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Application of RP-HPLC Method for the Simultaneous Determination of Nirmatrelvir and Ritonavir in Tablets Dosage Form

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Abstract: Present study describes a fast, simple, and sensitive procedure for the simultaneous determination of Nirmatrelvir and Ritonavir in tablets. The present work was designed to analyze these compounds in pharmaceutical and clinical labs being economical for use. The method consisted of using a Zorbax Eclipse XDB C18 column (dimensions: 150 mm × 4.6 mm, 5 µm) with a mobile phase composition of 0.025 M KH_2PO_4 (pH=2.5) and CH_3CN using gradient mode. The UV detection was performed at 240 nm. The linearity was assessed over the range of 5–15 µg/ml for Nirmatrelvir and 30-90 for µg/ml for Ritonavir. The parameters such as accuracy, precision, linearity (>0.999), and sensitivity were satisfactory. The results of the method were validated statistically according to ICH guidelines.

Keywords: Nirmatrelvir, Ritonavir, RP-HPLC, ICH guidelines, Validation

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Introduction

COVID-19, also known as SARS-CoV-2 infection, is a highly contagious viral disease caused by the "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)". With around 6.8 million deaths globally, COVID-19 has had devastating worldwide impact. SARS-CoV-2 rapidly spread worldwide after the initial cases of this primarily respiratory viral disease were reported in Wuhan, Hubei Province, China, in late December 2019. It is currently regarded as the biggest worldwide health emergency since the 1918 influenza pandemic. Even so, on March 11, 2020 the World Health Organisation (WHO) formally declared it a global pandemic (Sharma *et al*., 2021; Gudima *et al*., 2023). The capability of the virus to cause disease can be changed by genetic modifications in

its genome. A single amino acid alteration can have a major effect on ability of virus to avoid the immune system and hinder the advancement of vaccination against the virus (Giovanetti *et al*., 2021). Several novel variants of concern (VOCs) including Alpha (B.1.1.7), Beta (B.1.51), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) have surfaced since the start of the SARS-CoV-2 epidemic. These variants are associated with increased rates of transmission and more severe illness (Aleem *et al*., 2022).As of June 25, 2024, the SARS-CoV-2 Omicron variants KP.2, KP.3, and LB.1 are highly prevalent in the United States. Forecasts indicate that KP.3 is expected to account for approximately 33% of new COVID-19 cases in the United States.It is imperative that effective antiviral medications be developed to prevent the risks associated with the ongoing COVID-19 pandemic, that has become the result of infections induced by the new coronavirus SARS-CoV-2. The primary strategies for combating COVID-19 involve immunization to prevent the onset of the disease and the treatment of individuals who have been affected. Understanding the transmission pathways, molecular structure, life cycle characteristics, and cellular infection processes of SARS-CoV-2 has enabled us to enhance the effectiveness of treatment through the utilization of diverse types of drugs. The treatments mentioned include convalescent plasma, monoclonal antibodies, and interferons (Alexandridi *et al*., 2022). In addition to these, novel antiviral medications such as fusion inhibitors, protease inhibitors, and nucleoside analogues, including Remdesivir, Molnupiravir, and Nirmatrelvir/Ritonavir, have been extensively researched and approved as treatments for SARS-CoV-2 (Couzin-Frankel *et al*., 2021). The FDA and EMA have granted conditional marketing authorizations for Paxlovid® as an antiviral therapy for COVID-19 (Louis *et al*., 2023). Paxlovid® is composed of two distinct active compounds, nirmatrelvir (NIR) and ritonavir (RIT), which are presented in individual tablets.

The IUPAC name for NIR is "(1R,2S,5S)-N-((1S) -1- cyano -2- ((3S)-2-oxopyrrolidin-3-yl) ethyl)-3-((2S) -3, 3- dimethyl- 2- ((2,2,2-trifluoro-acetyl) amino)butanoyl)-6,6-dimethyl-3-azabicyclo(3.1.0) hexane-2-carboxamide", and RIT is "1,3-thiazol- 5 - ylmethyl N- ((2S,3S,5S)-3-hydroxy-5-(((2S) – 3 – methyl – 2 -((methyl-((2-propan-2-yl-1, 3- thiazol –4 -yl)methyl) carbamoyl)amino) butanoyl) amino)-1, 6-diphenylhexan-2-yl) carbamate" (Fig. 1). Paxlovid® demonstrates significant efficacy in mitigating the advancement of COVID-19 to a severe state in high-risk patients (Hammond *et al*., 2022; Najjar-Debbiny *et al*., 2023). The treatment provides significantly more positive therapeutic outcomes, with a demonstrated 89% reduction in hospitalisation or fatality rates (Vandyck *et al*., 2021). Nirmatrelvir acts as an inhibitor of the main protease of SARS-CoV-2, preventing the processing of virus polyproteins and effectively stop the virus from replicating. Ritonavir, a well-known inhibitor of HIV-1 protease, primarily works as an inhibitor of the enzyme cytochrome P4503A4. This prevents the premature metabolic deactivation of nirmatrelvir when the two are co-administered (Louis *et al*., 2023). Being an oral medication, Paxlovid® can be conveniently given to patients who are not hospitalized. However, the combination of this treatment with other prescribed medications is restricted due to its ability to inhibit enzymes and may cause drug-drug interactions. Additionally, for patients with moderate renal impairment (estimated glomerular filtration rate between 30 and 60 ml/min), it is essential to adjust the dosage of nirmatrelvir. Paxlovid® should not be administered to patients with a glomerular filtration rate (eGFR) below 30 ml/min or patients undergoing renal replacement treatment. These recommendations exclude therapy with Paxlovid® for major high-risk populations. In response to this issue, specialized treatment programs have been developed for individuals undergoing dialysis and suffering from severe renal impairment (Hiremath *et al*., 2022). Ensuring the quality of pharmaceuticals is crucial in the pharmaceutical sector. It is important to ensure that drugs are accessible and have a consistent and effective therapeutic impact (Sumalatha *et al*., 2024; Sai *et al.*, 2024; Harshita *et*

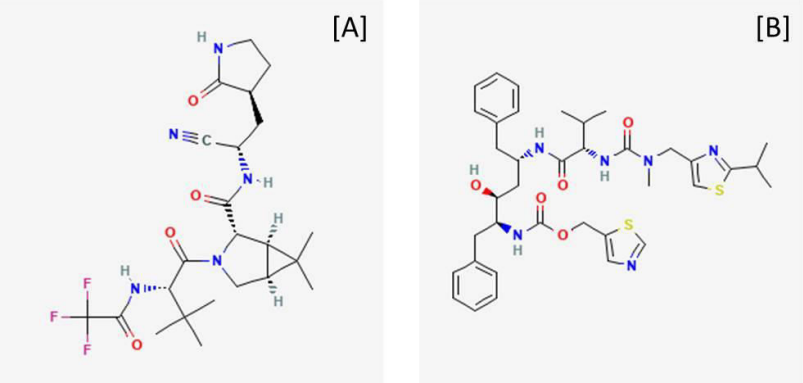

Fig. 1: Structure of Nirmatrelvir (A), and Ritonavir (B)

al., 2024). This underscores the need for precise and robust techniques. Recently, UPLC-MS/MS system was used for monitoring NIR and RIT in human plasma (Guyon *et al*., 2022), and a method using "LC-MS/MS" for monitoring NIR and RIT in biological samples also been reported (Liu *et al*., 2022). These techniques have been previously described and used in pharmacokinetic applications related to NIR and RIT. A few analytical methods have been published for estimating NIR and RIR content in tablet formulation or bulk material (Santosh Kumar *et al*., 2023; Sisubalan *et al*., 2024). However, these methods lack sensitivity and accuracy when estimating the NIR and RIT at low concentrations. Additionally, these methods are expensive, time consuming, and not suitable for routine lab analysis. Considering these factors, the objective of this study was to establish and verify an RP-HPLC technique for quantifying NIR and RIR in tablet formulation and bulk material.

Materials and Methods

Instruments:

The study was conducted using the Shimadzu HPLC system (Model No. LC-2030C) coupled with a UV detector (Model No. SPD-M20A). Data acquisition was done using Empower software version 2. The experimental setup consisted of a "Zorbax Eclipse XDB C_{18} column" (dimensions: 150 mm × 4.6 mm, 5 µm). The samples were introduced via a Rheodyne injection valve equipped with a 20 µL sample loop. Weighing was done using an analytical balance from Mettler Toledo.

Chemicals and materials:

The Paxlovid™ tablets (strength 150 mg NIR and 100 mg RIT), were procured from a local drug vendor. The reference standards of NIR and RIT were procured from Zenerapharma (Hyderabad, India).Throughout the analysis, all HPLC-grade solvents were employed, including potassium dihydrogen orthophosphate ($KH2PO_4$,), acetonitrile (CH_3CN), methanol, and water from Merck Ltd. (Mumbai, India).

Chromatographic conditions:

A gradienet mode was employed using a mixture of mobile phase A and B composed of a 0.025 M KH_2PO_4 (pH=2.5) and CH_3CN (Table 1). The analysis was performed at room temperature (25°C) with a mobile phase flow rate of 1.0 ml/min. Each trial required the injection of 20 µl of the sample into the HPLC system. The UV detector was configured to detect the NIR and RIT in the effluents from the column at a wavelength of 240 nm.

Preparation of 0.025 M Potassium Dihydrogen Orthophosphate Buffer (*pH 2.5*):

1000 ml of water were used to dissolve 3.5 g of potassium dihydrogen orthophosphate (KH_2PO_4).

Table 1: Time programming of gradient of elution

Time (min)	**Mobile phase A (% v/v)** ($0.025\ M\ KH_2PO_4$ buffer)	**Mobile phase B (% v/v)** (CH_3CN)
0	80	20
4	20	80
10	20	80
12	80	20
15	80	20

Then, using diluted ortho phosphoric acid, the pH of the solution was brought to 2.5. Next, a 0.45 micrometre membrane filter was used to filter the mixture.

Mobile Phase preparation:

The mobile phase consists of a mixture of mobile phase (A) composed of 0.025 M KH_2PO_4 (pH=2.5) and mobile phase (B) consists of CH_3CN. Both the solvents were degassed and filtered through a 0.45 µ membrane filter under vacuum. The gradient programme selected for method development is displayed in Table 1.

Diluent preparation:

Acetonitrile and water in the ratio of 50:50v/v

Preparation of Standard Solution:

The standard NIR and RIT stock solutions were prepared at concentrations of 1500 µg/ml and 1000 µg/ml by dissolving 150 mg of NIR and 100 mg of RIT in 100 ml of mobile phase. The resulting solution was filtered and diluted with more HPLC mobile phase to achieve a working concentration of 10 µg/ml (NIR) and 60 µg/ml (RIT). The linearity studies (NIR – 5 µg/ml to 15 µg/ml and RIT – 30 µg/ml to 90 µg/ml) and for building the calibration curves of NIR and RIT had been performed.

Preparation of sample stock solution:

A fine powder of Paxlovid™ tablets containing NIR and RIT was created by weighing and grinding 20 tablets. A precise amount of the fine powder, containing 1500 µg/ml of NIR and 1000 µg/ml of RIT, was then transferred into a separate volumetric flask (100 ml capacity) with 50 ml of HPLC mobile phase. The mixture was sonicated for 30 min, and then brought to a total volume of 100 ml with the mobile phase. The resulting solution was filtered and diluted with more HPLC mobile phase to achieve a working concentration of 10 µg/ml (NIR) and 60 µg/ml (RIT). The quantities of NIR and RIT were determined by analyzing the peak response data from the chromatograms.

Analytical method development:

In order to design a method for HPLC simultaneous analysis of NIR and RIT, several parameters have to be optimised while maintaining a set of constants. Mobile phase composition, column selection, and flow rates were carefully modified to obtain optimal chromatographic separation. Nevertheless, in order to maintain uniformity and facilitate validation, several parameters were kept constant: detector type, injection volume (20 µl), oven temperature (25 ± 2 ºC), and elution mode. A spectrum has been recorded for each set of chromatographic conditions at the specified detection wavelength. Throughout the method's development, additional factors considered were peak height, column pressure, accuracy, resolution, analysis time, and solvent efficiency per run.

Validation:

After acceptable chromatographic conditions were established, the method was validated following the ICH Q2 requirements (ICH). Additionally, the

stability of reagents and solvents was investigated as well.

Evaluation of system suitability:

System suitability studies were conducted to confirm the reliability of the HPLC system. This was assessed by injecting six duplicate standard solutions into the HPLC equipment. Column efficiency, plate count, and tailing factor were measured through six injections. The maximum acceptable relative standard deviation (RSD) for the area under the curve of the six repeated standard injections was set at 2.0%. The minimum column efficiency requirement was established at not less than 2000 theoretical plates. The maximum allowable tailing factor for both analyte peaks was set at no more than 2.0.

Specificity and selectivity:

Verifying the method's specificity to isolate the target drugs from impurities was crucial. This process involved a two-step injection under optimized chromatographic conditions. Initially, a blank solution without any drug sample was injected into the column to establish a baseline and ensure no interferences from the mobile phase or the system itself. Subsequently, a 20 µl injection of the drug solution containing both NIR and RIT was introduced. Analyzing the resulting chromatogram allowed for the evaluation of the chosen conditions for their effectiveness in separating the target drugs from any potential impurities in the sample.

Linearity:

Determination of linearity is done by preparation of the concentrations in the ranging from 5 to 15 µg/ml for Nirmatrelvir and 30 to 90 µg/ml for Ritonavir were injected and recorded the equations and calibration curves were calculated by plotted peak verses concentrations the proposed method of linearity assessed by using straight line calibration curve.

Precision:

A critical aspect of any analytical method is its precision, which reflects the consistency of results obtained from multiple measurements. To ensure a comprehensive evaluation, this study assessed precision across various levels as outlined by ICH validation guidelines. System precision, method precision (including repeatability), and intermediate precision were all investigated.

Method precision (*Repeatability*):

The precision of the procedure was determined by preparing six samples from a single batch. The percentage assay of the six samples was computed. The method's precision was assessed by determining the relative standard deviation (% RSD) of the results.

Intermediate precision (*IP*):

Intermediate precision refers to the capability of a procedure to consistently generate accurate results in controlled laboratory settings. This includes variations in factors such as different days, analysts, systems, and columns. Six samples were produced according to the test technique using the identical batch of formulation and then injected. The percentages of these samples were analyzed, and the robustness of the method was assessed by determining the percentage relative standard deviation (% RSD) of the results.

Accuracy or recovery studies:

The validity of an analytical procedure is determined by how closely the observed values align with either a conventional true value or an accepted reference value. Placebo samples were supplemented with standard solutions at different concentration levels, specifically 50%, 100%, and 150% of the working concentration. These samples were then evaluated using the method indicated. According to ICH guidelines, the average percentage recovery for the analytes at each concentration level should fall within the range of 98-102%. Additionally, the relative standard deviation (RSD) of the percentage recovery for the analytes at every level should not exceed 2.0%.

Sensitivity:

The limits of detection (LOD) and quantification (LOQ) of the method were established to

determine the lowest analyte concentrations that can be measured with confidence. LOD represents the minimum detectable amount, while LOQ signifies the lowest level that can be quantified with precision and accuracy. These values were determined based on signal-to-noise (S/N) ratios. In HPLC methods, an S/N ratio of 3 is typically indicative of LOD, while a ratio of 10 suggests LOQ. The specific concentrations and corresponding peak areas used to calculate LOD and LOQ.

Robustness:

The robustness was assessed by changing three parameters from the optimized chromatographic settings. These parameters included adjusting the mobile phase composition by ± 5%, making small changes to the flow rate by ± 0.2 ml/min, detection wavelength (±2), and modifying the column temperature by ± 5 °C.

Ruggedness:

The method's robustness was validated through multiple analysts analyzing the sample on different days using different tools.

Application of the developed method:

The approach can be assessed by injecting consecutive injections of standard and sample solutions, each with concentrations of approximately 10 µg/ml of NIR and 60 µg/ml of RIT, respectively. The making of standard and sample solutions was discussed in the materials and methods section earlier. % assay was computed using the following formula:

% Assay =TPA/SPA*WS/DS*DT/WT*PP/100*AWT/Label Claim

where, TPA is the test sample (tablet) peak area, SPA is standard solution peak area, WS is the weight of standard substance in mg, WT is the weight of sample (tablet) powder in mg, DS and DT are the dilution factor of standard and test solutions, PP is the percentage purity of standard, and AWT is the average weight of the tablets in mg.

Mobile phase stability:

To assess mobile phase stability, a 1:1 (w/w) mixture of NIR and RIT were prepared and evaluated at various time points. The stability of the mobile phase was monitored at intervals of 6, 12, 24, and 48 h.

Results and Discussion

Chromatographic Separation:

The method optimization was achieved by

employing a trial and error approach to create a chromatogram with desirable resolution (*R*), efficiency, acceptable number of USP plates, and tailing factor. Multiple iterations of this technique were conducted, involving modifications to the mobile phase composition, columns, and flow rate. The optimized procedure involved using an "Zorbax Eclipse XDB C_{18} column (dimensions: 150 mm × 4.6 mm, 5 µm)" with a mobile phase consisting of a mixture of mobile phase (A) composed of 0.025 M KH_2PO_4 (pH=2.5) and mobile phase (B) consists of CH_3CN.RP-HPLC with a gradient elution mode was utilized for chromatographic separation with a flow rate of 1.0 ml/min. The gradient program used for eluting both drugs is detailed in Table 1. The trial and error procedure yielded an optimized conditions for a well-separated peaks for both NIR (retention time: 3.88 min) and RIT (retention time: 7.71 min). The chromatograms of standard, sample and blank solutions are shown in Figure 2.

Method validation:

System suitability:

Upon injecting a solution with a concentration of 100%, the data obtained from the chromatograms indicated that the system suitability parameters, including a relative standard deviation (% RSD) of less than or equal to 2, a USP tailing factor of less than or equal to 2, and a USP plate count greater than 2000, met the acceptance criteria outlined in Table 2 according to the Q2 specifications of the ICH guidelines.

Linearity:

To assess the linearity of the HPLC method for quantifying NIR and RIT, a calibration curve was created using standard solutions at six

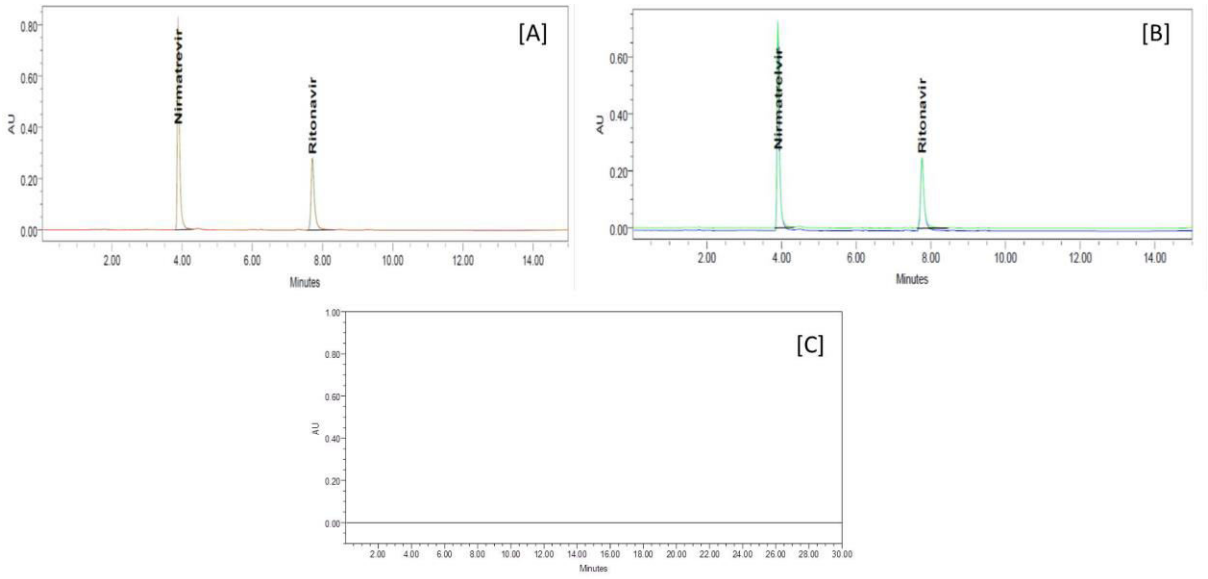

Fig. 2: Chromatograms of standard sample (A), test sample (B), and blank solution (C).

Table 2: System suitability parameters

S. No.	Parameters	NIR	RIT	Limits
1	Relative retention time (minutes)*	3.88	7.71	-
2	% RSD of retention time	0.23	0.16	Not more than 2
3	Peak area*	3458486	9036579	-
4	% RSD of peak area	0.91	0.43	Not more than 2
5	Theoretical plates	25045	36087	More than 2000
6	Tailing factor	1.5	1.7	Less than 2
7	Resolution	14.03	23.12	More than 2

*Mean of six determinations

Table 3: Regression analysis data

S. No.	Drug	Values of X and Y variables						R^2
1.	NIR	Variable	1	2	3	4	5	0.999
		X	5	7.5	10	12.5	15	
		Y	1833489	2589563	3458237	4319871	5194086	
2.	RIT	X	30	45	60	75	90	0.999
		Y	4576129	6715079	8953138	11193298	13431257	

concentration levels (5-15 µg/ml for NIR and 30-90 µg/ml for RIT) relative to the target test concentration. Each concentration level was analyzed in triplicate, and the correlation coefficient (R^2) was determined. A high correlation coefficient (typically above 0.99) indicates a linear relationship between the analyte concentration and the detector response. As shown in Figure 3 and Table 3, the correlation coefficient obtained was 0.99, confirming a strong linear relationship between the concentrations of NIR and RIT and the detector response, thus verifying the linearity of the HPLC method within the tested concentration range.

Precision:

The system precision investigation reported a % RSD of 0.64 and 0.75% for NIR and RIT,

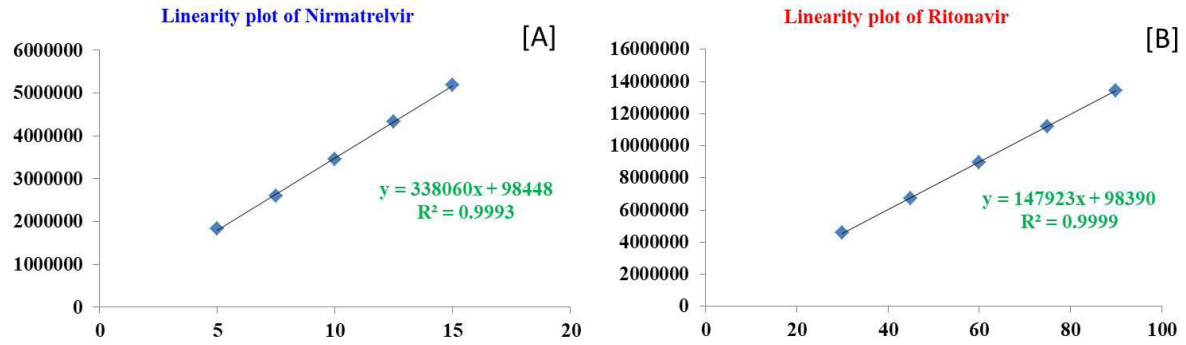

Fig. 3: Linearity graph of NIR (A) and RIT (B).

Table 4: Results of system precision

S. No.	Peak area of NIR	Peak area of RIT
Injection 1	3452937.8	8919249
Injection 2	3445857.9	8913770
Injection 3	3406079.3	8863756
Injection 4	3471840.2	9007758
Injection 5	3458827.5	9046120
Injection 6	3451642.2	8971890
Mean	3447864	8953757
SD	22293.77	81119
%RSD	**0.64**	**0.75**

Table 5: Results of method precision

Sample No.	Weight of NIR in mg	Weight of RIT in mg	Sample peak area of NIR	Sample peak area of RIT	% Assay for NIR	% Assay for RIT
1	150.3	100.1	3453424	8940864	98.5	99.5
2	150.1	100.4	3346548	8735495	98.6	98.9
3	150.1	100.32	3407917	8686481	99.1	98.1
4	149.73	100.41	3471782	8827603	98.9	99.2
5	149.74	100.36	3459144	8865198	98.7	98.6
6	149.74	100.64	3352165	8992452	99.3	99.2
Mean			3415163	8841349	99	99
SD			55377.16	117202.7	0.31	0.50
%RSD			**1.62**	**1.32**	**0.312**	**0.509**

respectively, based on six replicate injections. The % assay values of 12 sample solutions were determined to be within the specified range (less than 2%) during the precision and intermediate precision studies. Tables 4, 5 and 6 display the results for system, method and intermediate precision, respectively.

Accuracy:

The accuracy of the HPLC method for determining NIR and RIT was evaluated through recovery studies. Samples were spiked at different concentration levels, and the percentage recovery (% recovery) was calculated for each level. The acceptable recovery range typically falls between

Table 6: Results of intermediate precision

Inter-day	Sample name	RT	Peak area	RT	Peak area
Day 1	Injection1	3.87	3442897	7.71	8880642
	Injection 2	3.88	3444021	7.72	8873980
	Injection 3	3.89	3448859	7.71	8892401
Day 2	Injection 1	3.88	3473939	7.70	8959402
	Injection 2	3.90	3469510	7.69	8950598
	Injection 3	3.87	3485673	7.72	9000808
Day 3	Injection1	3.92	3452912	7.76	8919182
	Injection 2	3.90	3445027	7.77	8913729
	Injection 3	3.89	3440607	7.68	8863754
Mean		3.88	3455938	7.71	8917166
SD		0.016	16270.5	0.029	45522.82
%RSD		**0.41**	**0.470**	**0.38**	**0.510**

Table 7: Results of accuracy study

Level	Concentration (µg/ml)	Amount Recovered	Percentage Recovery
Nirmatrelvir			
50	5	4.91	98.7
	5	4.93	
	5	4.96	
100	10	10.03	99.9
	10	9.96	
	10	9.97	
150	15	15.15	100.4
	15	15.11	
	15	14.91	
Ritonavir			
50	30	29.72	99.3
	30	29.56	
	30	30.12	
100	60	59.44	99.4
	60	60.13	
	60	59.29	
150	90	90.16	99.6
	90	89.7	
	90	89.04	

Table 8: Results of robustness study

S. No.	Description	Variation	% Assay of precision		% Assay		Difference in assay	
			NIR	RIT	NIR	RI	NIR	RIT
1	Wavelength minus	238	99.3	98.6	99.6	98.7	0.3	0.1
2	Wavelength plus	242	100.2	99.3	99.8	99.2	0.4	0.1
3	Flow rate minus	0.8 ml/min	98.4	99.7	99.3	98.5	0.9	1.2
4	Flow rate plus	1.2 ml/min	99.1	99.1	98.4	99.3	0.7	0.2
5	Temperature minus	20 °C	99.3	98.2	98.7	98.7	0.6	0.5
6	Temperature plus	30 °C	99.6	99.2	99.7	99.6	0.1	0.4

Table 9: Results of ruggedness study

S. No.	Description	% Assay of precision		% Assay		Difference in assay	
		NIR	RIT	NIR	RI	NIR	RIT
1	Different column	98.5	99.2	99.1	99.8	0.6	0.6
2	Different system	99.6	98.1	101.2	99.3	2	1.2
3	Different analyst	100.4	99.6	98.7	98.8	1.7	0.8

Table 10: Analysis of marketed formulation

Paxlovid™ (Nirmatrelvir)		Paxlovid™ (Ritonavir)	
Labeled amount (mg/tablet)	Amount found (mg/tablet)	Labeled amount (mg/tablet)	Amount found (mg/tablet)
150	149.54	100	99.2
	149.38		100.85
	151.82		99.79
	149.65		101
	150.76		98.97
	148.98		99.12
Mean	150	**Mean**	100
SD	1.06	**SD**	0.90
%RSD	0.708	**%RSD**	0.902

98.0% and 102.0%. As indicated in Table 7, the recovery values for both NIR (ranging from 98.7% to 100.4%) and RIT (ranging from 99.3% to 99.6%) were within this acceptable range. These results demonstrate that the HPLC method used provides accurate quantification of NIR and RIT within the tested concentration range.

Sensitivity:

The method's sensitivity is demonstrated by the LOD and LOQ values, which were found to be 0.26 µg/ml and 0.6 µg/ml for NIR and 1.2 µg/ml and 3.6 µg/ml for RIT, respectively.

Robustness:

The robustness of the chromatography was evaluated by systematically adjusting four parameters from the optimized conditions. These parameters included the mobile phase composition (within a range of ± 5%), making slight changes to the flow rate (within a range of ± 0.1 ml/min), and adjusting the column

temperature (within a range of ± 5 °C). Minor variations in the operating parameters did not have a significant impact on the retention time of the desired peak, tailing factor (not exceeding 2.0), plate count (not falling below 2000), resolution (not less than 2.0), and %RSD for six repeated injections (not exceeding 2.0). These values all fell within an acceptable range. The results suggest that the developed approach is highly robust, as shown in Table 8.

Ruggedness:

The ruggedness of the procedure was verified through the analysis of the sample by multiple analysts using different instruments on different days. The findings presented in Table 9 indicate that there were no notable alterations observed in the chromatograms. This suggests that the established RP-HPLC technology is rugged and resistant to variations.

Application of the developed method:

The percentage assay of the NIR and RIT tablets, which was determined to be 100% ± 15, shows that the examined tablets have a percentage purity that is within the acceptable range according to the ICH recommendations. The findings are shown in Table 10.

Mobile phase stability:

At the time of the mobile phase stability study, the mobile phase appeared clear and particle-free, and the findings satisfied the acceptance requirements for system appropriateness and system repeatability, indicating that the mobile phase is stable for up to two days at bench top (RT) storage.

Conclusion

A novel HPLC method was developed for the simultaneous quantification of NIR and RIT, offering a simple and efficient approach for high-throughput analysis. This method achieves rapid separation within a short analysis time, making it ideal for quality control applications. Furthermore, the method exhibits excellent specificity, eliminating interference from common excipients present in pharmaceutical formulations, ensuring accurate determination of the target analytes. Validation studies confirmed the method's robustness, demonstrating its stability and repeatability even under varying experimental conditions and slight chromatographic modifications. These combined advantages make the proposed HPLC method a valuable tool for routine quality control analysis of NIR and RIT in both bulk drug substances and tablet dosage forms.

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