Research Article

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Analytical Method Development and Validation for Nirogacestat Using UV Spectrophotometry

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ABSTRACT

The primary aim of the current investigation was the Analytical progression and validation of a UV-spectrophotometric analytical approach for the quantification of Nirogacestat in both bulk and pharmaceutical dosage forms, in alignment with ICH regulatory standards. A straightforward, expeditious, reliable, and economically viable UV-spectrophotometric technique was established for the quantitative determination of Nirogacestat employing acetonitrile as the solvent medium. The absorbance of the analyte solution was recorded at 269 nm relative to a suitable blank. Method validation was performed pursuant with prescribed parameters, encompassing linearity, accuracy, precision and robustness. The λmax of Nirogacestat in acetonitrile was identified at 269 nm. The analyte demonstrated compliance with Beer-Lambert's law across the concentration interval of 2.5–15.0 µg/mL, exhibiting a correlation coefficient(R^2) of 0.999. The percentage recovery ranged from 99.3% to 100.1% reflecting substantial accuracy. Precision assessments resulted %RSD values below 2.0, substantiating both repeatability and intermediate precision. Robustness evaluations involving minor alterations in wavelength exhibited negligible deviation, thereby affirming reliability. Additionally, forced degradation experiments substantiated the stability-indicating characteristics. The delineated UV-spectrophotometric technique is straightforward, analytically sensitive, and reproducible. It is eminently suitable for routine quality assessment and quantitative evaluation of Nirogacestat in bulk drug substances and tablet formulations.

Keywords: Ultraviolet spectroscopy, validation, nirogacestat, method development, assay

1. Introduction

Nirogacestat is an orally active, selective gamma secretase inhibitor with potent antineoplastic activity. [1] It is primarily utilized for the treatment of desmoid tumors, a rare, aggressive form of soft tissue tumor that can't be surgically removed. Chemically, it is known as 2S.-2-[[(2S)-6,8-difluoro-1,2,3,4-tetrahydronaphthalen-2-yl]amino]-N-[1-[1-(2,2-dimethylpropylamino)-2-methylpropan-2-yl]imidazol-4-yl] "Pentanamide chemical structure is given in Figure 1." has a chemical formula of $C_{27}H_{41}F_2N_5O$and a molecular weight of 489.66 g/mol. Nirogacestat functions by inhibiting gamma secretase, an intramembrane protease responsible for activating Notch receptors.[2] Inhibition of Notch signaling interferes with tumor cell proliferation and survival.[3]The inhibition also affects downstream pathways involved in oncogenesis. Nirogacestat binds and blocks gamma secretase, thereby preventing Notch receptor activation, which is essential in the pathophysiology of desmoid tumors.[4] It has a terminal half-life in the vicinity of 23 hours and reaches peak plasma concentrations (Cmax) at around 1.5 hours, this achieves steady state within 6 days of administration. The most common adverse effects reported includes diarrhea, nausea, fatigue, hypophosphatemia, rash, and ovarian dysfunction. [5] Although various LC-MS/MS methods have been reported for the pharmacokinetic profiling of Nirogacestat [6], To date, there are no complete analytical method development and validation studies for UV–visible spectrophotometry. The UV– visible spectrophotometry is widely used to characterize and measure the amount of compounds according to their UV– visible absorbance and has been successfully applied. Hence it is a reliable analytical tool [7]. Besides, the establishment of powerful analytical approaches, which are properly calibrated and validated, is crucial in guaranteeing linearity, accuracy, precision and reproducibility in the routine pharmaceutical quality control [8,9]. Therefore, in the current study, a quick, specific and systematically validated UV– visible spectrophotometric method was developed for the predictive modelling of Nirogacestat in pharmaceutical formulations.

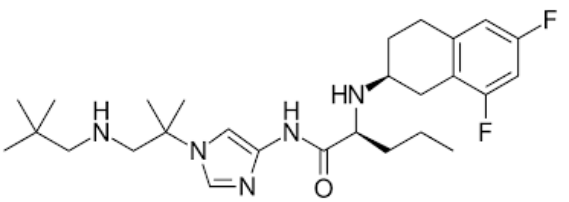


Figure 1. Molecular structure of Nirogacestat

2. Materials and Methods

2.1. Chemicals and reagent

Standard solution of Nirogacestat and Acetonitrile were used and these were procured from Thermo Fisher, India.

2.2. Instrumentation

The Shimadzu analytical balance (model BL-220H) was employed for precise weighing. Spectral measurements were conducted using a Shimadzu UV-1800 double beam spectrophotometer equipped with a 1 cm path length, operated through Shimadzu UV-Probe software (version 2.21), by employing a 10mm matched quartz cuvette procedures.

2.3. Formulation of standard solution

A precisely weighed quantity of 10 mg of the pure drug was Quantitatively introduced into a 100 mL volumetric flask. The drug was thoroughly solubilized in acetonitrile, the volume was adjusted to the mark with the appropriate solvent to create a stock solution that has a 100 µg/mL concentration.

Measured volumes of standard stock solution were pipetted out ranging from 5 ml to 50ml and subjected to serial dilution optimally with acetonitrile to get the final concentration of standard solutions (10µg/mL).

2.4. Formulation of working stock solution

Accurately weigh and decant 10mg of Nirogacestat working standard into a 100 ml clean dry volumetric flask. Added diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

2.5. Calibration curve

This was subjected to scanning using a Shimadzu 1700- UV Visible spectrophotometer where the operational range was 200-400 nm, with acetonitrile employed as the blank. [10] The peak absorbance was pinpointed at a wavelength of 269nm. To generate different concentrations spanning from 2.5 to 15 µg/ml, portions were formulated using distilled water as the solvent. These samples were then assessed at the specified wavelength of 269 nm to ascertain their respective absorbance values. "The collected data was subsequently employed to orchestrate a calibration curve which is given in Figure 2A." The linearity overlap spectra is given in Figure 3.

3. Results and Discussion

3.1. Method validation

The engineered method for the estimation of Nirogacestat was validated in adherence with ICH guidelines across various parameters, including linearity, accuracy, precision, robustness, and forced degradation.

3.2. Linearity

Fresh aliquots were systematically constituted from the standard stock solution within the quantitative strength range of 2.5–15.0 µg/mL. The absorbance of each concentration was identified at 269 nm under zero-order conditions, utilizing acetonitrile as the blank. The analyte exhibited linearity across the specified concentration range for the method. The correlation coefficient (R^2) was determined to be 0.999, indicating optimized linear association. [11] "The UV spectrum of Nirogacestat is given in Figure 2B."

3.3. Accuracy

The accuracy of the engineered method was substantiated through recovery evaluations conducted at three distinct quantitative strength levels 50%, 100%, and 150% respectively, where each of them performed in triplicate. Accuracy was articulated in terms of percentage recovery. The %RSD for the accuracy of Nirogacestat was observed to be below 2, and the recovery values were around 99.6%. This is in accordance with ICH guidelines and the statistical outcomes were falling under the acceptable limits. The accuracy is depecited in Table 1. [12]

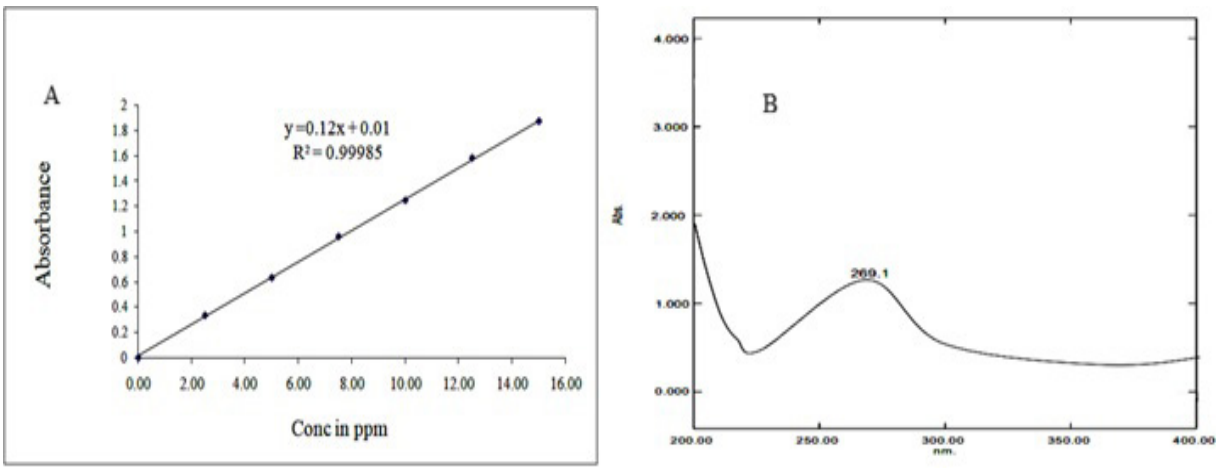


Figure 2. A) Calibration of First order B) UV spectrum of Nirogacestat

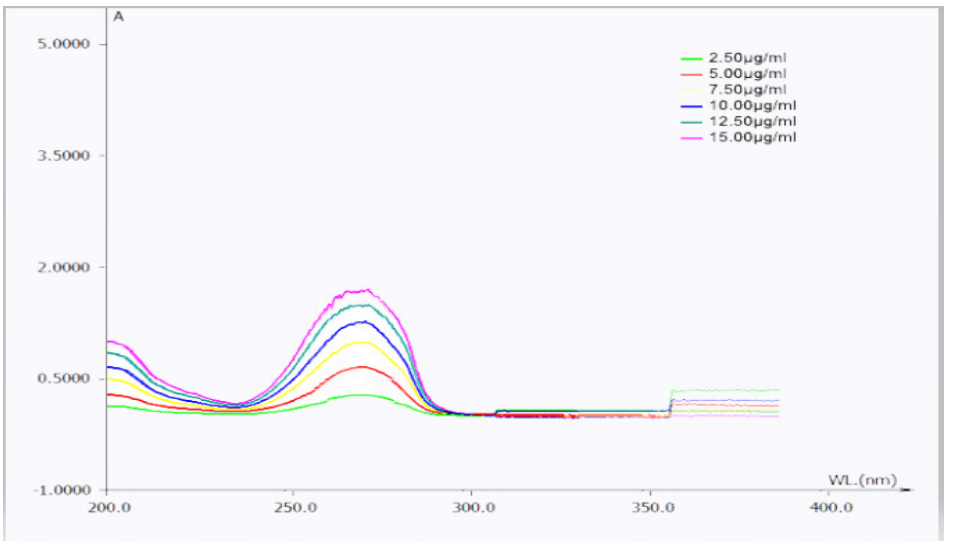


Figure 3. Linearity overlap spectra of NIRO

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Table 1. Acurracy data of Method

Level of addition	Formulation amount	Amount added	Experimental amount [n=3]	% recovery	% mean recovery ±SD
50%	10	5	4.96	99.20	98.20±0.005
100%	10	10	9.93	99.30	99.30±0.19
150%	10	15	14.96	99.73	99.73±0.22

S.D: Standard deviation

3.4. Precision

The precision of the proposed methodology was subjected to exhaustive validation through intra-day and inter-day variability studies. For intra-day evaluation, six independent aliquots of Nirogacestat were taken and they were prepared at a quantitative strength of 10 µg/mL. [13] Further these were analyzed in triplicate within a single analytical cycle, with absorbance values systematically recorded. For inter-day assessment, six aliquots of the same concentration were prepared and analyzed in triplicate across three successive days. Precision was quantitatively externalized as the percentage relative standard deviation (%RSD). The %RSD values obtained for both intra-day and inter-day precision remained less than 2.0, thereby demonstrating the method's high degree of repeatability and intermediate precision. As per ICH regulatory criteria, %RSD values within this threshold substantiate the methodological reliability and robustness. The precision data of method and intermediate precision is shown in Table 2 & Table 3. respectively [14]

3.4.1. Intraday precision

Table 2. Intraday Precision of Nirogacestat

		Intraday precision		
S.No	Conc(µg/ml)	Absorbance		
		Morning	Afternoon	Evening
1	10	1.231	1.232	1.224
2	10	1.243	1.242	1.218
3	10	1.221	1.225	1.239
4	10	1.235	1.228	1.213
5	10	1.229	1.227	1.244
6	10	1.217	1.221	1.257
mean		1.224	1.229	1.232
SD		0.009	0.007	0.015
%RSD		0.765	0.589	1.255

S.D: Standard deviation; RSD: Relative standard deviation

3.4.2. Intermediate precision

Table 3. Intermediate precision of Nirogacestat

		Intermediate precision		
S.No	**Conc(µg/ml)**	**Absorbance**		
		Day-1	Day-2	Day-3
1	10	1.212	1.202	1.225
2	10	1.215	1.232	1.268
3	10	1.231	1.205	1.249
4	10	1.245	1.228	1.233
5	10	1.219	1.217	1.224
6	10	1.217	1.231	1.257
mean		1.223	1.219	1.242
SD		0.012	0.013	0.016
%RSD		1.024	1.089	1.330

S.D: Standard deviation; RSD: Relative standard deviation

3.4.3. Robustness

The robustness of the analytical framework was evaluated by performing the assay at two distinct wavelengths (±5 nm from the optimized value). The corresponding absorbance values were recorded and the outcomes were presented as percentage relative standard deviation (%RSD) these are given in Table 4. [15] The %RSD values remained confined to the established acceptance thresholds, thereby substantiating the method's reliability under deliberate minor variations in analytical conditions.

3.5. Sensitivity

The sensitivity of the proposed UV–visible spectrophotometric method was assessed by calculating the limit of detection (LOD) and limit of quantification (LOQ) as per ICH Q2(R2) guideline. The LOD is the lowest concentration at which the analyte can be "detected" with reliable detection, while the LOQ is the lowest concentration at which the analyte can be "quantified" with acceptable precision and accuracy.

The equation of the calibration curve was used to determine the LOD and LOQ values in the following way:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Here, σ is the standard deviation of the response, and S the slope of the calibration curve. [16]. Calculated LOD and LOQ for Nirogacestat was 0.116 µg/mL and 0.354 µg/mL, respectively. These low values suggest that the proposed analytical method has a satisfactory level of sensitivity for identification and quantification of Nirogacestat in pharmaceutical preparations and can be used in the routine QC analysis.

Table 4. Robustness results

Parameter	**Concentration 10 (µg/mL)**	**% Assay**
		Method-A
Robustness Change in λ_{max} (± 5nm)	λ+ : 274 nm	100.0
	λ - : 264 nm	100.2

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3.6. Assay of Nirogacestat tablet

A precisely weighed quantity of Nirogacestat, commensurate to 50 mg, was quantitatively introduced into a 100 mL volumetric flask. The substance was completely solubilized in acetonitrile and the volume was made upto the mark using the corresponding solvent. The resulting solution was subsequently subjected to membrane filtration through Whatman filter paper No. 40. A 5 mL aliquot of the filtrate was progressively diluted to 50 mL with distilled water to attain a final quantitative strength of 10 µg/mL. [17] This solution was analyzed employing UV spectrophotometry at 269 nm. The concentration of the drug was quantified and reported as Assay percentage. Results of UV spectrophotometric examination of the formulation in Table 5.

3.7. Degradation Studies:

3.7.1. Formulation of stock

Weighed and quantitatively decanted 50 mg of the Nirogacestat sample with analytical precision into a 100 mL Volumetric flask that has been cleaned and dried. Then, added diluent and sonicated to ensure complete dissolution. Finally, to obtain the stock solution, used the appropriate solvent to adjust the volume upto the mark. [18]

3.7.2. Acid degradation

Accurately pipetted out 5 mL of the previously prepared stock solution into a 50 mL volumetric flask and subsequently added of 1 mL of 1 N HCl. This flask was subsequently retained under controlled conditions at 60 °C for 1 hour, after which the solution was neutralized using 1 N NaOH and the volume was adjusted to 50 mL with the diluent. The impending solution was passed through a microporous membrane by a 0.45 µm syringe filter and quantitatively introduced into appropriate vials for analysis.

3.7.3. Alkali degradation

Poured 5 ml of the prepared stock solution into a 50 ml volumetric flask, then added 1 ml of 1N NaOH. Following this, the content in the volumetric flask was neutralised with 1N HCl and filled to 50ml with diluent after being maintained at 60ºC for an hour. Then this solution was filtered through 0.45 micron syringe filters. Later the vials were filled with this solution.

3.7.4. Peroxide degradation

Pipetted One millilitre of 10% w/v hydrogen peroxide was added to a 50 millilitre vacuum flask containing the prepared stock solution. The volume was then raised by diluting. The vacuum flask was then kept at 60°C for 1 hour under controlled circumstances. For 15 minutes, the vacuum flask was kept at ambient temperature. The solution was passed over a microporous membrane, then decanted into bottles using 0.45 micron syringe filters.

3.7.5. Reduction degradation

With analytical precision pipetted 5 mL of the prepared stock solution into a 50 mL vacuum flask, followed by the inclusion of 1 mL of 10% w/v sodium bisulfite solution. The volume was then made upto the mark with the diluent. The flask was subsequently upheld at 60 °C for 1 hour and then allowed to stand at ambient temperature for 15 minutes. The resulting solution was passed through a microporous membrane by a 0.45 µm syringe filter and quantitatively introduced into appropriate vials for analysis.

3.7.6. Hydrolysis degradation

Transferred 5 mL of the previously produced stock solution to a 50 mL vacuum flask using an analytical pipette. Add 1 mL of HPLC-grade water. The volume was then adjusted to the proper level with the indicated diluent. The flask was maintained at 60 °C for 1 hour and then equilibrated at ambient temperature for 15 minutes. The resultant solution was passed through a microporous membrane by a 0.45 µm syringe filter and quantitatively put into appropriate vials for analysis.

Table 5. Results of UV spectrophotometric examination of the formulation.

Marketed Product	Label content (mg)	Found Amount (mg)	Recovery ± RSD %
OGSIVEO	50.00	49.93	99.93±0.12

3.7.7. Photolytic degradation

A defined quantity of the Nirogacestat sample was exposed to controlled light conditions in a photostability chamber for a duration of 3 hours. Following the exposure temporal window, the sample was attenuated in accordance with a diluent and was subsequently subjected to UV analysis.

3.7.8. Thermal degradation

An accurately measured quantity of the Nirogacestat sample was placed in a clean Petri dish and was subjected to thermal stress in a hot air oven maintained for 3 hours at 105 °C. After thermal exposure, the sample was attenuated using the specified diluent and analyzed via UV. All the degradation data is given in Table 6.

Table 6. Forced degradation results of Nirogacestat

S.No	Deg	Absorbance	% deg
1	Control	1.238	0
2	Acid	1.114	10.0
3	Alkali	1.083	12.5
4	Peroxide	1.063	14.1
5	Reduction	1.130	8.7
6	Thermal	1.225	1.0
7	Photolytic	1.230	0.6
8	Hydrolysis	1.215	1.8

4. Conclusion

A simple, rapid, robust, accurate, precise and cost effective UV–visible spectrophotometric method for quantitative analysis of Nirogacestat in pharmaceutical formulations was successfully developed and validated. The method showed good linearity over the concentration range of 2.5-15.0 µg/mL (R2 = 0.999) and had good accuracy, precision, robustness and specificity as per ICH guidelines. The absence of interference of the formulation excipients confirmed the selectivity of the method and the λmax value (269 nm) allowed the reliable and reproducible quantification of Nirogacestat. In addition, forced degradation studies under acidic, alkaline, oxidative, reductive, thermal, photolytic and hydrolytic conditions confirmed the stability indicating ability of the proposed method by being able to detect the change in drug stability under stressed conditions. The developed UV-visible spectrophotometric method is advantageous over the more sophisticated chromatographic methods including HPLC and LC–MS/MS, as it requires less sample preparation, is low in operational cost, less time involved in analysis and can be easily implemented without compromising the analytical performance. Due to these benefits, the proposed method is seen to be a practical and economical approach for routine quality control, assay determination and stability study of Nirogacestat in pharmaceutical formulations. This validated analytical method can also be useful in formulation development, quality assurance and in future study on the field of pharmaceutical research on Nirogacestat, in laboratories of which availability of advanced chromatographic instrumentation is limited.

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Conflict of Interest

The authors have no conflict of interest.

Author Contributions

Concept - V.D., M.M.; Design: V.D., M.M.; Sources - V.D., R.P.; Materials - V.D., M.M.; Data Collection and/or processing - V.D.,J.M.,R.P.; Analysis and/or interpretation-R.P., M.M.; Literature review-V.D.,J.M.; Manuscript writing- V.D., J.M.; Critical review-V.D., R.P, M.M.

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