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A NOVEL VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES OF CINACALCET HYDROCHLORIDE API

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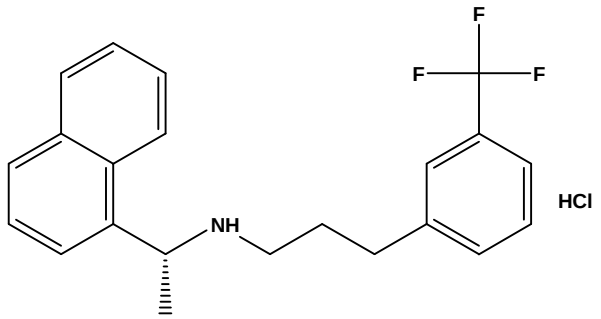
ABSTRACT
This article reports validated stability-indicating RP-HPLC method for the related substance of cinacalcet hydrochloride developed by separating its related substances and degradants on an Ascentis Express ES-CN (150mm × 4.6mm × 2.7µm) column using 10 mM aqueous solution of sodium perchlorate (pH 2.5 with perchloric acid) - acetonitrile as the mobile phase in a gradient mode of elution at a flow rate of 0.8 mL/min at 50°C. The column eluents were monitored by a photodiode array detector set at 215 nm with an autosampler temperature of 5˚C. The developed method was validated for system suitability, solution stability, specificity, linearity, range, accuracy, precision (repeatability), limit of detection, limit of quantitation and robustness according to ICH guidelines Q2 (R1). The forced degradation study of cinacalcet hydrochloride was carried out under acidic condition, basic condition, neutral condition, thermal condition, photodegradation, and oxidation conditions and the degradation products were separated. The linear range of the method was 0.4-1.5 µg/ml and limit of quantification of the Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7 and Impurity-8 were found to be 0.07, 0.15, 0.04, 0.18, 0.17, 0.13, 0.14 and 0.07 µg/ml respectively. The method was successfully applied to separate the degradation products and related substances of cinacalcet hydrochloride.

KEYWORDS: Cinacalcet Hydrochloride; Impurities; RP-HPLC; ICH guidelines; Stability Indicating; Method Development and Validation.

INTRODUCTION
The aim of the pharmaceutical industries is to care for the well-being of the public via empowering patients to obtain adequate medication at adequate quality and quantity at a reasonable price. Thus, pharmaceutical care and effectiveness are two important concerns in drug treatment. An active moiety's safety is determined by its pharmacological toxicological summary and the adverse effects in the bulk and dosage forms caused by impurities, which means a drug product's safety depends not only on the toxicological characteristics of the active moiety but also on the impurities contained therein. It is therefore important to characterize the products totally before human consumption. Impurity monitoring and control generally ensure active moiety's quality and safety. A significant component of the current guidelines issued by the ICH (International Conference of Harmonization) is analytical monitoring of impurities in new active ingredients.[1] Forced degradation studies investigations deliver evidence to support the identification of probable degradants; API degradation pathways alone and in the drug invention, any likely enantiomeric or polymorphic substances and difference between excipient interferences and drug-related degradation, drug molecule intrinsic stability and validation of stability-indicating analytical methods (SIAM). Impurity profiling and forced degradation studies are therefore one of the bases for NDA as well as IND registration document.[2]

Chemically cinacalcet hydrochloride is [(1R)-1-(naphthalen-1-yl)ethyl]({3-[3-(trifluoromethyl)phenyl]propyl})amine hydrochloride. Cinacalcet exists as a calcimimetic agent that shows an effect on the parathyroid calcium-sensing receptor. 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 levels of calcium in patients with parathyroid carcinoma. The main regulator of parathyroid hormone (PTH) secretion is the calcium-sensing receptor on the surface of the chief cell of the parathyroid gland. It directly acts by reducing the levels of parathyroid hormone by increasing the sensitivity of the calcium-sensing receptors to extracellular calcium activation, resulting in PTH secretion inhibition. The decrease in PTH is associated

with a reduction in serum calcium levels simultaneously. Cinacalcet hydrochloride with different brand names such as Sensipar, Mimpara is available in the market.[3]



Cinacalcet Hydrochloride

Fig. 1: Structure of cinacalcet hydrochloride.

The present article describes (I) optimization of reverse-phase HPLC method for the eight related substances of cinacalcet hydrochloride which are Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7 and Impurity-8, (II) method validation studies and (III) the degradation behaviour of CNC in different stress conditions.

An exhaustive literature survey showed that numerous liquid chromatographic (HPLC and UPLC) analytical[3-13] and bio-analytical[14-20] methods are existing for the estimation of cinacalcet hydrochloride in API as well as solid dosage form individually. Few Capillary electrophoresis,[21,22] spectrophotometric[23,24] and gas chromatography-mass spectroscopy[25] methods were also reported for impurity detection and degradation product detection. However, no HPLC method has been reported to date for simultaneous detection of the eight related substance along with cinacalcet hydrochloride API in a single method. There is, therefore, a need to develop a rapid, sensitive, accurate and reproducible HPLC method to detect the related substance of cinacalcet hydrochloride in API.

Table 1: Chemical names of CNC and related substances.

Substance name	IUPAC name
Cinacalcet hydrochloride	[(1R)-1-(naphthalen-1-yl)ethyl]({3-[3-(trifluoromethyl)phenyl]propyl})amine hydrochloride
Impurity-1	1- (Naphthalen-1-yl) ethanamine
Impurity-2	3-(3-(trifluoromethyl) phenyl) propan-1-amine
Impurity-3	(Z)-1-(naphthalen-1-yl) ethan-1-one oxime
Impurity-4	1-(naphthalen-1-yl) ethan-1-one
Impurity-5	3-[3-(Trifluoromethyl)phenyl]propan-1-ol
Impurity-6	3-[3-(triflurомethyl) phenyl] propyl methane sulphonate
Impurity-7	(R)-N-(1-(Naphthalen-1-yl) ethyl)-3-(3-(trifluoromethyl) phenyl) propan-1-amine N-oxide
Impurity-8	(1- Naphthalene -1 -ylethyl)-[3-(3-trifluoromethylcyclohexyl) -propyl]-amine.

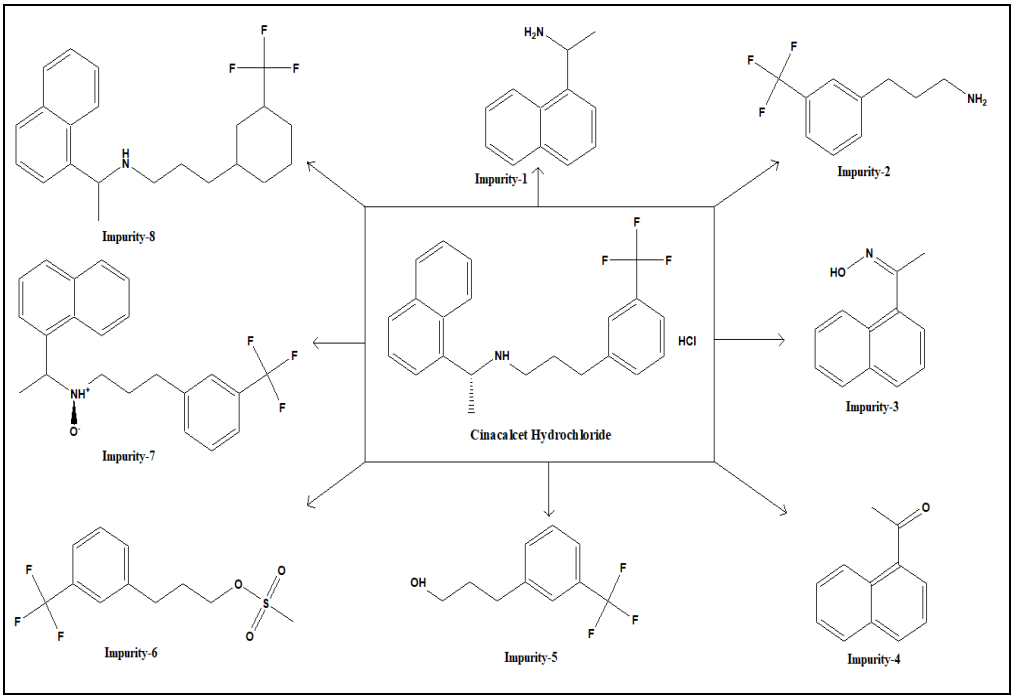


Fig. 2: Cinacalcet hydrochloride and its related substances.

EXPERIMENTAL METHODS

Materials

Cinacalcet hydrochloride API and related substances were received from CIPLA Ltd., Mumbai, India as gift sample. High-purity water was used for mobile phase preparation (Millipore Milli-Q Plus purification system). Acetonitrile (HPLC grade, Qualigens), sodium perchlorate (A.R. grade, Merck) and 70% perchloric acid (A.R. grade, Rankem) were used in this research project.

Apparatus

The HPLC system used was Agilent technologies 1260 series, equipped with PDA detector and chromeleon software. Other instruments used in this research were U.V. spectrometer (Perkin Elmer), FTIR spectrometer (Perkin-Elmer), sonicator (Dolphin), pH meter (LAB INDIA), electronic balance (Mettler-Toledo).

Chromatographic conditions

HPLC methods were carried out using an Ascentis Express ES-CN column (150mm × 4.6mm × 2.7µm) operated at 50°C and autosampler temperature of 5˚C with a flow rate of 0.8 mL/min using a mobile phase containing 10 mM aqueous sodium perchlorate (pH 2.5 adjusted using perchloric acid) - acetonitrile in a gradient mixture of solvents. The HPLC gradient program has been set as: time (min)/% mobile phase A/% mobile phase B: 0.01/80/20, 03/80/20, 15/60/40, 20/60/40, 25/20/80, 27/20/80, 27.1/80/20 and 35/80/20. The samples were injected at 5µl injection volume and were detected at 215 nm using the PDA detector.

Methods

Preparation of 10 mM sodium perchlorate buffer pH 2.5

1.40 gm of sodium perchlorate was transferred to a volumetric flask of 1000ml and dissolved in 800 ml of Millipore water, adjusted the pH up to 2.5 using perchloric acid and enough quantity of Millipore water was added to produce 1000ml.

Diluent preparation

A buffer-acetonitrile mixture was prepared as a diluent in the ratio of 1:1.

Preparation of stock solution

10 mg of Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7, and Impurity-8 was accurately weighed individually and transferred to 100 ml of volumetric flask and volume was made up to the mark with acetonitrile to obtain a concentration of 100 µg/ml of each impurity individually.

Preparation of the standard and system suitability solution

A standard working solution for related substance was prepared by taking 1 ml aliquot from a stock solution of each 100 µg / ml of impurity and transferred to 10 ml volumetric flask and volume were made up to the mark with the diluent to produce 10 µg/ml mixture of impurity. Furthermore, 1 ml aliquot was pipetted out and transferred to 10 ml volumetric flask and volume made up to the mark with diluent to achieve a concentration of 1 µg/ml mixture of impurity.

Preparation of sample solution

A sample solution for related substance was prepared by taking 1 ml aliquot from eight stock solutions of each 100 µg / ml of impurity and transferred to a 10 ml volumetric flask and volume were made up to the mark with the diluent to produce 10 µg/ml mixture of impurities. Furthermore, 1 ml aliquot was pipetted out from the 10 µg/ml mixture of impurities and transferred to a volumetric flask of 10ml containing previously weighed 10 mg of cinacalcet hydrochloride API. The volume was made up to the mark with diluent to obtain a concentration of 1 µg/ml mixture of related substances and 1000 µg/ml of cinacalcet hydrochloride. This produces 0.1% concentration of related substances with respect to cinacalcet hydrochloride.

METHOD VALIDATION

Specificity and Selectivity

Method specificity has been performed by injecting blank, cinacalcet hydrochloride standard, individual related substance and spiked solution (related substances spiked with cinacalcet hydrochloride at the specification level) to confirm the retention time of related substances. The retention time of the related substances and cinacalcet hydrochloride is similar to that of the spiked solution.

Linearity and range

The linearity of related substances was determined by preparing a calibration curve of each impurity. The responses of impurities were found to be linear in a concentration range of 0.4-1.5 µg/ml and the linear regression was found to be more than 0.95 ($r^2 > 0.95$).

Accuracy

The mean % recovery at 50%, 100%, and 150% for related substances was found to be in the range of 95-105% and the % RSD was found to be less than 2.0.

Precision

Repeatability and intermediate precision studies were carried out by injecting six replicates of a standard mixture of related substances and the %RSD values were found to be <2%, thus indicating that the developed method was precise.

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

LOD and LOQ were determined from the slope of calibration plot and standard deviation of the standard solution by use of the equations

LOD = 3.3×Standard deviation / Slope and

LOQ = 10×Standard deviation / Slope

Robustness

No significant outcome on system suitability factors such as tailing factor, % RSD, or theoretical plates were observed when minor but deliberate chromatographic condition changes were made. Therefore, the method was found to be robust in terms of variability in the conditions applied

Solution stability

A standard mixture of related substances and spiked sample (related substances at 0.1% of the cinacalcet hydrochloride) were prepared using diluent and kept at room temperature for 48 hours. During the study period, the mobile phase prepared was kept constant. Samples were picked up at a time interval of 0 hours, 24 hours and 48 hours and injected. The consistency in the % of each impurity was examined at each time interval by calculating the impurity content. The mobile phase analysis was shown by injecting the newly prepared sample solution at a time interval of 0 hours, 24 hours and 48 hours. The % RSD of these samples were found to be less than 2.0 indicating the stability of solution and method.

RESULT AND DISCUSSION

Optimization of chromatographic conditions

Preliminary studies revealed that the drug is freely soluble in acetonitrile, methanol, 95% ethanol and slightly soluble in water. A standard solution (100µg/ml) of cinacalcet in acetonitrile was scanned in the range of 200-400 nm and the maximum absorption was found at 223 nm (Fig. 3). Under method development related substances were not giving maximum absorbance at 223 nm whereas 215nm was the wavelength at which each related substance and cinacalcet hydrochloride were showing optimum absorbance. Hence, 215nm was selected as the detection wavelength for the analysis of the drug. The chromatographic method was selected on the basis of physicochemical characteristics of the drug like solubility, nature of the drug and molecular weight. Since the drug is polar in nature, a reversed-phase chromatographic method has been selected for the analysis. During the course of method development, both isocratic and gradient mode of elution were tried. Under isocratic mode, water: acetonitrile, water: methanol were tried in different ratio and no separation was observed. Under gradient mode of elution various mobile phase systems consist of a mixture of various buffers like acetates, phosphates at different pH with acetonitrile and methanol were tried in different compositions at various gradient ratio. A mixture of 10 mM aqueous Sodium perchlorate (pH 2.5 with perchloric acid) - acetonitrile in gradient mode gave symmetric peak with good peak shape and optimum retention time. Hence mobile phase consisting of 10 mM aqueous Sodium perchlorate pH 2.5 with perchloric acid - acetonitrile in gradient mode was considered as the optimum mobile phase. Several flow rate (i.e. 0.8 ml/min, 1.0 ml/min and 1.2 ml/min) were tried and the flow rate was selected as 0.8 ml/minute. Various column such as C-8, C-18, phenyl, cyano was used from which Ascentis Express ES-CN gave optimum results and selected for method. The above-optimized conditions were used for further analysis of the drug.

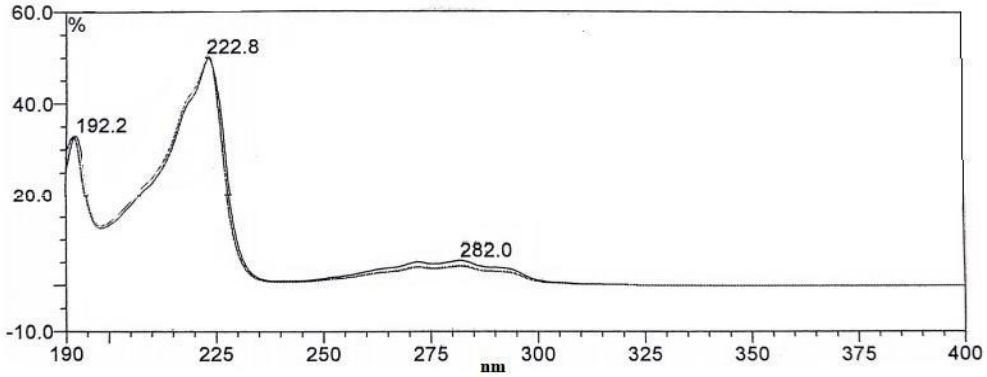


Fig. 3: UV spectrum of a standard solution of CNC (100 µg/ml).

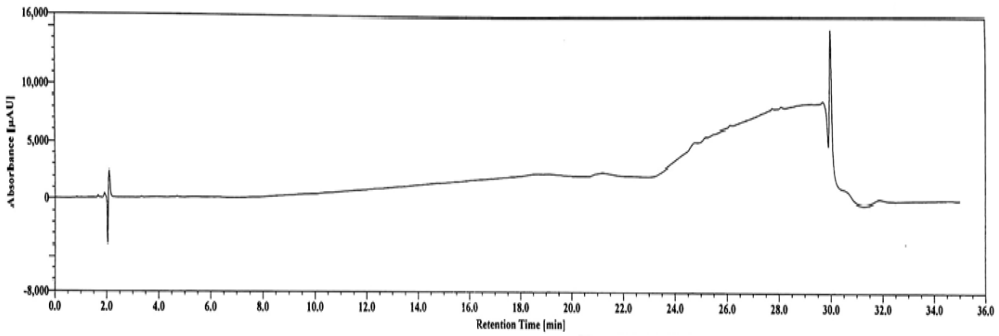


Fig. 4: Chromatogram of lank.

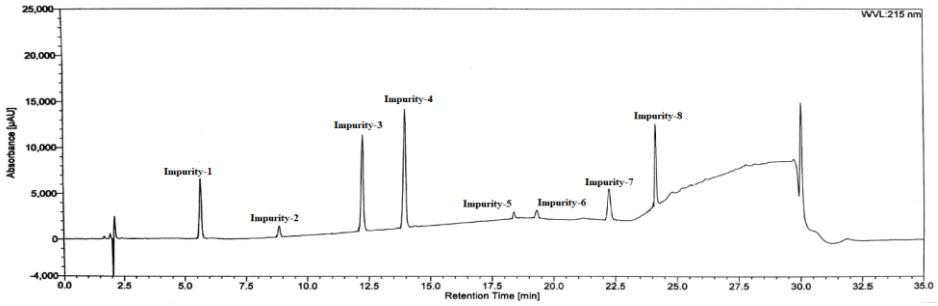


Fig. 5: Chromatogram of a standard solution of related substances.

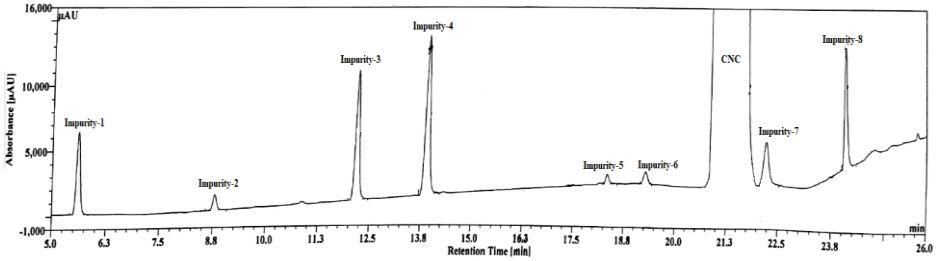


Fig. 6: Chromatogram of related substances along with cinacalcet hydrochloride.

System suitability

In order to check system performance, system suitability parameters were conducted. System precision was determined with six replicate injections of the standard preparation. All the important characteristics were measured, including the retention time, resolution, tailing factor and number of theoretical plates (Table. 2). All of these parameters of system suitability covered the performance of the system, method, and column.

Table 2: Data for system suitability of the method.

Compound	Retention time (RT)	USP Resolution (Rs)	USP Tailing factor	No. of theoretical Plates (*N*)
Impurity-1	5.5	NA	1.1	19684
Impurity-2	8.7	19.9	1.1	47207
Impurity-3	12.1	20.3	1.1	81688
Impurity-4	13.8	9.4	1.1	83167
Impurity-5	18.3	26.9	1.2	274581
Impurity-6	19.2	5.5	1.2	155090
Cinacalcet hydrochloride	21.5	-	-	-
Impurity-7	22.2	13.9	1.1	152107
Impurity-8	24.1	10.8	1.1	660494

Specificity

At the retention time of related substances and cinacalcet hydrochloride, no interference was observed due to blank.

Linearity and range

The calibration curve between peak area and respective concentrations was designed. The calibration curve over the range of 0.4-1.5 µg/ml was linear and the correlation coefficient was found to be within limits. Results of linearity are shown in Table 3.

Table 3: Data For Linearity of The Method.

	Trend line equation	Range	Regression coefficient	Slope	Intercept
Impurity-1	y = 39790x + 394.61	0.3-1.5	0.9930	39790	394.61
Impurity-2	y = 6884.7x + 128.19	0.3-1.5	0.9938	6884.7	128.19
Impurity-3	y = 74628x + 128.71	0.3-1.5	0.9935	74628	128.71
Impurity-4	y = 87004x + 1399.1	0.3-1.5	0.9935	87004	1399.1
Impurity-5	y = 3098.7x + 88.591	0.3-1.5	0.9935	3098	88.591
Impurity-6	y = 5033.3x - 19.732	0.3-1.5	0.9929	5033.3	-19.732
Impurity-7	y = 27429x + 547.02	0.3-1.5	0.9926	27429	547.02
Impurity-8	y = 42042x + 973.9	0.3-1.5	0.9919	42042	973.9

Accuracy

Accuracy was calculated by % recovery studies in three concentrations i.e., 0.5,1.0 and 1.5 (50%, 100% and 150%) µg/ml by the standard addition method. The results of the accuracy in terms of % recovery was found within the range of 95-105% and are shown in Table 4.

Table 4: Data for % recovery.

Compound	Spike level (%)	Amount Added (µg)	Amount recovered (µg)	% Recovery	% RSD (n=3)	Acceptance criteria
Impurity-1	50	0.46	0.45	99.03	0.3	% RSD ≥ 2.0
	100	0.92	0.89	96.28	1.0	
	150	1.39	1.35	97.14	1.0	
Impurity-2	50	0.45	0.45	99.27	1.6	
	100	0.91	0.87	95.77	1.8	
	150	1.36	1.32	96.97	0.4	
Impurity-3	50	0.41	0.39	96.39	0.4	
	100	0.82	0.77	96.01	1.0	
	150	1.23	1.18	96.40	1.3	
Impurity-4	50	0.48	0.46	96.22	0.9	
	100	0.97	0.94	96.56	1.0	
	150	1.46	1.45	99.41	1.3	
Impurity-5	50	0.47	0.48	103.37	1.3	
	100	0.94	0.90	95.74	1.5	
	150	1.42	1.45	102.07	1.9	
Impurity-6	50	0.479	0.476	99.37	0.6	
	100	0.95	0.98	102.91	1.2	
	150	1.43	1.49	103.99	1.8	
Impurity-7	50	0.43	0.43	100.04	1.3	
	100	0.86	0.83	95.58	1.3	
	150	1.30	1.25	96.25	0.1	
Impurity-8	50	0.40	0.42	104.14	0.8	
	100	0.81	0.85	103.7	1.1	
	150	1.22	1.27	104.03	1.3	

Precision

The precision of the method was determined in terms of intraday, inter-day precision and expressed in terms of % RSD. Obtained % RSD for intraday and inter-day precision was found to be less than 2%, which is within the levels of acceptance criteria. The repeatability of the method was expressed in terms of %RSD. The results of precision and repeatability are shown in Table 5.

Table 5: Data for Precision and Repeatability.

Compound	% RSD (n=6)		
	Intraday	Inter-day	Repeatability
Impurity-1	0.46	0.76	0.35
Impurity-2	1.45	1.44	1.07
Impurity-3	0.28	0.45	0.13
Impurity-4	0.19	1.65	0.91
Impurity-5	1.59	1.38	1.14
Impurity-6	1.02	1.11	0.85
Impurity-7	0.73	1.35	0.84
Impurity-8	0.31	0.74	0.45

Limit of detection (LOD)

Limit of detection of Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7 and Impurity-8 were calculated and were found to be 0.02, 0.05, 0.01, 0.06, 0.05, 0.04, 0.04 and 0.02 µg/ml respectively.

Limit of quantification (LOQ)

Limit of quantification of Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5, Impurity-6, Impurity-7 and Impurity-8 were calculated and were found to be 0.07, 0.15, 0.04, 0.18, 0.17, 0.13, 0.14 and 0.07 µg/ml respectively.

Robustness

The method's robustness was determined by altering parameters such as flow rate (±0.2 ml/min), temperature (±2˚C) and pH of mobile phase A (±0.2 units). Samples were analysed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in Table 6.

Table 6: Data for robustness.

Compound	% RSD								
	Temperature (±2˚C)			Flow (±0.2 ml/min)			pH (±0.2 units)		
	RT	Rs	Theoretical plates	RT	Rs	Theoretical plates	RT	Rs	Theoretical plates
Impurity-1	0.52	0.88	0.36	0.84	0.43	0.21	1.32	0.95	1.42
Impurity-2	1.35	1.65	1.70	0.93	1.06	1.01	1.22	1.30	1.69
Impurity-3	0.97	1.02	0.99	0.68	0.87	0.72	0.48	0.66	0.69
Impurity-4	0.59	0.54	0.61	0.81	0.79	0.76	1.13	1.20	1.17
Impurity-5	1.48	1.46	1.52	1.33	1.43	1.46	1.61	1.65	1.73
Impurity-6	1.82	1.79	1.75	1.25	1.32	1.28	1.64	1.72	1.75
Impurity-7	0.85	0.83	0.87	0.43	0.35	0.42	0.79	0.62	0.75
Impurity-8	0.49	0.52	0.79	0.77	0.83	0.84	0.53	0.61	0.53
Acceptance criteria	% RSD should be less than 2.0								

Solution stability

During the solution and mobile phase stability experiments using related substance method, no significant changes were observed in the content of cinacalcet hydrochloride related substance. This experiment data concluded that the sample solution and mobile phase used during the method were stable for at least 48 hours.

Degradation study

The cinacalcet hydrochloride API was subjected to different stress conditions purposely. The % mass was calculated for a number of a degradants formed and total mass balance was calculated. It was observed that there was no significant degradation under alkaline, acidic, neutral, photolytic, and thermal conditions whereas under oxidative conditions the significant degradation was observed and it was found to be 12.39 % a degradation. The result of a degradation study is tabulated below (Table 7).

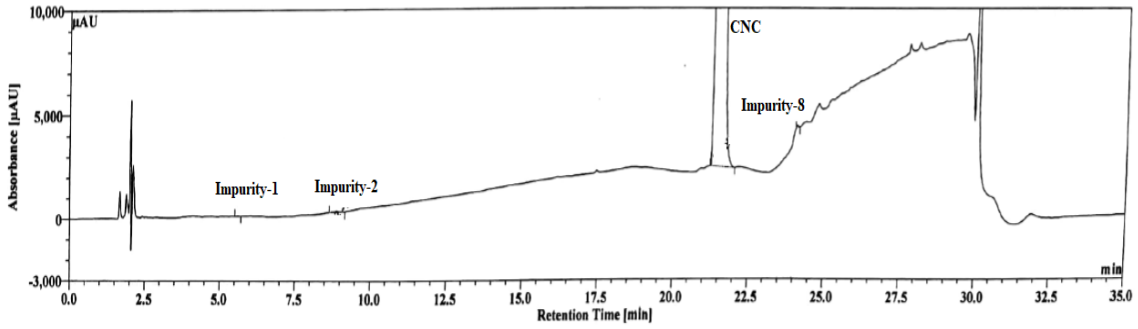


Fig. 7: Acid degradation.

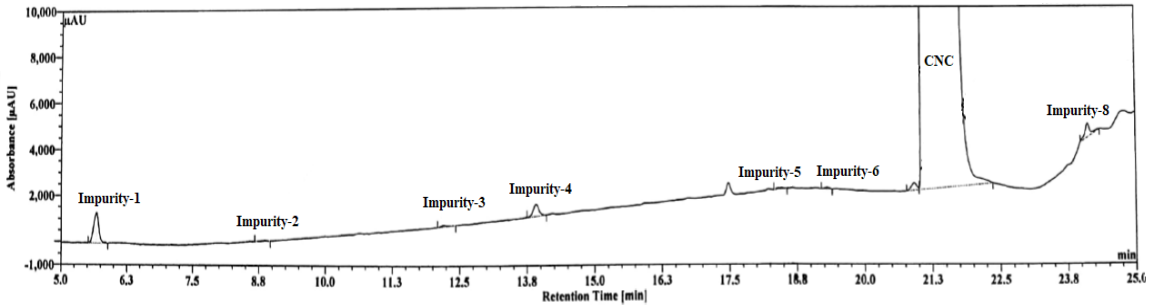


Fig. 8: Alkaline degradation.

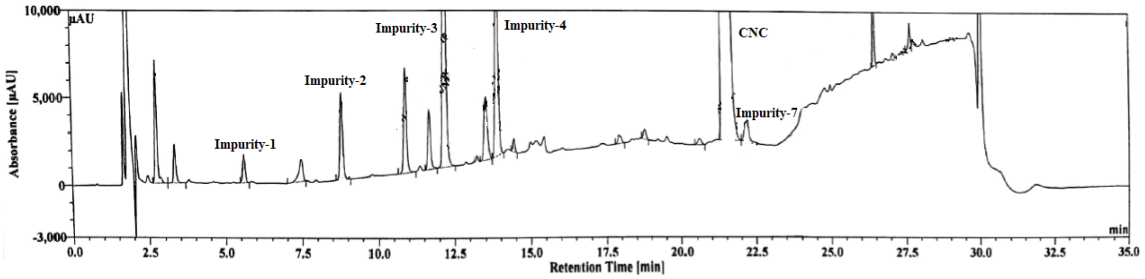


Fig. 9: Oxidative degradation.

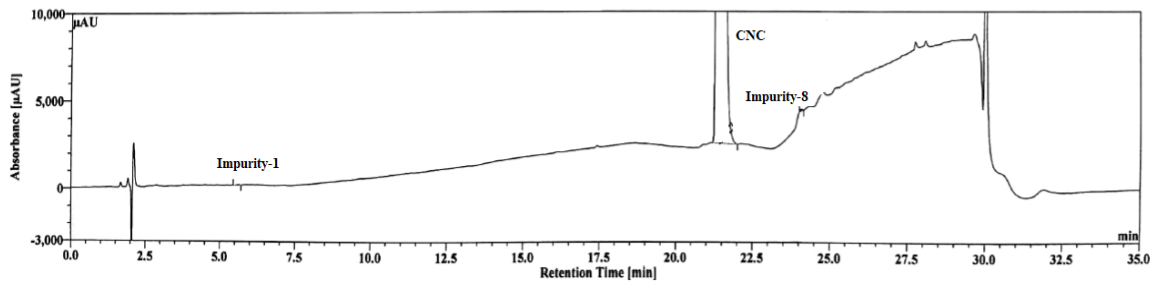


Fig. 10: Hydrolytic degradation.

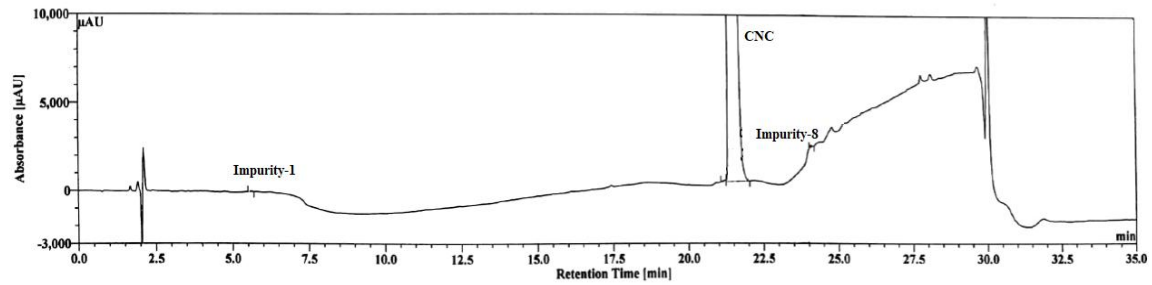


Fig. 11: Photolytic degradation.

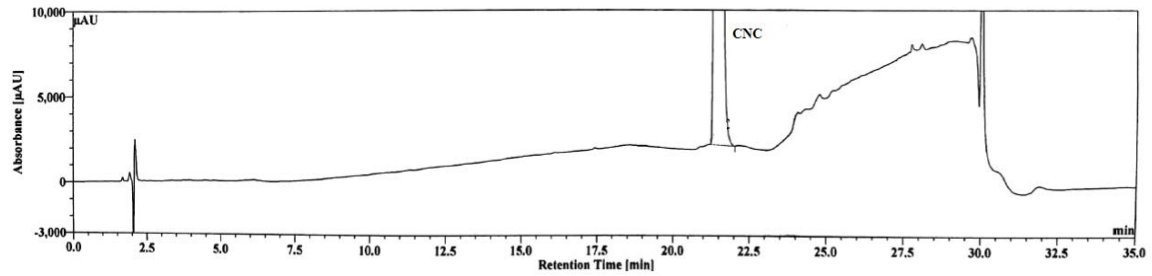


Fig. 12: Thermal degradation.

Table 7: Data for degradation study.

Stress condition	No of degradants	Assay (%)	Impurities (%)	Mass balance (assay + Total impurities) (% w/w)
Acidic degradation	3	99.97	0.022	99.99
Alkaline degradation	8	99.92	0.079	99.99
Oxidative degradation	17	87.60	12.39	99.99
Hydrolytic degradation	2	99.98	0.013	99.99
Photolytic degradation	2	99.98	0.018	99.99
Thermal degradation	0	100	0	100

CONCLUSION

As there are very few methods for the estimation of cinacalcet hydrochloride 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 for the detection of related substances of cinacalcet hydrochloride was developed and validated. The method is sufficiently sensitive to detect the related substance of cinacalcet hydrochloride in API compared to the literature studies. 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 related substances in API.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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