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Assessment of Nirmatrelvir Stability in Pharmaceutical Formulations Using a Validated UV Spectrophotometric Method

Durga Panikumar Anumolu*, Vegesna Bhanvee Varma, Ambati Akshara, Dubbudu Saketh Reddy, Syed Sara Afreen.

[1]Gokaraju Rangaraju College of Pharmacy, Department of Pharmaceutical Analysis, Osmania University, Hyderabad-500090.

Abstract: The estimate of Nirmatrelvir in bulk and tablet dosage forms has been accomplished by the development and validation of a straightforward, accurate, and exact UV-spectrophotometric approach. When methanol was used as the solvent, the maximum absorption was discovered to be 248 nm. In terms of specificity, linearity, accuracy, precision, robustness, ruggedness, and detection and quantification limitations, the technique was verified in accordance with ICH criteria. With a correlation value of 0.9992, linearity was noted in the 10–50 µg/mL range. The average accuracy, according to recovery studies, was 100.04%. The accuracy investigations' %RSD values were less than 2.0%, proving the method's repeatability. Studies on forced deterioration validated the method's capacity to indicate stability. In quality control labs, this verified technique may be applied successfully to regular Nirmatrelvir analysis.

Keywords: UV-Spectrophotometry, Accuracy, Linearity, Stability-indicating, Forced Degradation, Nirmatrelvir, and Method Validation.

Introduction: A new antiviral medication called nimmatrelvir is used to treat COVID-19 in conjunction with ritonavir. The creation of a reliable and easy-to-use analytical technique is required due to the growing need for routine Nirmatrelvir analysis. UV-spectrophotometry is a commonly used method because of its dependability, cost, and ease of use. According to ICH Q2(R1) requirements, the objective of this work is to develop and verify a UV-spectrophotometric technique for the quantitative measurement of nimatrelvir (1).

Experimental:

Table 1: REAGENTS AND MATERIALS

S. No.	Name	Grade	Make/company
1.	Water	AR	SD-Fine chemicals limited
2.	Methanol	AR	SD-Fine chemicals limited
4.	Propanol	AR	SD-Fine chemicals limited
5.	Nirmatrelvir Purity : 99.5 %	N/A	
7.	Nirmatrelvir Tablets-150mg	N/A	

Table 2 : INSTRUMENTS USED

S.No.	Name of Instrument	Software	Model	Make/company
1.	UV-Spectrophotometer	UV win Lab ES	Lamda 365+	Perkin Elmer
2.	Weighing Balance	N/A	Gold-300P	Phoenix
3.	Sonicator	N/A	2K13016	LIFE CARE

Instrumentation: UV-Visible spectrophotometer (Perkin Elmer Lambda 365+), UV WinLab ES software, 10 mm quartz cells, analytical balance (Phoenix Gold-300P), and Life Care sonicator were used.

Methodology:

Solubility studies:

Nirmatrelvir solubility tests were conducted utilizing a range of solvents. The methodology described by Higuchi and Connors, 1965 (2), was followed for conducting phase solubility investigations. The drug's solubility was examined in hydrochloric acid, ethanol, methanol, and distilled water. The surplus medication was poured into 10 mL conical flasks with stoppers, and the volume was adjusted with the appropriate solvents. The mixture was shaken for 24 hours at 37±5 0C in a thermostatic shaker bath. The 5 mL aliquots were taken out and passed through a 0.45-µm Whatman filter paper so that the UV spectrophotometer could measure the drug concentration.

Determination of absorption maxima: Finding the absorption maxima: 3.0 mL of a standard solution (100 µg/mL) was placed in a cuvette and scanned with a fixed slit width (2nm)between 400 and 200 nm. Based on the spectrum, the diluent was optimized; Nirmatrelvir's asymmetric factor was used.

Preparation of solutions for Assay:

Preparation of standard solution: To create the standard solution, 100 mg of Nirmatrelvir were precisely weighed in a 100 mL volumetric flask with 30 mL of diluent. The flask was then sonicated for about 30 minutes, and the volume was adjusted using the same diluent to reach the desired final concentration of 1000 µg/mL. To get a final concentration of 100 µg/mL, 10 mL of the aforementioned solution was pipetted out into a 100 mL volumetric flask, and the volume was adjusted with diluent. To get a final concentration of 10 µg/mL, 10 mL of the aforementioned solution was pipetted out into a 100 mL volumetric flask, and the volume was adjusted with diluent. (3–13)

Preparation of sample solution:

Weighing 20 pills allowed us to calculate their average weight. A fine powder was created by crushing the pills. A 100 mL standard volumetric flask was filled with 256 mg of powder, which was precisely weighed and equivalent to 150 mg of Nirmatrelvir. Add around 30 mL of diluent, dissolve, and use the diluent to get the desired volume. Following 30 minutes of sonication and marking the solution as the sample stock solution, 10 mL was pipetted out into a 100 mL volumetric flask, and the volume was adjusted with diluent to reach the final concentration of 100 µg/mL. To obtain the final concentration, 10 mL was pipetted out into a 100 mL volumetric flask and the volume was adjusted using diluent to 10 µg/mL.

Method Validation:

The developed approach was validated in accordance with accepted practices.

DEGRADATION STUDIES:

Stress testing is necessary to clarify the intrinsic stability properties of the active ingredient, according to the International Conference on Harmonization (ICH) guideline titled Stability testing of novel medicinal substances and products. The purpose of this work was to use the suggested approach to conduct stress degradation experiments on Nirmatrelvir.

Preparation of stock:

Using a mortar and pestle, weigh ten tablets precisely. Then, transfer the equivalent of 100 mg of Nirmatrelvir (marketed formulation = 240.4 mg of tablet powder) into a 100 ml clean, dry volumetric flask. Add about 30 ml of diluent, sonicate for up to 30 minutes to completely dissolve it, and then use the same solvent to bring the volume up to the desired level. After that, a 0.44-micron injection filter is used to filter it. 10 mL was pipetted out of this and put into a 100 mL volumetric flask, which was then diluted with diluent until it reached the desired level. (A stock solution).

Degradation via hydrolysis in an acidic environment

The pipette 30 ml of 0.1N HCl was added to 10 ml of the aforesaid solution in a 100 ml volumetric flask. Following six hours at 60ºC, the volumetric flask was neutralized with 0.1 N NaOH and diluted to a level of 10 ml. Use 0.22-micron syringe filters to filter the solution, then scan it at 248 nm.

Degradation via hydrolysis in an alkaline environment

30 ml of 0.1N NaOH was added after 10 ml of the aforesaid solution was pipetted into a 100 ml volumetric flask. After six hours at 60ºC, the volumetric flask was neutralized with 0.1N HCl and diluted to a level of 10 ml. Use 0.22-micron syringe filters to filter the solution, then scan it at 248 nm.

Oxidative degradation

The pipette Above the stock solution, 10 ml The solution was transferred from a 100 ml volumetric flask to a 30 ml volumetric flask. In a 100 ml volumetric flask, 10 ml of 3% w/v hydrogen peroxide was introduced, and the volume was adjusted with diluent. After that, the volumetric flask was left at room temperature for fifteen minutes. Use 0.45 micron syringe filters to filter the solution, then scan it at 248 nm.

Thermal induced degradation

A sample of nirmatrelvir was obtained in a petridish and stored for 24 hours at 1100 C in a hot air oven. After that, the material was extracted, diluted with diluents, scanned at 248 nm, and examined.

Photo degradation: Pour 10 ml of the stock solution into a 100 ml volumetric flask, leave it in the sun for 24 hours, and then add diluent to bring the volume up to the proper level. Use 0.45 micron syringe filters to filter the solution, then scan it at 248 nm.

RESULTS:

Solubility Studies: Solubility was examined in ethanol, methanol, distilled water, and HCl. Methanol was chosen as the diluent since it demonstrated the highest solubility.

Determination of λ max: It was determined by scanning the standard solution (100 µg/mL) between 200 and 400 nm. The absorbance was highest at 248 nm. (Figure 1)

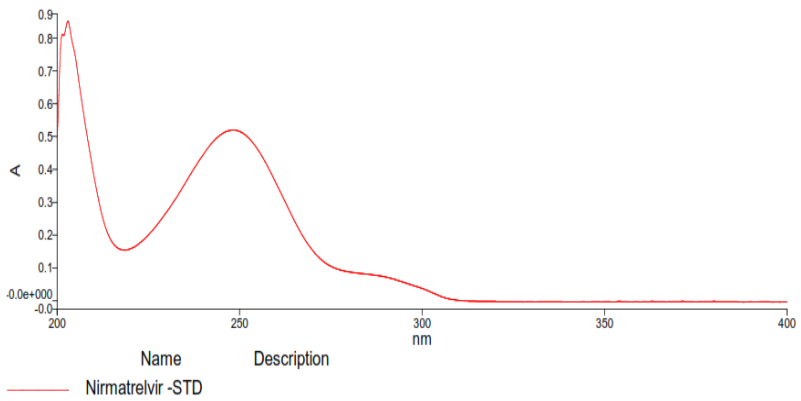

Fig.1 - Spectrum of Nirmatrelvir using Methanol

Method Validation:

Since the absorbance maxima at 248 nm for the standard and sample solutions were similar, suggesting that there was no interference from excipients or solvents, the UV spectroscopic technique created for the quantification of Nirmatrelvir was determined to be specific. With a regression equation of y = 0.0171x – 0.0097 and a correlation coefficient (R^2) of 0.9992 (FIG-2), linearity was demonstrated across the concentration range of 10–50 µg/mL, indicating a good linear connection between concentration and absorbance. Recovery experiments evaluating accuracy at 50%, 100%, and 150% levels revealed a mean recovery of 100.04%, demonstrating the method's dependability (Table-02). With %RSD values continuously below 2.0%, precision experiments showed that both system and technique accuracy were within acceptable bounds, indicating strong repeatability. The technique proved resilient to little changes in temperature and wavelength, and its robustness was validated by consistent outcomes from several labs, instruments, and analyzers. The established LOD and LOQ values of 0.210 µg/mL and 0.467 µg/mL, respectively, demonstrated the method's sensitivity and made it appropriate for routine analysis of nimatrelvir in pharmaceutical dosage forms and bulk.

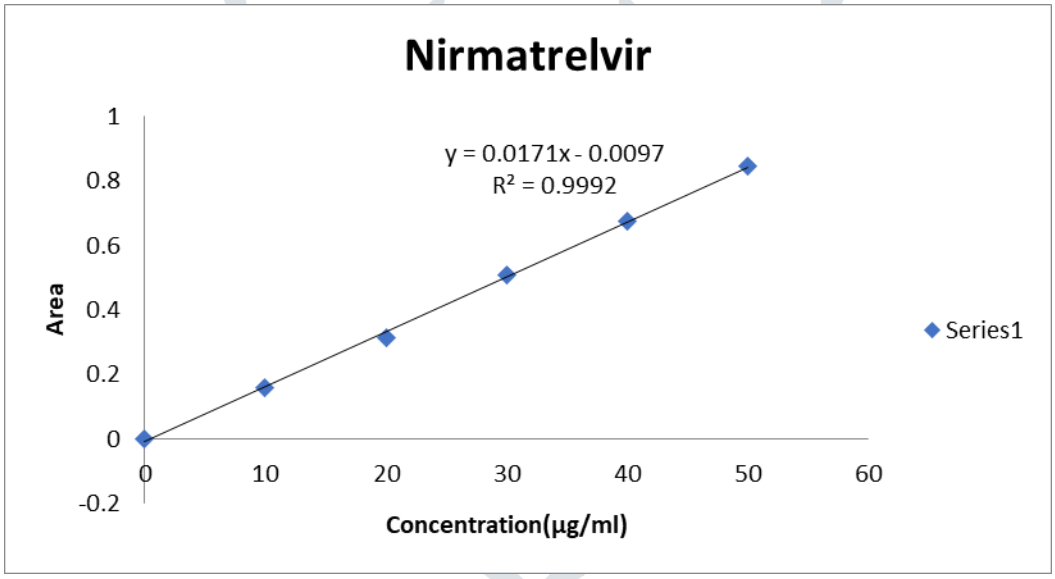

Fig. 2- Calibration Curve for Nirmatrelvir (10-50 µg/mL).

Table-3: Accuracy Data of Nirmatrelvir

S.No	Recovery level	Absorbance	% Recovery	Average Recovery
1.	**50%**	0.170 0.172 0.167	101.57%	100.04%
2.	**100%**	0.505 0.509 0.511	100.0%	
3.	**150%**	0.754 0.744 0.756	98.55%	

Forced Degradation Studies: Stability-indicating nature was confirmed by controlled degradation under stress conditions (acid, base, oxidative, thermal, and photolytic).

Table 4. Forced Degradation Results

	Absorbance		**% Degraded**
Standard	0.507	100	
Acid	0.498	98.22	1.78
Base	0.489	96.45	3.55
Peroxide	0.496	97.83	2.17
Thermal	0.475	93.69	6.31
Photo	0.493	97.24	2.76

Discussion: The new approach showed outstanding accuracy, precision, and linearity. The approach can separate the analyte from its breakdown products, according to the forced degradation investigations. These findings support the method's applicability for regular quality control analysis.

Conclusion: To conclude, the proposed work outlines the creation of a novel UV spectrophotometric approach and validates its measurement of Nirmatrelvir using a straightforward diluent. The approach provides an excellent linearity response. After validation, the technique was determined to be straightforward, sensitive, accurate, and exact. The percentage recovery indicates that the formulation's excipients are not interfering with the process. The suggested approach may be utilized for regular examination of the dosage form of nimatrelvir since it conforms with ICH recommendations.

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