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Development of Validated Stability-indicating HPLC Method for the determination of Cinacalcet hydrochloride and its impurities

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Abstract: A reversed phase high performance liquid chromatographic method was developed for the determination of Cinacalcet hydrochloride in bulk drug and its related substances. The purpose of the present study was to develop a simple, cost effective, validated and stability-indicating method for the quantitative determination of Cinacalcet hydrochloride (CNC) and its impurities. The chromatographic separation was achieved in binary gradient mode on a C18 stationary phase and the elution was monitored through a PDA detector at a wave length of 210 nm. The proposed method was validated with respect tospecificity, precision, sensitivity, linearity, range, accuracy and robustness studies. The developed method was found to be sensitive to determine the content of all the impurities including degradation products. Degradation studies were carried out on CNC in acidic, basic as well as oxidation and photolytic media. While there was considerable degradation in oxidative atmosphere, it was rather less in other media. The mass balance study in each case was found more than 94.6% providing the stability indicating power of the method. Under the optimum conditions the system suitability parameters such as tailing factor and resolution were found to be within acceptable level. Linearity studies were demonstrated a good correlation between concentration and responses of impurities with a regression coefficient of greater than 0.98.

Keywords: Cinacalcet hydrochloride; RP-HPLC; Degradation studies; Validation; Stability Indicating.

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1. Introduction

Cinacalcet hydrochloride described chemically as(*R*)-*N*-[1-(1-naphthyl)ethyl]-3-[3-trifluoromethyl) phenyl] propan-1-amine hydrochloride has an empirical formula $C_{22}H_{23}ClF_3N$ and a molecular weight 393.873 g/mol.Cinacalcet hydrochloride is a selective calcimetic agent, which acts on a calcium-sensing receptor of the parathyroid gland. This principal negative regulator of parathyroid hormone release increases its selectivity to activation by extracellular calcium thus decreasing parathyroid hormone levels [1, 2]. Cinacalcet hydrochloride is effective in Clinical setting and it has been approved for the treatment of secondary hyperthyroidism in patients with chronic kidney disease placed on dialysis [3] and for the treatment of elevated levels in patients with parathyroid carcinoma [4].

To the best of our knowledge, based on an extensive literature survey, only a few methods exist for analysis of Cinacalcet hydrochloride [5-8]. The first two methods were thin-layer [5] and HPLC [5,6]; these methods were employed for the qualitative enantiomeric separation of Cinacalcet hydrochloride enantiomers in laboratory-made racemic mixtures. The third method was HPLC with UV detection for detection of impurities in presence of Cinacalcet hydrochloride in bulk drug manufacturing [7]. The fourth method was liquid chromatography-coupled with tandem mass spectrometric detector (LC-MS-MS)[8]. A RP-HPLC method was reported using RI detector for the determination of impurities in CNC related compound of Meprobamate[9]. The proposed

method was validated in accordance with guidelines described in ICH guidelines and USP [10-12]. The present study described a simple reliable and sensitive method for the determination of Cinacalcet hydrochloride.

2. Experimental

2.1 Method development and Optimization

The study was aimed at developing a RP-HPLC method capable of eluting and resolving Cinacalcet hydrochloride and its impurities. Initial trials were done on Inertsil ODS 3V column (250 mm x 4.6 mm id., particle size 5 µm) with a mobile phase consisting of solvent A as a mixture of 10 mM of Disodium hydrogen orthophosphate (Na_2HPO_4) and 10 mM of Sodium hydrogen orthophosphate (NaH_2PO_4)and solvent B as a 70:30 mixture of acetonitrile and water. Elution was performedwith a linear binary gradient (time, (min)/ % solution B): 0/20, 3/20, 8/80, 25/80, 30/20, 35/20 at flow rate 1.0 $mL.min^{-1}$. Longer retention times and poor peak shape of Cinacalcet hydrochloride was problem with the above method. Different columns such as Zorbax XDB C-18, Hypersil BDS C18 and X-Terra RP-8 columns (250 mm x 4.6 mm id., particle size 5 µm) and different buffers such as potassium dihydrogen phosphate, Trifluoroacetic acid, Ammonium acetate were also tried with different gradient methods to achieve the best chromatographic separation. But longer retention times and poor peak shapes were still unavoidable. With the addition of 0.1 % trifluoroacetic acid to the mobile phase, imp-C and imp-D were co-eluting and retention times were long. The peak shapes significantly improved but imp-B and imp-C are co-eluting. Though better peak shapes were observed with Zodiac-C18 and obtained using a mobile phase consisting of solution A as 10 mM of KH_2PO_4alone some impuritieswere co-eluting with Cinacalcet hydrochloride peak and hence the method required further modifications. Addition of 10mM of K_2HPO_4 into the mobile phase solution A by keeping the solution B as the same above, showed an improvement in peak shape as well as good separation between impurities and CNC. The percentage of acetonitrile played a key role in the retention times and resolution between impurities. After many logical trials, the final chromatographic conditionswere established as described in the following section using these optimized conditions, Cinacalcet hydrochloride and its known impurities were well separated with a USP resolution of greater than 2.0.

2.1.1. Chemicals

Samples of Cinacalcet hydrochloride and its related substances (Fig-1 and Fig-2) were obtained from in-house laboratory, Symphony Pharma Life Sciences Pvt. Ltd., Hyderabad, India. HPLC grade Acetonitrile, analytical reagent grade Potassium dihydrogenphosphate and Dipotassium orthophosphate were purchased from Merck, Darmstadt, Germany. High purity water was prepared using water purification system from TKA Instruments. Japan. All impurity samples used in this study were of greater than 98.0% purity and CNC was of >99.5% purity.

Fig-2.Chemical structures of impurities (Imp-A to Imp-E) are shown below (Fig.2a-2e)

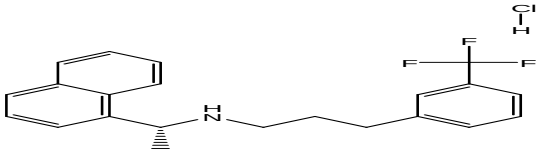
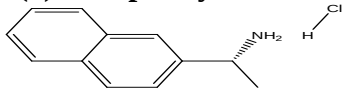


Figure.I. Chemical structure of Cinacalcet hydrochlorideis(CNC)

Chemical Name: (R)-N-[1-(1-naphthyl) ethyl]-3-[3-(trifluoromethyl) phenyl] propan-1-amine Hydrochloride

Molecular Formula: $C_{22}H_{23}ClF_3N$Molecular Weight: 393.873 g/mole

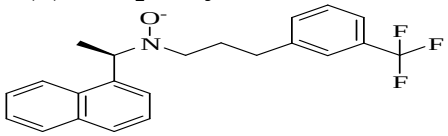
(a) Impurity-A:



Chemical Name: (R)-(+)-1-(2-Naphthyl) ethylamine Hydrochloride

Molecular Formula: $C_{12}H_{14}ClN$Molecular Weight: 207.699 g/mole

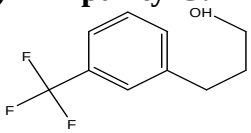
(b) Impurity-B:



Chemical Name: N-(1-Naphthalen-1-yl-ethyl)-N-[3-(3-trifluoromethyl-phenyl)-propyl]-hydroxylamine-N-oxide

Molecular Formula: $C_{22}H_{22}F_3NO_2$Molecular Weight: 389.411 g/mole

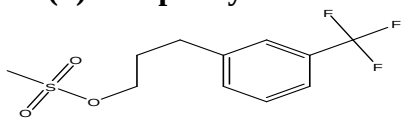
(c) Impurity-C:



Chemical Name: 3-(trifluoromethyl) benzyl propanol

Molecular Formula: $C_{11}H_{13}F_3O$Molecular Weight: 218.216 g/mole

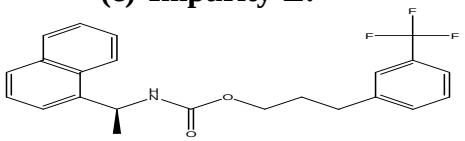
(d) Impurity-D:



Chemical Name: 3-(3-(trifluoromethyl) phenyl) propyl methanesulfonate

Molecular Formula: $C_{11}H_{13}F_3O_3S$Molecular Weight: 282.279 g/mole

(e) Impurity-E:



Chemical Name: 3-(3-(trifluoromethyl) phenyl) propyl-1-(naphthalen-5-yl) ethylcarbamate

Molecular Formula: $C_{23}H_{21}F_3NO_2$Molecular Weight: 400.414 g/mole

2.2.Equipment

The HPLC system, used for method development, forced degradation studies and method validationsconsisted of Waters 2695 binary pump with an auto sampler and a 2996 photo diode array detector. The output signal was monitored and processed using Empower software, (Waters Corporation, MA, USA) on a Pentium computer (Digital equipment Co). Water bath equipped with temperature controller was used to carry out degradation studies for all solutions. Photo stability studies were carried out using a photo stability chamber (Mack Pharmatech, Hyderabad, India).

2.3. Chromatographic conditions

The chromatographic column was used to Zodiac-C18 column (250 x 4.6 mm, 5 µm particlesize). The mobile phase A consisted of a mixture of 10 mM Potassium dihydrogen phosphate (KH_2PO_4) and 10 mM Di-potassium orthophosphate (K_2HPO_4) and mobile phase B consistedof 70:30 mixture of acetonitrile and water. The flow rate was kept at 1.0 mLmin^{-1}. The HPLC gradient program was set as: time (min) vs. % solution B: 0/30, 3/30, 20/85, 35/85, 40/30 and 45/30. The column temperature was maintained at 40°C and the detection was monitored at a wavelength of 210 nm. The injection volume was 10 µL. A mixture of equal volumes of Acetonitrile and Methanol (50:50) (v/v) was used as diluent.

2.4.Preparation of System suitability solution

As a first step, impurities stock solution was prepared by dissolving approximate quantities of each impurity in diluent and diluting with the same solvent to obtain a concentration of 75 µg.mL^{-1} of each impurity.

In the next step, System suitability solution was prepared by adding 1mL of impurity stock solution to a 50 mL solution of CNC standard solution contains 1 mg.mL^{-1} concentration.

2.5.Analytical Method Validation

The developed chromatographic method was validated for specificity, precision, sensitivity, linearity, accuracy, robustness, solution stability and mobile phase stability parameters.

2.5.1. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used.

The specificity of the Cinacalcet hydrochloride concentration 1.0 mg/mL in the presence of its impurities concentrations 0.0015 mg/mL namely imp-A, imp-B, imp-C, imp-D and imp-E and degradation products were determined by developed HPLC method. Forced degradation studies were performed on Cinacalcet hydrochloride

to provide an indication of the stability indicating property and specificity of the proposed method [13-14]. The stress conditions were employed for degradation study included light (carried out as per ICH Q1B), acid hydrolysis (0.1N HCl), base hydrolysis (0.1N NaOH) and oxidation (3% H_2O_2). For the purpose of degradation in the sample was kept 1.2 million Lux hours at whereas for acid, base and peroxide hydrolysis the test period was 48 hrs. Peak purity of stressed samples of Cinacalcet hydrochloride was checked by using 2996 Photo diode array detector of Waters Corporation, MA, USA.

2.5.1.1 Degradation of Acidic solution

The drug was exposed to 0.1N HCl at 70°C for 3 h Cinacalcet hydrochloride has shown significant sensitivity towards the treatment of 0.1N HCl. The drug gradually undergone degradation with time in 0.1N HCl and prominent degradation was observed (2%).

2.5.1.2 Degradation of Basic solution

The drug was exposed to 0.1N NaOH at 70°C for 3 h Cinacalcet hydrochloride has shown significant sensitivity towards the treatment of 0.1N NaOH. The drug gradually undergone degradation with time in 0.1N NaOH and prominent degradation was observed (4%).

2.5.1.3 Degradation of Oxidative conditions

The drug was exposed to 3% hydrogen peroxide at 70°C for 3 h Cinacalcet hydrochloride has shown significant sensitivity towards the treatment of 3% hydrogen peroxide and the drug gradually undergone prominent degradation (3%).

2.5.1.4 Degradation of Photo Stress Stability conditions

The drug was exposed to 1.2 Million Lux hours. Cinacalcet hydrochloride has shown significant sensitivity towards the illumination of UV light and the drug gradually undergone prominent degradation (5%).

The assay studies were conducted for stress samples (at 40µg.mL^{-1}) against a qualified reference standard of Cinacalcet hydrochloride. The mass balance results (%assay + % sum of all impurities + % sum of all degradants) were calculated for all stressed samples and found to be more than 97%. The purity and assay of Cinacalcet hydrochloride was unaffected by the presence of its impurities and degradation products, which thus confirms the stability-indicating power of the developed method.

2.5.2. Precision

The precision of the related substance method was checked by injecting six individual preparations of (1000 µg.mL^{-1}). Cinacalcet hydrochloride spiked with 0.15 % each imp-A, imp-B, imp-C, imp-D and imp-E (1.5µg.mL^{-1}). Intra-day Precision study was also performed.

The intermediate precision (ruggedness) of the method was also evaluated using different analyst, different column and different instrument in the same laboratory.

2.5.3. Sensitivity (LOD and LOQ)

Sensitivity was determined by establishing the Limit of Detection (LOD) and Limit of Quantification (LOQ) for imp-A, imp-B, imp-C, imp-D and imp-E estimated at signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study was also carried out at the LOQ level by injecting six individual preparations of imp-A, imp-B, imp-C, imp-D and imp-E calculated the RSD for the areas of each impurity.

2.5.4. Linearity and Range

Linearity study for related substance method was performed for impurities. The solutions were prepared at seven concentration levels from LOQ to 200 % of the permitted maximum level of the impurity. Linear regression analysis showed a good correlation between the concentrations used and the responses obtained. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses.

Linearity was checked for three consecutive days in the same concentration range for this method and calculated the RSD value of the slope and Y-intercept of the calibration curve. Upper and lower levels of range were also established.

2.5.5. Accuracy

The accuracy study of the related substance method was evaluated in triplicate from 80% to 120% of analytical concentration. The mean recovery of CNC and its impurities were calculated in Table-V.

2.5.6 Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution (Rs) between Cinacalcet hydrochloride (CNC) and its imp-A, imp-B, imp-C, imp-D and imp-E were evaluated. The flow rate of the mobile phase was 1.0

mL min^{-1}. To study the effect of flow rate on the developed method, 0.2 units of flow rate was changed (i.e 0.8 and 1.2 $mL.min^{-1}$). The effect of column temperature on the developed method was studied at 35°C and 45°C instead of 40°C. In the all above varied conditions, the components of the mobile phase were held constant.

2.5.7. Solution stability and Mobile Phase Stability

The solution stability of Cinacalcet hydrochloride and its processrelated impurities were carried out by leaving both spiked sample and un-spiked sample solution in tightly capped volumetric flask at room temperature for 48 hrs. imp-A, imp-B, imp-C, imp-D and imp-E was determined at every 6 hrs. intervals, up to the study period.

Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h Interval. Content of imp-A, imp-B, imp-C, imp-D and imp-E was checked in the test solutions. Mobile phasewas prepared and kept constant during the study period.

3. Results and Discussion:

3.1. Specificity

The peak purity test confirmed that the Cinacalcet hydrochloride peak was homogeneous and pure in all the stressed samples analysed. The mass balance of the stressed samples was close to 94.6%. The developed HPLC method was found to be specific in the presence of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E and its degradation products and confirms its stability indicating powerof the method. Results of forced degradation studies are shown in Table-I.

Table-I:

	Impurities formed (%)						Assay	Mass balance†
	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Total		degradation
Acid hydrolysis	0.24	ND	ND	0.53	0.15	2.31	98.81	101.12
Base hydrolysis	0.05	ND	ND	0.21	0.11	1.63	96.07	97.70
Oxidative degradation	1.37	0.04	ND	0.09	0.43	2.33	92.26	94.59
Photolytic degradation	0.07	0.09	ND	0.16	0.07	0.67	97.61	98.28

ND: Not detected†Mass balance: (%assay + % sum of all degradants)

3.2. System suitability

The system suitability parameters of Resolution and Tailing factor are shown in Table-II.

Table-II:

Sample	Name	USP resolution	(RSD)	USP tailing	(RSD)
1	Imp-A	2.27	(2.13)	1.11	(3.74)
2	Imp-B	7.12	(1.51)	1.12	(3.28)
3	Imp-C	4.59	(2.98)	1.08	(3.28)
4	Imp-D	3.15	(3.15)	1.15	(2.19)
5	Imp-E	2.29	(2.24)	1.07	(4.18)
6	CNC	7.55	(2.88)	1.16	(2.87)

3.3. Precision

The results of precision and Intermediate precision were found to be well within acceptable limits (<2%).

3.4 Sensitivity (LOD and LOQ)

The determined LOD, LOQ values for Cinacalcet hydrochloride and its impurities from A-E were reported in Table-III.

Table-III:

Parameter	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
LOD (µg.mL^{-1})	0.04	0.04	0.03	0.03	0.04
LOQ (µg.mL^{-1})	0.12	0.11	0.09	0.12	0.11

3.5.Linearity and Range

Linear calibration plot was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for imp-A, imp-B, imp-C, imp-D and imp-E. The correlation coefficient obtained was greater than 0.98 for all five impurities. The results show an excellent correlation existed between the peak area and concentration of imp-A, imp-B, imp-C, imp-D and imp-E.

At all concentration levels, standard deviation of peak area was significantly low and RSD was below 1.0%. Analysis of residuals indicated that residuals were scattered within ±2% with respect to 100 % concentration response.

Linearity was checked for related substances over the same concentration range on three consecutive days and the RSD of the slopes and Y-intercept of the calibration plots were with in 2.3 and 5.0 respectively. The range of the method was found from LOQ to 0.3% of the analyte concentration (100 $\mu g.mL^{-1}$). The results are represented in Table-IV.

Table-IV:

Parameter	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E
Regression equation (γ)					
Slope (b)	10374292	94218762	42875531	31857794	26472089
Intercept (a)	31	-433	-113	-7	-106
Correlation coefficient	0.9897	0.9893	0.9886	0.9815	0.9824
Repeatability (RSD)	0.3	0.2	0.6	0.5	1.3
Intermediate precision (RSD)	0.2	0.4	0.5	0.6	1.1

* Linearity range is LOQ to 200% with respect to specification limit, 0.15%

3.6.Accuracy

The percentage recovery of Cinacalcet hydrochloride in bulk drug samples ranged from 99.4% - 99.8%. The recovery of the five impurities in Cinacalcet hydrochloride active pharmaceutical ingredients ranged from 97.2 to 100.7%. The percentage recovery of the impurities and Cinacalcet hydrochloride are listed in Table-V.

Table-V:

Concentration Levels*	CNC Mean (RSD)	Imp-A Mean (RSD)	Imp-B Mean (RSD)	Imp-C Mean (RSD)	Imp-D Mean (RSD)	Imp-E Mean (RSD)
80%	99.4 (0.41)	100.4 (1.11)	100.3 (0.47)	97.2 (0.86)	99.9 (0.23)	99.3 (0.82)
100%	99.8 (0.46)	100.7 (1.16)	100.7 (0.31)	99.5 (0.54)	99.1 (0.11)	99.5 (1.56)
120%	99.6 (0.46)	100.2 (1.13)	100.6 (0.34)	99.7 (0.61)	99.7 (0.16)	99.9 (1.19)

*Amount of five impurities spiked with respect to specification limit, 0.15%

3.7.Robustness

Close observation of the analysis results for deliberately changed chromatographic conditions (different flow rate and different column temperature) revealed that the resolution between closely eluting impurities, namely imp-A, imp-B, imp-C, imp-D and imp-E were always greater than 2.0. The resolution values are presented in Table-VI.

Table-VI:

Flow rate ($mL\ min^{-1}$)	Temperature (°C)	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	CNC
1.0	40	2.27	7.12	4.59	3.15	2.29	7.55
0.8	40	2.16	6.24	4.12	3.52	2.37	7.24
1.2	40	2.22	7.05	4.37	3.38	2.41	7.62
1.0	35	2.24	6.64	4.28	3.11	2.26	7.57
1.0	45	2.08	6.72	4.47	3.27	2.46	7.38

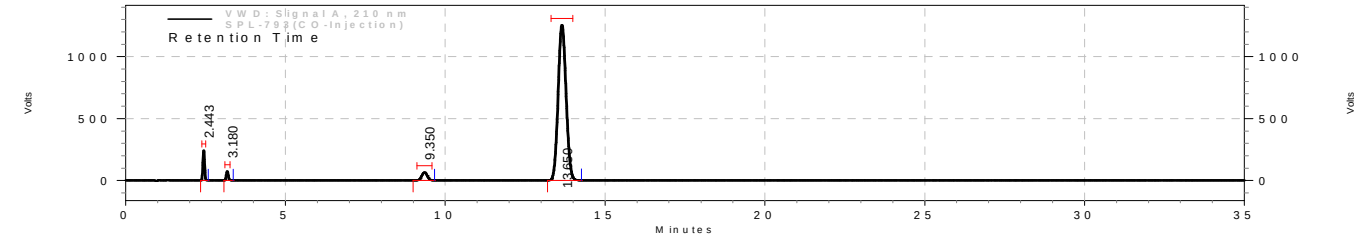
*USP resolution were calculated between two adjacent peaks

3.6.Solution stability and Mobile Phase Stability

The RSD of Cinacalcet hydrochloride during solution stability and mobile phase stability experiments were within 1.0%. No significant changes were observed in the content of imp-A, imp-B, imp-C, imp-D and imp-E during solution stability and mobile phase stability experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during related substance determination were stable up to the study period of 48h. The optimized and degradation studies chromatograms were shown below.

Figure-3: Different column trials and forced degradation chromatograms

A



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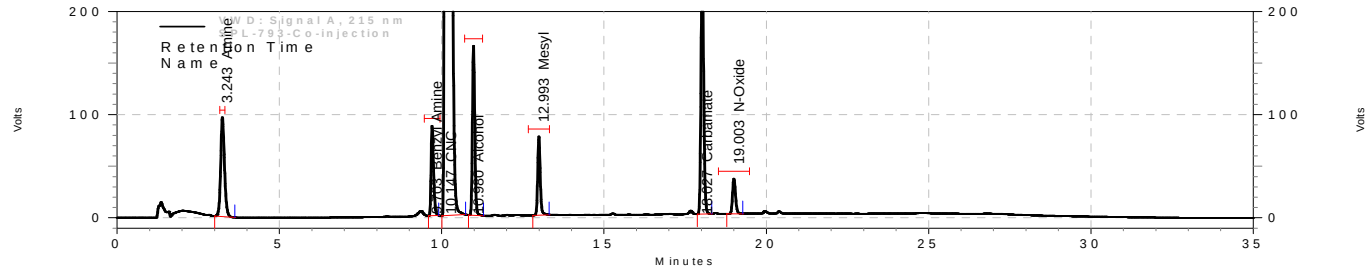

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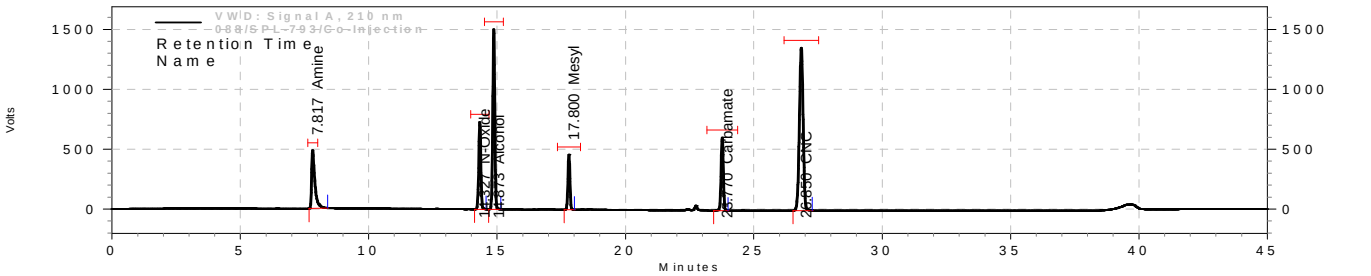

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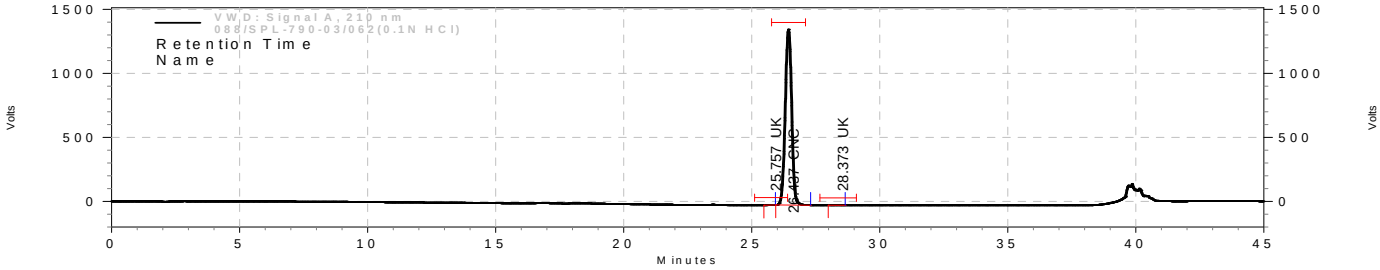

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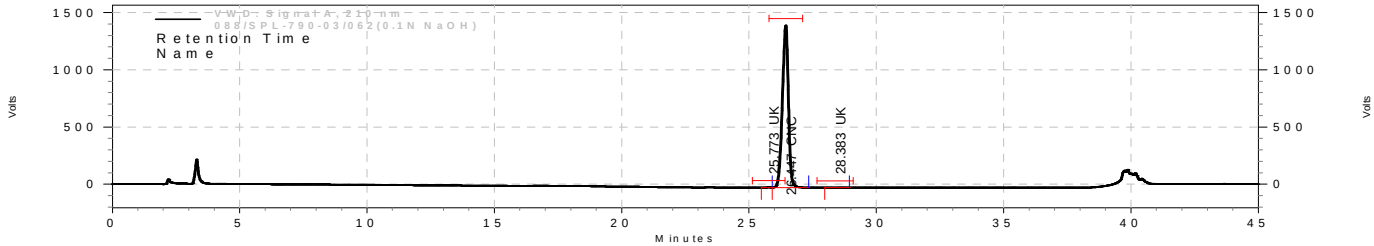

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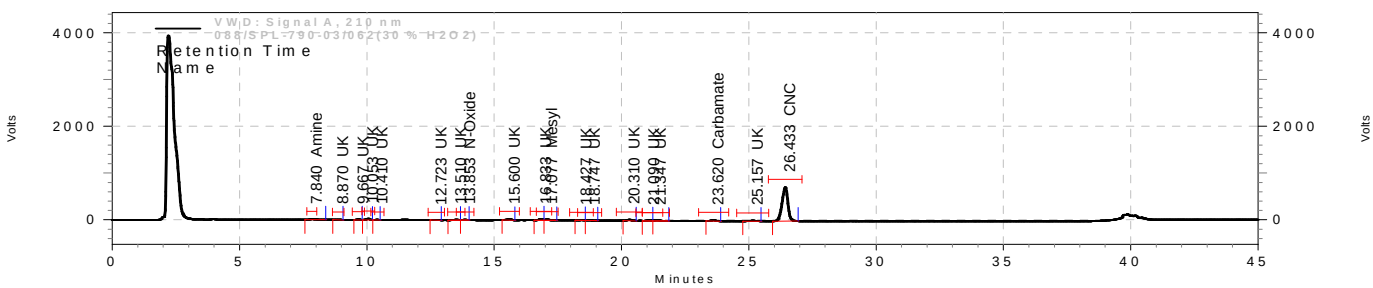
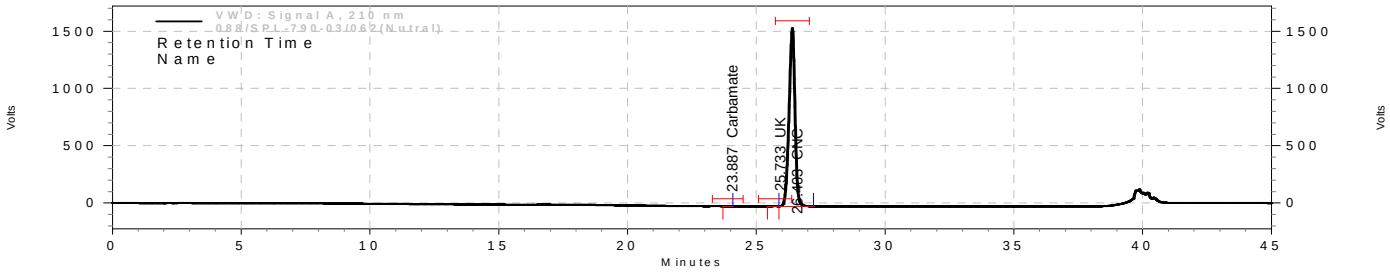


G



A. CNC spiked chromatogram with an Inertsil ODS-3V column (250 x 4.6 mm, 5.0 µm)
B. CNC spiked chromatogram with an Zorbax XDB C-18 column (250 x 4.6 mm, 5.0 µm)
C. CNC spiked chromatogram with impurities A, B, C, D and E (Final conditions)
D. Acid degradation chromatogram
E. Base degradation chromatogram
F. Oxidative degradation chromatogram
G. Photo degradation chromatogram

4. Conclusion

The developed RP-HPLC method for the quantitative analysis of Cinacalcet hydrochloride and related substances in active pharmaceutical ingredients was found to bespecific, precise, accurate, linear and robust. Satisfactory results were obtained during validation of the method. This method exhibited excellent performance in terms of sensitivity and speed. The method is found to be stability-indicating and can be used for routine analysis of production samples and to check the stability of samples.

5. Acknowledgments

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