic system method parameters the standard solution was injected for each of the changes made to access the robustness of proposed analytical method.

Change in flow rate of mobile phase by (± 10%):
Effect of Change in flow rate of Mobile phase is monitored in the range of ±10%. The results are presented in Table 8.

Table 8: Robustness results for variation of Flow rate

System suitability parameter	Effect of Flow rate(ml/min)			Acceptance criteria
	0.8	1.0	1.2	
Resolution	2.06	2.22	2.10	NLT 2.0

Change in column oven temperature by (± 5°C):
Effect of the column oven temperature is monitored at a range of $\pm 5^0$C. The results are presented in Table 9.

Table 9: Robustness results for variation of Tempearature

System suitability parameter	Effect of Temperature			Acceptance criteria
	30	35	40	
Resolution	2.15	2.22	2.25	NLT 2.0

The system suitability parameters found complying as per the acceptance criteria, hence the method is robust.

3.2.8. Mobile phase stability:
Sufficient quantity of mobile phase is prepared, observed visually and analyzed at initial, after 24th hour and after 48th hr at room temperature and summarised results are given below.

Table 10: Stability of Sample solution

Sampling time	Resolution (between S-isomer and Cinacalcet)	Turbidity/Particles
Initial	2.2	Not observed
After 24th hr	1.98	Not observed
After 48th hr	1.59	Not observed
Acceptance criteria	NLT 1.50	Should not show any turbidity/particles

CONCLUSION

The parameters covered under Analytical method validation study of S-isomer in Cinacalcet API are Specificity, Method precision, Linearity and Range, Accuracy, Robustness and Forced degradation studies. All these parameters considered for validations meet predefined acceptance criteria.

So the method is proposed for quantitative estimation of assay for in-process blends, finished drug products and stability studies. Proposed analytical method is suitable for intended applications.

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