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A VALIDATED GC-MS METHOD FOR THE DETERMINATION OF 3-(TRIFLUOROMETHYL) CINNAMALDEHYDE CONTENT IN CINACALCET HYDROCHLORIDE DRUG SUBSTANCE

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ABSTRACT
A specific GC-MS method has been developed and validated for the determination of 3-(Trifluoromethyl) cinnamaldehyde (TFMCA) content in Cinacalcet hydrochloride (CCHC) drug substance. Efficient chromatographic separation was achieved on DB-1 column (30 m x 0.32 mm, 1.0 µm), consists of 100% dimethyl polysiloxane material as stationary phase by passing helium as carrier gas. Dichloromethane (DCM) is used as diluent and TFMCA, CCHC were dissolved in DCM and monitored by gas chromatography electron ionization mass spectrometry (GC-EI-MS) with selective ion monitoring (SIM) mode. The mass fragments (m/z) selected for TFMCA quantification was m/z 131 and m/z 199 was the qualifier ion for the analysis. The performance of the developed method was assessed by evaluating specificity, linearity, sensitivity, precision and accuracy. The established limits of detection (LOD) and limits of quantification (LOQ) values for TFMCA was 0.0166 µg mL-1 and 0.0332 µg mL-1 respectively. The correlation co-efficient value of linearity experiment was 0.9997 and the average recovery was 111.7%. The results proved that the method is suitable for the determination of TFMCA content in Cinacalcet hydrochloride and method can be successfully applied for the quality control analysis of Cinacalcet hydrochloride drug substance.

KEYWORDS: Gas chromatography, Cinacalcet hydrochloride, Validation, Development, Content.

1. INTRODUCTION
Cinacalcet hydrochloride (CCHC) is the first food and drug administration approved calcimimetic drug, which act by increasing the sensitivity of calcium-sensing receptors in the parathyroid gland.[1] Chemically, it is known as (R)-N-(3-(3-(Trifluoromethyl) phenyl) propyl-1-(1-naphthyl)ethylamine hydrochloride. The molecular formula of CCHC is $C_{22}H_{22}F_3N.HCl$ and the molecular weight is 393.9. Cinacalcet hydrochloride is an oral calcimimetic drug and used for the treatment of secondary hyperparathyroidism in patients on dialysis with end-stage renal disease and in patients with parathyroid carcinoma to reduce hypercalcemia. It is also effectively works for improving control of parathyroid hormone, serum calcium, phosphorus and calcium-phosphorus product.[2]

In the synthesis process of CCHC drug substance, 3-(Trifluoromethyl) cinnamaldehyde (TFMCA) was used as a key raw material. This organic residual impurity (TFMCA) may come through the manufacturing process of CCHC drug substance. Based on structural alert, TFMCA comes under genotoxic category, which can directly or indirectly damage DNA cell and causes mutagenesis. The European Agency for the Evaluation of Medicinal products (EMEA), United States Food and Drug Administration (USFDA) and ICH Q3A/B issued the guidelines and draft guidance on the limitation of genotoxic impurities in pharmaceutical ingredients [3-5]. As per threshold of toxicological concern (TTC) approach, TFMCA residual genotoxic impurity should be <4 µg g^{-1} based on the maximum daily dosage of cinacalcet 0.36 g. The levels of TFMCA in CCHC drug needs to be monitored and controlled with appropriate methods for the quality of the CCHC drug. In the available literature, few analytical procedures have been reported for the estimation of cinacalcet and its process related impurities in CCHC drug substance and CCHC tablet formulations, human urine and in human plasma samples.[6-15]

A stability-indicating RP-UPLC method was reported for the estimation of cinacalcet impurities in cinacalcet hydrochloride drug substance and its formulations by using Acquity BEH Shield RP18 column (100 mm x 2.1 mm, 1.7 µm) with the mobile phase containing pH 6.6 phosphate buffer and detection wavelength at 223 nm.[6] An accurate HPLC method was established for the determination of related substances in cinacalcet hydrochloride using Waters C_{18} column (250 mm × 4.6 mm, 5 µm) with the gradient mobile phase consisted of acetonitrile and phosphate buffer pH 6.5 and UV detection wavelength at 210 nm.[7] Another RP-HPLC method was developed and subsequently validated for the determination of cinacalcet hydrochloride and its process-related impurities under gradient conditions with UV detection was performed at 223 nm.[8] A content method was also presented for the determination of cinacalcet in its tablets by HPLC with the detection wavelength at 272 nm.[9] Further, a stability-indicating LC method was reported for the determination of cinacalcet hydrochloride drug as well as in tablet formulations.[10] A validated UPLC-MS/MS method was reported for the determination of cinacalcet in human plasma.[11] A quantitative RP-HPLC method with fluorescence detector was reported for the determination of cinacalcet metabolites in human urine[12] and also a rapid, economical micro method for quantifying cinacalcet plasma concentrations by LC-MS/MS.[13] In additionally, a sensitive and selective HPLC-MS/MS method was reported to determine the cinacalcet hydrochloride in human plasma.[14] Finally, the best electrophoretic method was presented for the determination of cinacalcet hydrochloride within 5 min in a deactivated fused silica capillary column and electrolyte solution consisted of phosphate buffer (50 mM, pH 6.4) and Methanol in the ratio of 95:5 v/v by capillary electrophoresis using a photodiode array detector at 220 nm.[15]

The above all reported analytical methods are described about for the determination of cinacalcet and its related substances but not for the determination of TFMCA content in CCHC drug. To the best of our knowledge, no report has been published on the analysis of 3-(Trifluoromethyl) cinnamaldehyde (TFMCA) in cinacalcet hydrochloride drug substance. The aim of this work is to develop a simple and sensitive method for the analysis of this impurity using the GC–EI-MS method with SIM mode.

2. MAERIALS AND METHODS

2.1 Instrumentation, reagents and chromatographic conditions

The analysis was carried on Agilent GCMS-5977A gas chromatograph equipped with an AOC-5000 combipal auto sampler and a data handling system with HPCHEM solution software. Agilent J&W DB-1, (30 m x 0.32 mm, 1.0 µm) column that consists of 100% dimethyl polysiloxane material was used as stationary phase. High purity helium gas was used as the carrier gas with the column flow 1.5 mL/min. The initial column oven temperature of 80°C is maintained for 2 min and then increased to 230°C at a rate of 10°C/min, followed by holding at 230°C for 5 min. The run time was fixed as 22 min. The injection volume is 1.0 µL with a split ratio set at 5:1. The capillary injector temperature was 250°C, MS Parameters (For Agilent model No. 5977A): MS source = 230°C; MS quad = 150°C; MSD transfer line = 250°C; Detector voltage = Delta EMV. The analytes are monitored by gas chromatography electron ionization mass spectrometry (GC-EI-MS) with selective ion monitoring (SIM) mode. The mass fragments m/z 131 was selected for quantification and m/z 199 was the qualifier ion for TFMCA. Timed MS Detector: The MS must be ‘Dector Off after 12.5 min. The samples of CCHC drug substance and TFMCA reference standard of was procured from APL Research Centre-II (A division of Aurobindo Pharma Limited). HPLC grade Dichloromethane was procured from Merck limited. The structures of Cinacalcet hydrochloride, 3-(Trifluoromethyl) cinnamaldehyde and Mass spectrum of 3-(Trifluoromethyl) cinnamaldehyde are shown in Fig. 1.

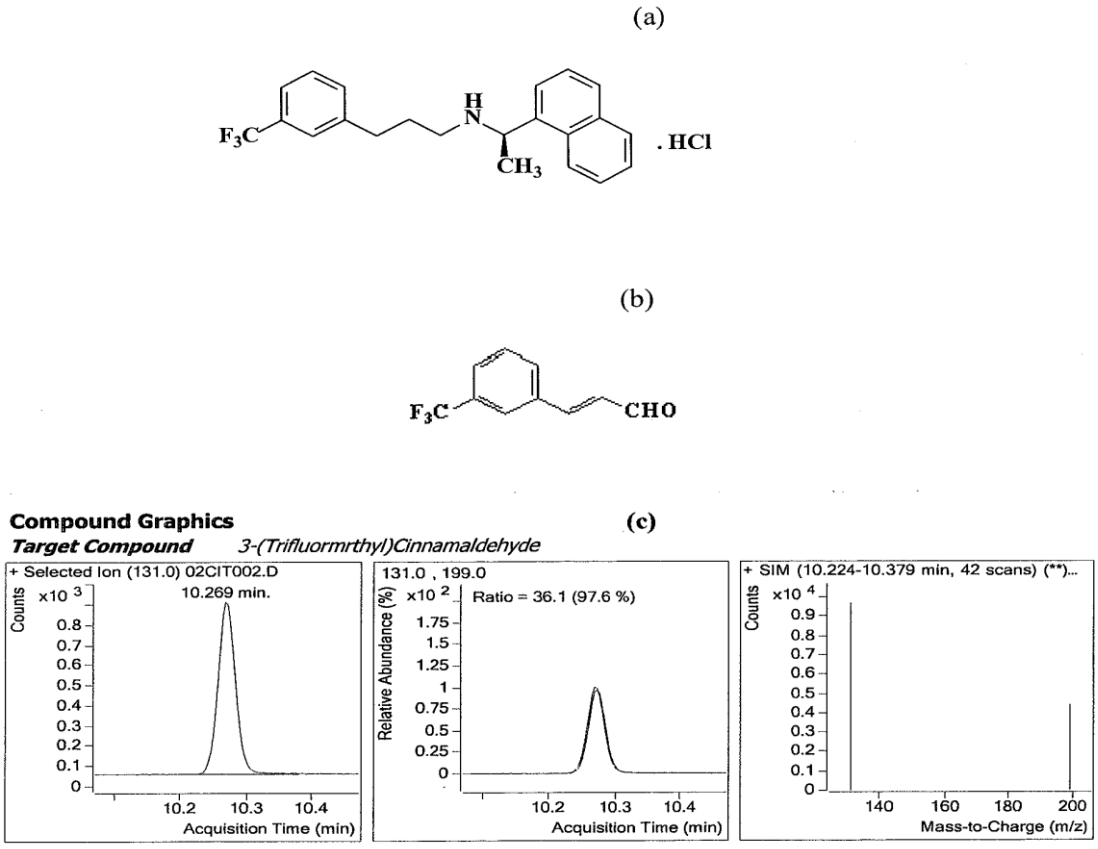

Fig. 1. The structures of (a) Cinacalcet hydrochloride, (b) 3-(Trifluoromethyl) cinnamaldehyde and and (c) Mass spectrum of 3-(Trifluoromethyl) cinnamaldehyde

2.2 Preparation of standard and sample solutions

Dichloromethane (DCM) solution was used as blank solution. A standard solution was prepared by appropriate weighing and respective dilutions of 3-(Trifluoromethyl) cinnamaldehyde reference standard in the dichloromethane to get a concentration of 0.198 µg mL^{-1} and used as standard solution. Further, 0.150 g of cinacalcet hydrochloride sample was dissolved in 3 mL of Dichloromethane and used for analysis.

Warning: Chlorinated solvents are not "less toxic"; they are generally considered cancer suspect agents. Hence, while handling Dichloromethane solvent use safety precautions: wear protective clothing, always wear personal protective equipment such as chemical splash goggles and safety gloves. Work in preferably in an environment with a fume extraction system.

3. RESULTS

3.1 Method validation

In order to determine the content of TFMCA in Cinacalcet hydrochloride drug substance, the method was validated as per the ICH guidelines [16] individually in terms of specificity, limit of detection, limit of quantification, linearity, accuracy and precision (system precision and method precision).

3.1.1 Specificity

Specificity is the ability of the method to measure the analyte response in presence of Cinacalcet hydrochloride drug substance. For specificity determination TFMCA solution was prepared and injected into GC-MS to confirm the retention times. Later on solutions of blank, control sample (Cinacalcet hydrochloride sample) and spiked samples (Cinacalcet hydrochloride sample spiked with TFMCA) were prepared as per methodology and injected into GC-MS to confirm any co-elution with analyte peak from respective blank. Moreover, from the spiked sample injection it is confirm that the TFMCA peak is well resolved and there is no other interference (co-elution) from the sample matrix indicated that the method is selective and specific for the determination of TFMCA content in Cinacalcet hydrochloride drug substance. Typical representative overlaid chromatograms of (a) Control sample and (b) Spiked sample are shown Fig. 2.

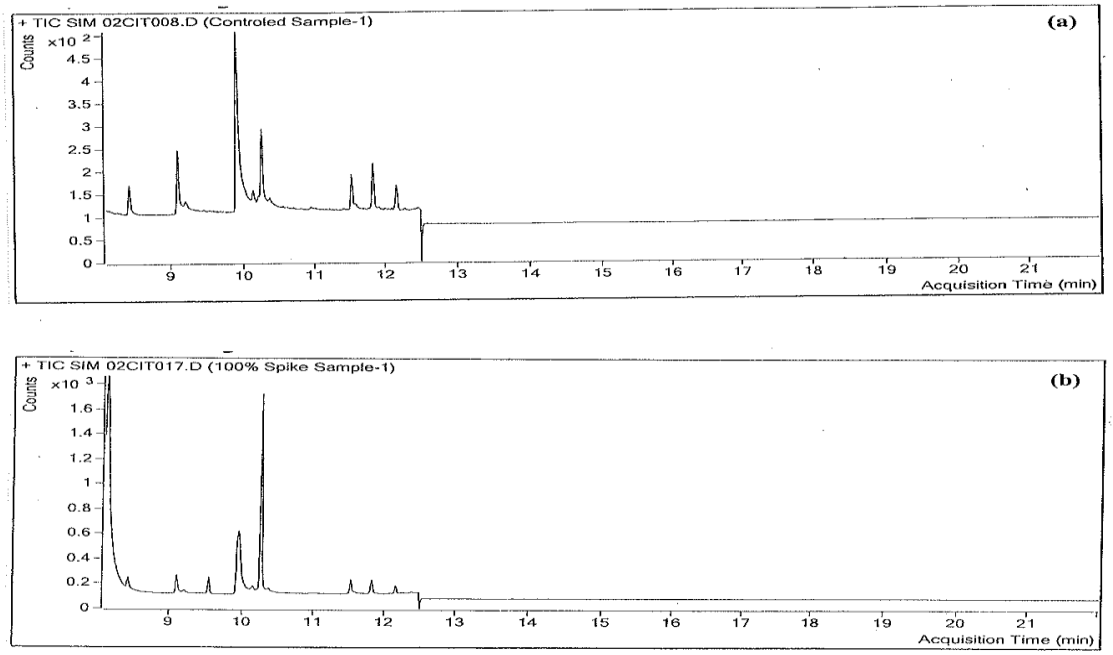

Fig. 2. Typical representative overlaid chromatograms of (a) Control sample and (b) Spiked sample

3.1.2 Limit of detection and limit of quantification (LOD and LOQ)

The LOD and LOQ values for TFMCA analyte was predicted from visual evaluation method (based on response). Each predicted concentration was verified for precision by preparing solution containing TFMCA around its quantification limit and detection limit concentrations, and injected six replicates in to GC-MS. The relative standard deviation [% RSD (n=6)] for LOQ precision of TFMCA was 1.7; for LOD precision 3.3 respectively. The overlaid GC-MS chromatograms of LOD solution and LOQ solution are shown in Fig. 3. The detailed precised LOD and LOQ values are shown in Table 1.

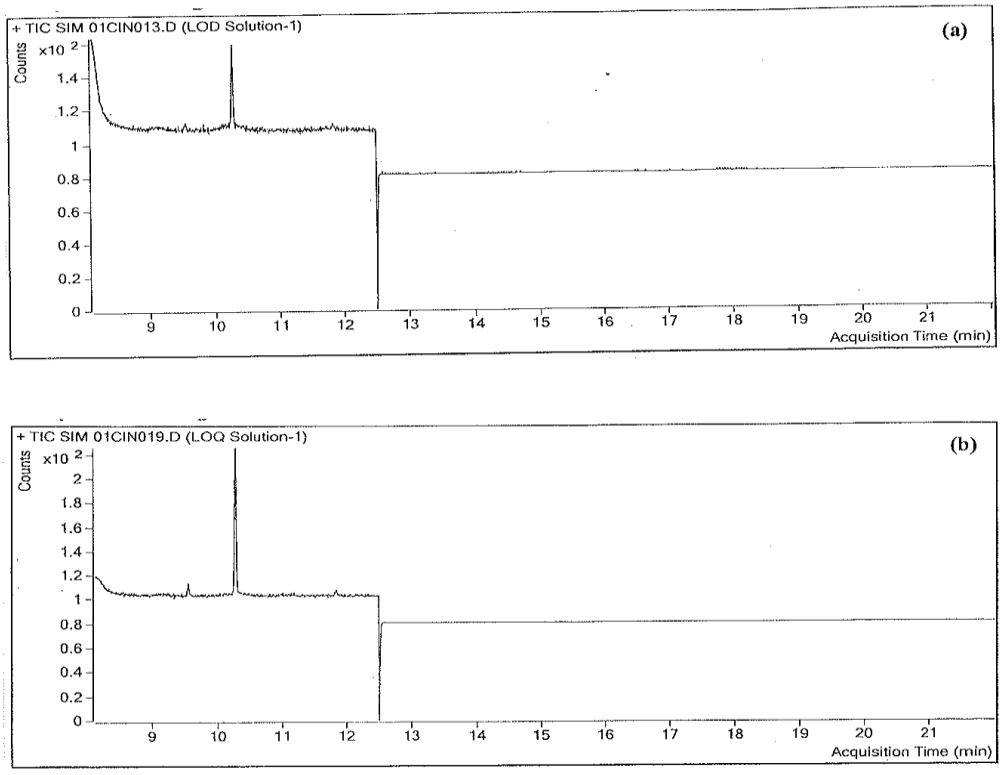

Fig. 3. Typical representative overlaid chromatograms of (a) LOD solution and (b) LOQ solution

Table 1: Statistical data of linearity, LOD/LOQ for 3-(Trifluoromethyl) cinnamaldehyde.

Statistical parameters	3-(Trifluoromethyl) cinnamaldehyde (TFMCA)
Slope	8543
Intercept	-2.3057
Residual standard on deviation response	21.9864
Correlation coefficient	0.9997
Concentration range (µg mL^{-1})	0.0198 - 0.2968
Limit of detection (µg mL^{-1})[a]	0.0166
Limit of quantification (µg mL^{-1})[a]	0.0332
Precision for Limit Of Detection (% RSD)	3.3
Precision for Limit Of Quantification (% RSD)	1.7

a: Precised LOD and LOQ values

3.1.3 Linearity

The linearity was evaluated by measuring GC-EI-MS response of TFMCA about seven different concentrations were prepared across the range concentrations were studied in the range of about 0.0198 µg mL^{-1} to 0.2968 µg mL^{-1} and injected each in single injection. The data were subjected to statistical analysis using a linear-regression model. The statistical parameters slope, intercept, residual standard on deviation and correlation coefficient values were calculated. The calculated statistical results were shown in Table 1.

3.1.4 Accuracy

Accuracy experiment was performed by spiking known amounts of TFMCA at LOQ level, 50%, 100% and 150% levels (with respect to 4.0 µg/g limit) into Cinacalcet hydrochloride drug substance. The samples were prepared, analyzed in triplicate and the percentage recoveries were calculated. The average % recovery values of four levels (LOQ, 50%, 100% and 150% levels) for twelve determinations for TFMCA was 111.7. The completely validated accuracy results were shown in Table 2.

Table 2: Accuracy data of 3-(Trifluoromethyl) cinnamaldehyde.

Identification	3-(Trifluoromethyl) cinnamaldehyde (TFMCA)			
	LOQ Level	Level-I (50% Level)	Level-II (100% Level)	Level-III (150% Level)
*Added (µg/g)	0.415	2.079	4.142	6.206
*Found (µg/g)	0.502	2.203	4.819	6.434
Recovery (%)	120.9	106.0	116.3	103.7
* % RSD	5.0	4.5	2.2	10.8

* Average of 3 replicates

3.1.5 Precision

The precision was the study of the method using reproducibility (method precision). The precision of the method was evaluated with replicate injections of standard and sample solutions. Standard solution was analyzed six times for checking the performance of the GC-MS system under test method conditions on the day tested (system precision). The repeatability of the method was studied by analyzing six sample solutions separately by adding TFMCA at known concentration levels. The precision results are shown in Table 3. A typical representative overlaid chromatograms of (a) blank and (b) standard solution are shown Fig. 4.

Table 3: Statistical data of precision.

S. No.	*Repeatability (System precision) Area in counts*	*Reproducibility (Method precision) (µg/g)*
	3-(Trifluoromethyl) cinnamaldehyde (TFMCA)	3-(Trifluoromethyl) cinnamaldehyde (TFMCA)
1	1840	4.73
2	1882	4.87
3	1818	4.85
4	1811	4.91
5	1813	4.87
6	1806	4.72
Mean	1828	4.83
SD	29	0.08
% RSD	1.6	1.7

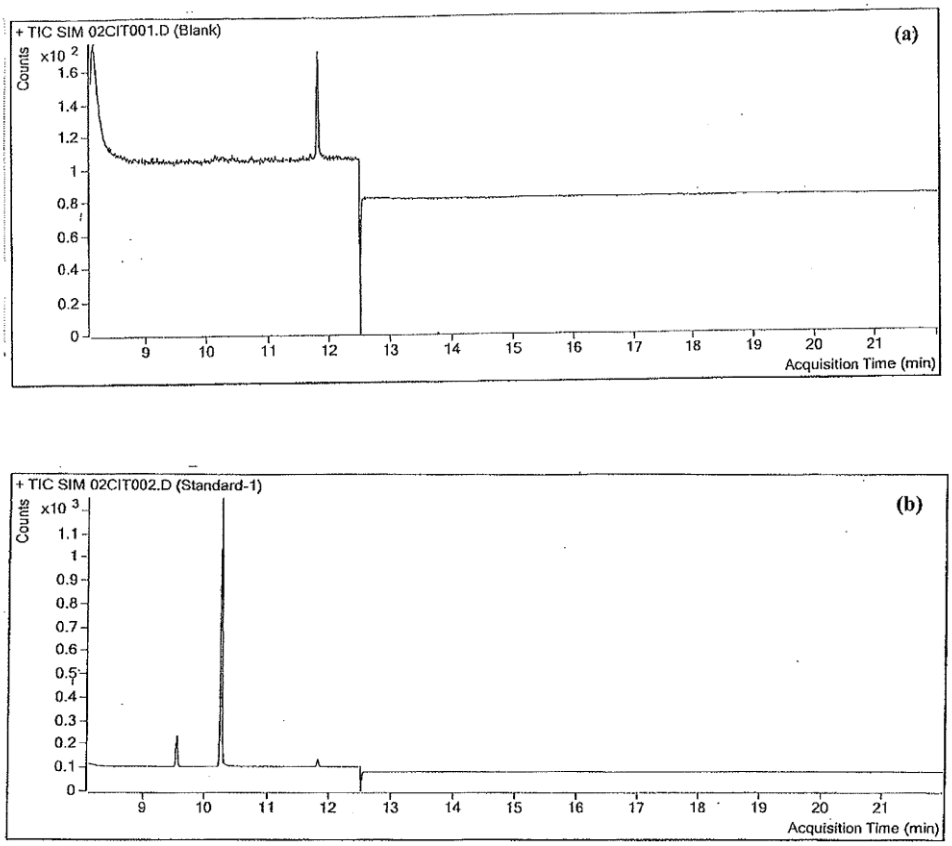

Fig. 4. Typical representative overlaid chromatograms of (a) Blank and (b) Standard

4. DISCUSSION

4.1 Method development and optimization

The objective of this work is, to evaluate the quantitative determination of 3-(Trifluoromethyl) cinnamaldehyde (TFMCA) content in Cinacalcet hydrochloride drug substance. In the synthesis process of Cinacalcet hydrochloride drug substance, TFMCA is used as key raw material and come through the manufacturing process of CCHC drug substance. Method development activity was initiated with solubility studies of Cinacalcet hydrochloride, TFMCA and taken an advantage of volatility and polar nature of Cinacalcet hydrochloride drug substance to develop the analytical chromatography method by gas chromatograph (GC) equipped with flame ionization detector (FID). We made few trails by changing different diluents and chromatographic conditions by GC with FID. Because of very poor response of TFMCA, we have chosen a gas chromatography electron ionization mass spectrometry (GC-EI-MS) with selective ion monitoring (SIM) mode. A well resolved chromatographic head space GC-EI-MS method was achieved by using DB-1, 30 m long with 0.32 mm i.d., 3.0 µm particle diameter column consists of 100% dimethyl polysiloxane as a stationary phase, helium as carrier gas, Dichloromethane as diluent and temperature of column oven is initial column oven temperature of 80°C is maintained for 2 min and then increased to 230°C at a rate of 10°C/min, followed by holding at 230°C for 5 min. Finally, satisfactory separation with better peak shapes were achieved and the method was used for validation study to evaluate its performance characteristics.

5. CONCLUSION

This study proved that a new analytical method is developed for the determination of 3-(Trifluoromethyl) cinnamaldehyde (TFMCA), a genotoxic impurity by GC-EI-MS with SIM mode at very low levels. Method validation data demonstrated that the developed method is a simple, user friendly, sensitive, specific, precise, linear and cost-effective as well as accurate for the estimation of trace quantities of TFMCA in Cinacalcet hydrochloride drug substance.

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