ECO-FRIENDLY AND STABILITY INDICATING SPECTROSCOPIC METHODS FOR CINACALCET HYDROCHLORIDE: METHOD DEVELOPMENT, VALIDATION, AND APPLICATION IN ITS PHARMACEUTICAL FORMULATIONS**

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The present paper explains the development and validation of four new eco-friendly and stability-indicating spectroscopic methods of estimating a calcimimetic drug, i.e., cinacalcet hydrochloride (CIN), in its different pharmaceutical formulations. In these methods, the response of the standard solution is measured in 0.1N HCl as a diluent (nonhazardous) at 279, 294, and 299 nm for UV, first-, and second-order derivative spectrophotometric tools, respectively. The AUC-spectrophotometric technique is carried out by calculating the area under the curve (AUC) between the wavelength regions ranging from 266 to 286 nm. The linearity graph of the methods is plotted by taking response [Absorbance/(dA/dλ)/($d^2A/d\lambda^2$) or AUC] values against the concentration and shows that the drug obeys Beer's law within the concentration range 2.5 to 200 μg/mL for all the methods. The drug is exposed to various stress conditions as recommended by the ICH Q1A-Q1E guideline. The different method validation parameters are within the acceptable limit as per the ICH Q2R(1) guideline. The greenness profile of the developed UV spectroscopic tools is evaluated using the National Environmental Methods Index, the Analytical Eco-Scale, and the Analytical GREEnness (AGREE) metric approach. All approaches prove the greenness of the methods concerning solvent, chemicals, energy consumed, and waste produced. Eco-friendly spectroscopic methods can determine the quantity of CIN in various pharmaceutical dosage forms without influencing the environment and analysts.

Keywords: *cinacalcet hydrochloride, stress degradation study, eco-friendly, AGREE, Analytical Eco-Scale, national environmental methods index.*

ЭКОЛОГИЧЕСКИ БЕЗОПАСНЫЕ И СТАБИЛЬНЫЕ СПЕКТРОСКОПИЧЕСКИЕ МЕТОДЫ ОБНАРУЖЕНИЯ ГИДРОХЛОРИДА ЦИНАКАЛЬЦЕТА

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Разработаны и проверены четыре экологически чистых и стабильных спектроскопических метода оценки кальциймиметического лекарственного средства цинакальцета гидрохлорида (CIN) в различных фармацевтических составах. Реакция стандартного раствора измерена в 0.1N HCl в качестве разбавителя (безопасного) при 279, 294 и 299 нм для УФ-спектрофотометрии и ее первой и второй производных соответственно. Спектрофотометрический метод AUC заключается в расчете площади под кривой (AUC) в диапазоне длин волн 266—286 нм. Графики линейности методов построены по значениям [абсорбция, (dA/dλ), ($d^2A/d\lambda^2$) или AUC] в зависимости от концентрации и показывают выполнение закона Бера в диапазоне концентраций 2.5—200 мкг/мл для всех ме-

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тодов. В ходе исследования препарат был подвергнут различным стрессовым воздействиям в соответствии с рекомендациями Международной конференции по гармонизации Q1A-Q1E. Проверочные параметры методов находятся в допустимых пределах согласно рекомендациям Международной конференции по гармонизации ICH Q2R(1). Экологичность разработанных УФ-спектроскопических инструментов оценена с использованием Национального индекса экологических методов, аналитической экологической шкалы и метрического подхода AGREE. Все методы доказали свою экологичность в отношении растворителей, химикатов, потребляемой энергии и образующихся отходов. Экологически безопасные спектроскопические методы позволяют определять количество CIN в различных лекарственных формах, не оказывая влияния на окружающую среду.

Ключевые слова: *цинакальцет гидрохлорид, исследование деградации под воздействием стресса, экологичность, AGREE, аналитическая экошкала, индекс национальных экологических методов.*

Introduction. Chemically, cinacalcet hydrochloride (CIN) is N-[(1R)-1-naphthalen-1-ylethyl]-3-[3-(trifluoromethyl) phenyl] propane-1-amine hydrochloride. It is used for the medication of hypercalcemia in people suffering from parathyroid carcinoma and secondary hyperparathyroidism for patients with chronic kidney disorder on dialysis [1, 2]. Analysis of any drug substance is a crucial part not only in formulation development but also in new drug development. A well-validated and eco-friendly method is essential during the investigation of drug(s) in bulk, in medication deliverance systems, in dissolution research (*in vitro*), and bio matrices (*in vivo*) without affecting the environment and analysts. Suppose that such methods for a particular need are not accessible. In that case, building a simple, precise, and accurate green method for assessing drugs in different pharmaceutical formulations becomes necessary.

A survey on literature reveals that CIN is analyzed by spectrophotometry [3–6], LC [7–11], HPTLC [12], LC-MS [13–17], and capillary electrophoresis (CE) with photodiode array detection [18]. Darwish et al. developed a colorimetric method using 1,2-naphthoquinone-4-sulphonate (NQS) as a colorimetric reagent. It is a complex and time-consuming procedure [3]. Manjula et al. [4], and Loni et al. [5], also developed a spectroscopic method where methanol or methanol/water was used as a diluent, which is not economical and cannot be used in dissolution studies. As per the EPA hazardous list (hazardous waste no. U154 and chemical abstract number 67-56-1), methanol is a hazardous solvent in terms of its inherent toxicity and disposal; hence, it is not safe for analysts and the environment [19]. Again, some reported methods [7–18] require highly sophisticated instruments (HPLC, LC-MS, and CE) that consume more energy and require trained personnel for the operation. Hence, the buildup of eco-friendly, cost-effective, and straightforward techniques for estimating CIN in various pharmaceutical formulations is called for.

In the present study, 0.1N HCl is selected as an eco-friendly, economical, and valuable diluent for dissolution studies. The required instrument is a UV-Visible spectrophotometer, which is readily available owing to its low price, it requires less energy to operate, and it requires cost-effective analytical reagent grade or spectrograde solvents. The proposed method is the UV spectroscopic or zero derivative method (method A or D^0). It is a straightforward method based on its application and analysis of the point of view. In method A, the UV absorption spectrum does not show an ideal shape, i.e., a bell-shaped curve. Hence, samples containing interfering elements such as degradation by-products, impurities, or excipients cannot be quantified precisely and accurately. Again, contaminants and degradation products are generally present in small quantities contrast to the drug. Hence, it is challenging to get an adequate response to prior treatment or extraction of the interfering component using the D^0 technique. A higher derivative method, namely second or third, is selected for superior resolution of interfering analytes. For the reasons stated above, derivative spectroscopy is recommended. Thus, the D^0 spectra are derivatized to obtain D^1 (method B: first-order derivative spectroscopic technique) and D^2 spectra (method C: second-order derivative spectroscopic technique). Again, method D (AUC or area under the curve method) is developed as the drug shows a broad spectrum. The selected wavelength range is between 266 and 286 nm for measuring AUC. Hence, to solve the above problems, the present study was carried out to develop and validate four stability-indicating and environmentally friendly UV-spectroscopic methods for CIN as per ICHQ2(R1), ICH Q1A-Q1E recommendations, and principles of green analytical chemistry [20–22].

Methodology. *Chemicals and solvents.* Our college provided the CIN reference standard (purity >98.7%). Its qualitative test is carried out by conducting its IR spectra. Hydrochloric acid (35%, w/w) of an analytical grade solution was brought from Merck Life Science Pvt. Ltd. (Mumbai, India) and employed to prepare a 0.1N HCl. Tablets of CIN were devised using a direct compression technique.

Instruments and software. UV-Vis double-beam spectrophotometer (UV-1800, Shimadzu, Japan) was used to develop methods. It is associated with computer-supported UV Probe spectra manager application software and 10-mm duplicate sample cells made of quartz. The different instrument-affecting variables are a wavelength scale of 400–200 nm; a rapid scanning speed; a sampling break of 1.0 nm; D^1, D^2 derivative modes (first- and second-order derivative, respectively); a bandwidth ($\Delta\lambda$) of 10 nm for both D^1 and D^2; a spectral slit width of 1 nm is used to obtain an absorption spectrum. All weight measurements were carried out precisely on an electronic balance (Wensar, New Delhi, India). The FT-IR spectrum of CIN was documented on IR Affinity-1 using KBr disks (Shimadzu, Japan) and handled under a dry air purge. The device stored the scan at a scanning speed of 2 mm/s with a resolution of 4 cm^{-1} over 400–4000 cm^{-1}.

Stock and calibration standard solution. CIN standard stock solution (200 μg/mL) was prepared during the study by solubilizing 20 mg of analyte in 0.1N HCl, sonicated for 5 min, and made up to 100 mL using the same solvent. A series of dilutions was created to devise the calibration standards covering 2.5–200 μg/mL, scanned over the 200 to 400 nm scale against solvent as blank, and observed λ_{max} at 279 nm (D^0). The acquired zero-order spectra were derivatized to obtain D^1, D^2 spectra, and the response ($dA/d\lambda$ and $d^2A/d\lambda^2$) of the spectra was calculated at 294 and 299 nm, respectively. The software used the wavelength range 266–286 nm to calculate AUC. Concentration (2.5–200 μg/mL) verses response linearity curves for various methods were plotted [23, 24].

Preparation of CIN tablets. CIN tablets were unavailable on the local market; hence, we prepared tablets on a laboratory scale for this study. Initially, the drug and selected excipients (Table 1) were strained by sieve no. 40 and then blended in dry by ensuing geometric dilution to establish consistent blending. The processed compounds of drug and additives were compressed into pills in a Minipress-II using a flat and round punch of 6 mm in diameter (Karnavati, India). Analysts accomplished a tablet group of 200 pills with diverse quality-control assessments.

TABLE 1. Drug and Diluent Composition for a Unit CIN Tablet Dosage Form

Constituents	Quantity, mg
Cinacalcet hydrochloride	30
Microcrystalline cellulose pH 102	38
Polyvinyl pyrrolidone K 30	20
Starch	10
Talc	1
Magnesium stearate	1
Aggregate weight	100

Evaluation tests. The various evaluation assessments followed the IP specifications [25, 26]. For the content uniformity experiment, 20 pills were put in the balance to obtain the average weight and ground into fine powder. The tablet powder comparable with 20 mg was taken in a 100-mL graduated container. At the beginning, 50 mL of 0.1N HCl was mixed, sonicated for 15 min, diluted up to the mark by 0.1N HCl, and strained using Whatman filter paper (no. 41). Proper dilution was carried out with 0.1N HCl from the sample solution. The quantity of CIN was found out using the linearity curves. The analysts determined the drug content and standard deviation (SD) of the prepared formulation.

The hardness value of CIN tablets was calculated utilizing a digital hardness tester. A tablet group (no. 10) was taken for every test, and the average hardness was determined by using the formula

$$\text{Mean hardness} = \frac{\text{Total hardness of 10 tablets}}{10}.$$

The standard hardness value is 5–8 kg/cm^2 for a conventional compressed tablet with hardly any deviations such as an effervescent pill, chewable tablets, dispersible tablets, etc. The score for sustained and controlled release pills is higher than 8–12 kg/cm^2.

A digital friabilator was used to carry out a friability test for the CIN tablets. Ten tablets were weighed and kept in the Roche friabilator, which revolved for 4 min at a rate of 25 rpm, and then their weight was remeasured. Ultimately, % friability was computed applying the formula

$$\text{Friability}(\%) = \frac{\text{Starting weight} - \text{Final weight}}{\text{Starting weight}} \times 100.$$

The highest percentage friability must be 0.5–1% for conventional compressed pills.

To conduct the weight uniformity test as per IP, 20 tablets were arbitrarily selected, weighed accurately using an electronic balance, and the weight difference (%) was resolved using the formula

$$\text{Weight uniformity} = \frac{\text{Average weight} - \text{Individual weight}}{\text{Average weight}}.$$

The allowable weight difference for pills weighing 130 mg or less should be 10%. The disintegration test was conducted by placing six tablets in six tubes (one each). A basket rack assembly was positioned in a 1 L beaker with water at 37±2°C so that CIN tablets were placed 2.5 cm below the upper level of the beaker and 2.5 cm above the bottom of the beaker through ascending and descending motion, respectively. The disintegration time was recorded when CIN tablets had disintegrated. As per IP, the disintegration time for an uncoated tablet is 15 min.

The dissolution study was conducted according to USP guidelines by taking six CIN tablets in 900 mL of 0.1 N HCl at a temperature of 37±0.5°C using the Dissolution Apparatus II (paddle method, 50 rpm). By maintaining sink conditions, 5 mL of the sample was removed at prearranged time intervals (0, 5, 10, 15, and 30 min) and strained via a Whatman filter paper. The samples were diluted with a medium and analyzed spectrophotometrically at 279 nm (D^0). The release studies were performed three times. The dissolution profile was assessed for the amount of drug liberated for the first 15 min (Q15).

Validation of the proposed methods. Validation is a technique of demonstrating written evidence that guarantees a specific action. It will persistently produce the favored result or a product that matches the defined demands and quality characteristics. The methods were validated for various criteria such as linearity, accuracy, specificity, precision, LOQ, LOD, and robustness following ICH Q2(R1) guidelines.

The Beer–Lambert range of CIN was examined over the concentration range from 2.5 to 200 μg/mL at seven points, and each solution was planned in triplicate. The standard curves for CIN were obtained by linear least-squares regression analysis by designing response (y) versus the concentration of the standard (x). The Beer–Lambert range was indicated as a correlation coefficient (R^2), and the value should be ≥0.9990. The sensitivity of the methods (LOQ and LOD) was calculated based on the SD of the slope and response of the standard curve.

The authors carried out the precision of the methods by analysis of calibration standards, which were sorted out based on the smile curve (concentration on the x-axis vs % RSD on the y-axis) at three concentrations (10, 50, and 100 μg/mL), and prepared all solutions in triplicate [27]. For the repeatability study, three duplicates of the reference samples were assessed on the same day. Three replications of the standard samples were evaluated for intermediate precision on three different days. The results are described in view of % RSD and one-way ANOVA at the 5% significance level to contrast the inter- and intraday data.

An accuracy study measured the percentage of standard drug recovered by assay method and was accomplished by adding three investigated quantities (40, 50, and 60 μg/mL; at three levels 120, 100, and 80% of sample solution) into a fixed sample solution of 50 μg/mL and prepared it in triplicate. Analysts predicted the percentage recovery of the added drug by recording the response and placing these values in the linear regression equation of linearity curves.

The authors measured the specificity of the methods by documenting the UV absorption spectra for the sample solution within the calibration range and compared it with the same strength of the standard solution. This study proves information related to the interference of excipients during sample analysis.

Two investigators conducted a robustness study by assessing aliquots from a calibration slot (50 μg/mL) in various research labs and employing two instruments under similar environmental and operational conditions. Outcomes recorded as % RSD and t test at the 5% significance level were used to correlate the data.

A stress degeneration study was carried out according to ICH Q1A-Q1E recommendations to support the stability-indicating power of the developed methods and disclose the intrinsic constancy of the active substance [28–30]. As per the guideline, the drug was exposed to multiple stressful conditions, resulting in remarkable drug degradation. The degradation percentage should remain between 5 and 30%, in contrast with nondegraded API [31]. This limit is determined; thus, significantly less degradation will happen without generating secondary products [32]. It was carried out by preparing and recording spectra of a drug solution within the linearity range. Initially, the drug solution was subjected to base hydrolysis by adding 1 mL of 0.1 M NaOH and positioning it for 30 min at 50°C. The acid hydrolysis was carried out by adding 1 mL of 0.1 M HCl and placing it for 30 min at 50°C. The reference drug was taken in a Petri dish and kept for 30 min at 50°C in a hot air oven for thermal degradation study. For the photolytic stress study, the reference drug was placed in a Petri dish and kept in a UV chamber for 30 min. Finally, the oxidative stress experiment was

carried out by adding 1 mL of 3% H_2O_2, holding at 50°C for 30 min in a water bath, and neutralizing by 3% sodium metabisulphite to find the stability of the analyte.

Results and discussion. The D^0 spectra (Fig. 1a) of CIN showed λ_{max} at 279 nm and the shape of the spectra was not bell-shaped (spectra are split at the top). Hence, to remove unwanted background absorption, subdue broad bands to sharp bands, and improve the specificity and sensitivity in qualitative and quantitative analysis of different analytes, D^1, D^2, and AUC methods are performed. In the D^1 and D^2 derivative spectra, the drug indicated three to four peaks at distinct wavelengths (Figs. 1b,c). But the experiment was conducted at 294 nm (D^1) and 299 nm (D^2), because at this wavelength, the Beer–Lambert law has shown an adequate range of linearity, deformed derivative spectra are not found, and the maximum wavelength of the peaks, as well as the zero-crossing point, remains invariable (Figs. 1e,f). The wavelength range 266–286 nm was selected for the AUC spectroscopic method by repeated observation to achieve the maximum linearity between the concentration and the AUC (Fig. 1d).

The identity of the CIN drug was confirmed by comparing the IR and reference spectra. IR spectra of the drug showed various vibrations such as ([–CH_3]: 2513.25, 2428.38 cm^{-1}; [–CF_3]: 1517 cm^{-1}; [–NH]: 2918–2962 cm^{-1}; Aromatics: (C=C): 1251, 1327, 1460 cm^{-1} (Fig. 2). This learning proves that CIN is unmixed and pure.

The solubility of CIN in various diluents such as methanol, distilled water, 0.1N NaOH, ethanol, phosphate buffer (pH 6.8), and 0.1N HCl was assessed by spectrophotometry. In methanol, the drug showed a clear solution with the maximum solubility (15.278 mg/mL), but 0.1N HCl was selected as a diluent (solubility = 8.71 mg/mL) because it was economical, eco-friendly, noncarcinogenic, and also useful for dissolution studies.

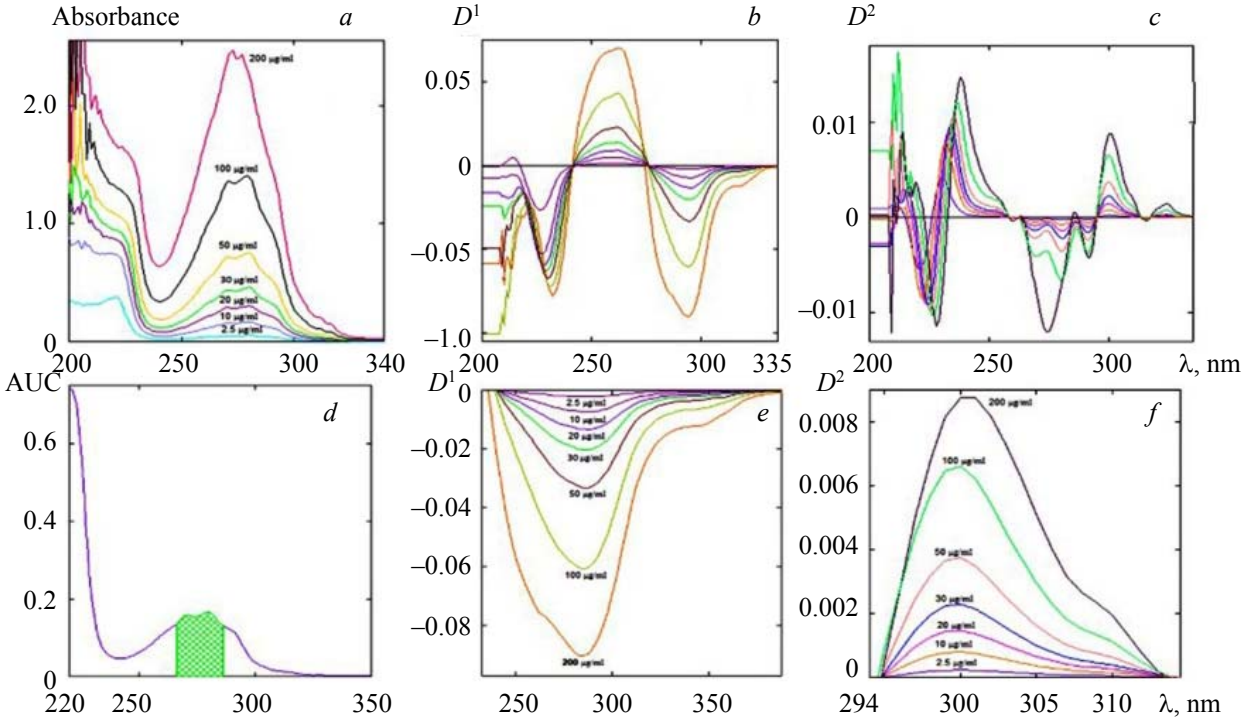

Fig. 1. Overlaid UV absorption spectrum of cinacalcet HCl in 0.1N HCl (2.5–200.0 μg/mL) (a) D^0, (b) D^1 normal view, (c) D^2 normal view, (d) AUC, (e) D^1 enlarged view, and (f) D^2 enlarged view.

From the standard plot of CIN (Fig. 3), it was noticed that there was a corresponding increase in response regarding concentration. All the proposed methods follow the Beer–Lambert law in concentrations ranging from 2.5 to 200 μg/mL. The R^2 score of 0.999 indicates that the proposed UV-spectrophotometric methods have good linearity over the suggested linearity range. The sensitivity of the methods (LOD and LOQ) was calculated, and the low value indicates that the techniques were sensitive (Table 2).

The intra- and interday precision scores of the four methods were acceptable with a % RSD value less than 2, which proves that the developed methods were reproducible. Again, one-way ANOVA was used to

statisticaly compare the intra- and interday data, and the resulting *p*-value was 0.999 (for four processes), which was greater than 0.05. F_{calc} values were less than F_{tab} value (5.143), indicating no distinction in the outcomes obtained in different days by different methods. The 5 and at 1% confidence levels were calculated for the processes, and the values were found to be less than 1.90 and 2.58 for 5 and 1%, respectively.

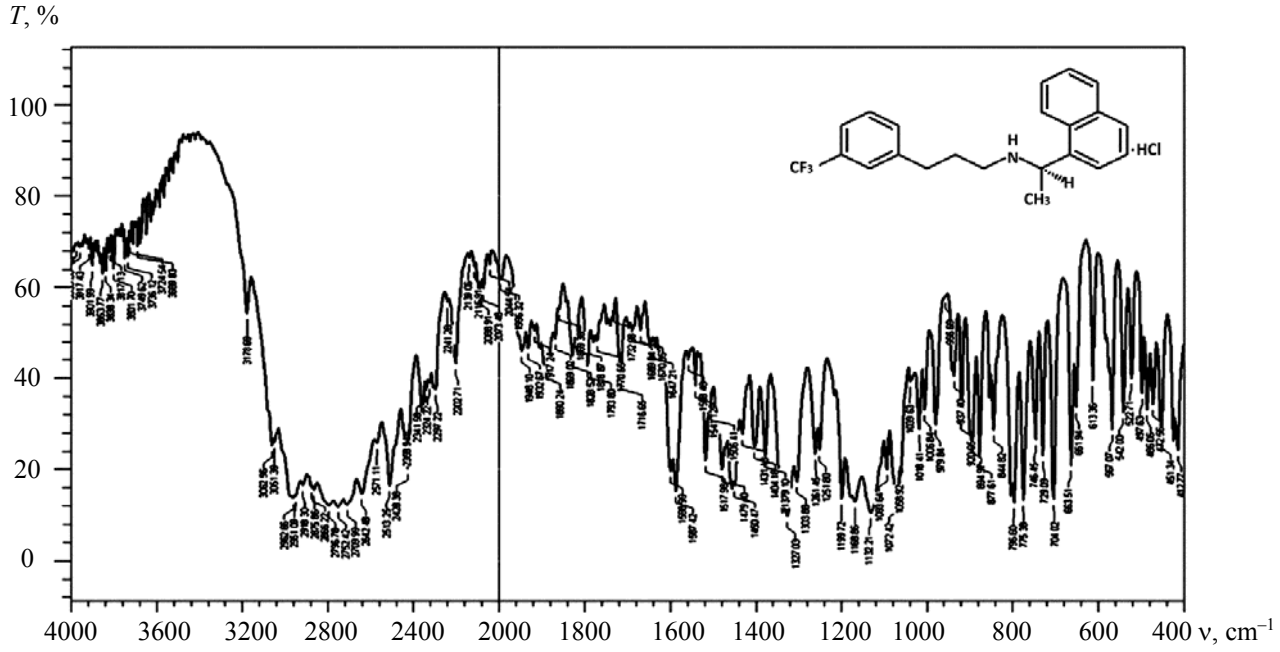

Fig. 2. FTIR spectra of cinacalcet HCl.

TABLE 2. Method Validation Parameters of CIN

Parameters	Method A	Method B	Method C	Method D
Linearity range, μg/mL	2.5−200.0	2.5−200.0	2.5−200.0	2.5−200.0
Detection wavelength, nm	279.0	294.0	299.0	266.0−286.0
Molar absorptivity±SD, L/mol · cm	6004.349± 453.594	–	–	–
Sandell's sensitivity (μg/cm^2/0.001 A)	0.066	–	–	–
Slope	0.013	−0.001	0.00007	0.261
Intercept	0.029	0.001	0.00006	0.396
Correlation coefficient (r^2)	0.999	0.999	0.999	0.999
LOD	0.486	0.459	0.514	0.408
LOQ	1.473	1.390	1.558	1.237
Precision (RSD, %) Intraday (n = 3) Interday (n = 3) F_{cal}	 0.154−0.939 0.203−0.941 0. 826×10^{-6}	 0.346−1.133 0.163−1.651 9. 888×10^{-5}	 1.322−1.894 0.675−1.917 0.00021	 0.486−1.878 0.800−1.762 0.00028
Accuracy	96.667–102.564	96.111–103.333	96.667–103.810	97.873–103.196
Assay results (100 mg/tab) % Label claimed ± SD (n = 5) %RSD	 98.978±0.121 0.122	 97.844±0.869 0.888	 99.046±0.842 0.850	 98.432±0.759 0.771
Ruggedness (50μg/mL; RSD, %) Analyst 1/Instrument 1 Analyst 2/Instrument 2	 0.641 0.674	 0.754 0.543	 0.562 0.582	 0.236 0.478

The various optical parameters of CIN were determined by the suggested methods and are presented in Table 2. The quality control variables for the prepared tablets were within the acceptable limit as per IP and USP recommendations, as shown in Table 3. The pills were analyzed, and the drug quantity was determined with the help of the developed methods and found to be nearer to the labeled claim (Table 2). To prove the specificity of the plans, absorption spectra of the sample overlapped with reference spectra, and both the spectra matched, reflecting that there was no interference from excipients.

TABLE 3. Quality Control Parameters for CIN Tablet

Sl. No.	Quality control test parameters		Results*,%
1	Weight variation, %		100±3.5
2	Drug content, %		99.5±2
3	Hardness, kg/cm²		5.1±0.2
4	Friability, %		0.3±0.002
5	Disintegration time, min		8±0.5
6	Cumulative % drug release at	15 min	27.7±2.6
		30 min	56.5±2.8
		60 min	97.4±2.3

*Mean ± SD, $n = 6$.

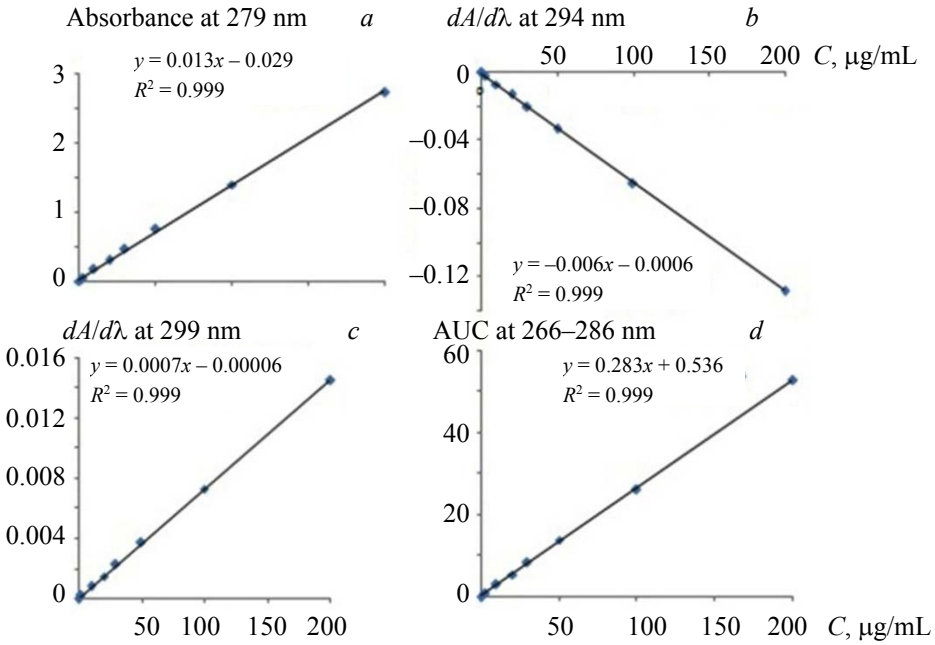

Fig. 3. Calibration curve of cinacalcet HCl in 0.1N HCl (2.5–200.0 μg/mL)
(a) D^0, (b) D^1, (c) D^2, and (d) AUC.

The accuracy of the developed methods was determined, and the outcomes are shown in Table 2. Percentage recovery shows that the developed methods were accurate, and also there was an absence of interference from the fillers. The ruggedness of the developed methods carried out and the results is presented in Table 2. The results obtained by two observers validate the ruggedness of the spectrophotometric techniques as the obtained % RSD value was less than 2.

The stress degradation experiments determined the stability-indicating capacity of the developed methods. The reference drug was subjected to different degradation conditions, and reported a decline in the drug concentration on the one hand and an increase in the level of degradation products from the other hand. The outcomes from this study are shown in Fig. 4, and the scores were within the range. The drug is more sensitive to photolytic degradation and less susceptible to alkaline medium. This experiment proved that the drug was stable under different stress conditions.

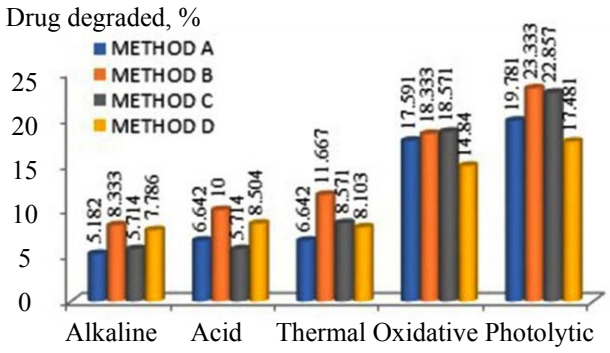

Fig. 4. Stability study data presenting % drug degradation under various stress conditions by methods A, B, C, and D.

Assessment of Greenness of the proposed and reported spectroscopic methods. There are various approaches to the evaluation of the greenness score of an analytical protocol, such as the National Environmental Methods Index (NEMI) [33, 34], Analytical Eco-Scale (AES) [35, 36], Analytical Method Greenness Score (AMGS) [37], Analytical GREEnness (AGREE) metric approach [38], Green Analytical Procedure Index (GAPI) [39], and White Analytical Chemistry (WAC) [40]. Each approach has its benefits, drawbacks, and evaluation procedure. The authors used the NEMI, AES, and AGREE metric policy in this study.

The NEMI approach has four color-coded (green and colorless) quadrants such as Persistent Bioaccumulative Toxic (PBT), Hazardous, Corrosive, and Waste as the criteria for the greenness profile. The first quadrant deals with the list of chemicals issued by the Environmental Protection Agency's (EPA's) Toxic Regulatory Inventory (TRI) list of PBT chemicals [41]. The solvent (0.1N HCl) used in these spectroscopic techniques has not been listed in PBT. Hence, the first quadrant is color-coded green. Loni et al. and Rao and Gowrisankar used methanol for method development [5, 6]. Methanol is not listed in PBT, hence the first quadrant is coded green. The second quadrant explains hazardous chemicals listed under the EPA's Resource Conservation and Recovery Act (RCRA) [42]. Hydrochloric acid (Gas) (Sl. No. 313) is in this list. However, the authors used minimal quantities (~7 mL) of concentrated Hcl under a fume hood by wearing gloves and a mask to avoid direct contact and inhalation. Further, analysts were not exposed continuously to HCl gas while running the experiment. Hence, the second quadrant is also coded green. Methanol (Sl. No. 373) is listed as a hazardous chemical under the RCRA by the EPA. Therefore, the second quadrant is colorless for the reported methods. The third quadrant deals with the pH of the solutions used for analysis, which should not be less than 2 or more than 12 to make this quadrant green. The diluent (For 0.1N HCl, pH 1) used in these methods was not within the range; thus, the third quadrant was colorless [33]. The pH of methanol used in the reported methods is within this range and is thus coded with green. Finally, the fourth quadrant deals with wastage, as the total wastage should not be more than 50 g or mL. In these methods, the wastage is negligible; thus, the fourth quadrant was coded green. But, in the case of the reported methods, wastage is more than 100 mL. The overall NEMI pictogram of the methods is depicted in Fig. 5.

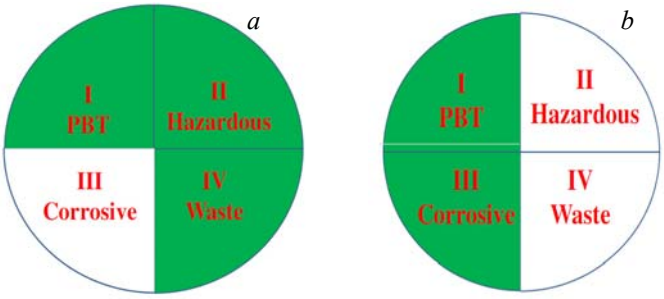

Fig. 5. Assessment of greenness of (a) developed spectroscopic methods, (b) reported method [5, 6] by NEMI.

The AES is another tool for calculating the greenness of analytical methods. The principle of AES is that an ideal green analysis has a score of 100. The score of the technique is calculated based on penalty points (PP). PP is calculated based on the four major classes quantity of reagents and hazard (physical, environmental, health) [43], energy (instrument) [44], occupational hazard, and waste. The first sub-total PP is calculated for reagents based on the quantity of reagents, the number of pictograms, and the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) signal word. Then, the total PP for the reagent is calculated as the amount of PP×Hazard PP. Subsequently, the AES is calculated as AES = 100 – total PP. Five PPs were generated by this advanced method, and the final AES score was 95 for the developed methods. The calculation results conclude that developed methods are in the excellent green analysis category (>75%) and show their efficiency toward sustainability. The AES score for the reported methods was 74, less than that of the developed methods and representing an acceptable green analysis rank. The overall calculation of AES is presented in Table 4.

TABLE 4. Calculation of AES Score for Proposed and Developed Methods

Parameter	Description		Limit		Sub-total PP		Total PP	
	A	B	A	B	A	B	A	B
Amount of reagents	0.1N HCl	Methanol	<10 mL (g)	>100 mL (g)	1	3	1×2=2	3×6=18
Hazard (physical, environmental, health)	1 pictogram and GHS-US word is Warning [45]	3 pictogram and GHS-US word is danger [46–48]	Less severe hazard	More severe hazard	2	6		
Energy (instrument)	UV-Vis Spectrophotometer	UV-Vis Spectrophotometer	≤0.1 kWh per sample	≤0.1 kWh per sample	–	–	0	0
Occupational hazard	Analytical process hermetization	Emission of vapors and gases to the air	–	–	–	–	0	3
Waste	~7 mL	>100 mL	1–10 mL (g)	>10 mL	–	–	3	5
Total PP							5	26
AES							95	74

Note. A = Developed methods, B = Reported methods [5, 6].

The AGREE metrics is the newest green computation method based on novel software covering all 12 (SIGNIFICANCE) green analytical principles and are converted into a zero to one scale [22]. The outcome is a circular graph showing the ultimate value, performance of the analytical procedure in each criterion, and the weights assigned by the user. The method's performance in each principle is represented by the intuitive red-yellow-green color scale. At the same time, the importance of each direction is reflected in the width of its corresponding segment. The total score of the developed methods was 0.9, presented in the middle of the pictogram with values close to 1 and the dark green color, suggesting that the evaluated procedures are greener (Fig. 6a). But the performance of the reported methods is less as the score is 0.7, as depicted in Fig. 6b.

Fig. 6. Assessment of greenness of (a) developed spectroscopic methods, (b) reported method [5, 6] by AGREE.

The output of the various assessment tools for greenness profiles such as the NEMI, AGREE metrics, and the ESA showed that the proposed methods are eco-friendly and hence can be employed for the routine analysis of CIN with a positive influence on the environment.

Conclusions. Four simple, accurate, precise, sensitive, robust, and green spectroscopic methods have been designed and validated to estimate cinacalcet hydrochloride. The designed methods can be used fruitfully to consistently analyze cinacalcet hydrochloride in different pharmaceutical medicaments without affecting the environment and the analysts. The developed spectrophotometric methods may not substitute the presently well-acknowledged techniques accessible for investigating the drug; they can still provide an alternative way for sophisticated analytical instruments such as HPLC that are not accessible for usual investigation and dissolution studies.

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