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A NEW ANALYTICAL RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF NIRMATRELVIR AND RITONAVIR IN BULK FORM AND PHARMACEUTICAL DOSAGE FORM

G. Vijaya Kumar[1]*, B. Sravanthi[2], Gaini Pavan[3]

[1*]Professor and Principal, Department of Pharmacy, KGR Institute of Technology & Management, Sy. No. 419, Rampally (V), Keesara (M), Medchal-Malkajgiri (Dist), Telangana – 501 302

[2]Associate Professor, Department of Pharmacy, KGR Institute of Technology & Management, Sy. No. 419, Rampally (V), Keesara (M), Medchal-Malkajgiri (Dist), Telangana – 501 302

[3]Department of Pharmaceutical Analysis, KGR Institute of Technology & Management, Sy. No. 419, Rampally (V), Keesara (M), Medchal-Malkajgiri (Dist), Telangana – 501 302

Abstract:

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Nirmatrelvir and Ritonavir in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Nirmatrelvir and Ritonavir were (1.788, 3.475min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Nirmatrelvir and 1.28µg/ml 3.84µg/ml for Ritonavir respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Nirmatrelvir and Ritonavir in tablets.

Keywords: *Nirmatrelvir and Ritonavir, RP-HPLC, Validation, Accuracy, Precision.*

Corresponding author:

Dr. G. Vijaya Kumar
Professor and Principal,
Department of Pharmacy
Mail id: vijaytanu71@gmail.com
Phone No.: 9849171547

QR CODE

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INTRODUCTION:

Nirmatrelvir (PF-07321332) is an orally bioavailable 3C-like protease (3CLPRO) inhibitor that is the subject of clinical trial NCT04756531. 3CLPRO is responsible for cleaving polyproteins 1a and 1ab of SARS-CoV-2. Without the activity of the SARS-CoV-2 3CLPRO, nonstructural proteins (including proteases) cannot be released to perform their functions, inhibiting viral replication [1]. In the US, Europe, and Canada, Nirmatrelvir, in combination with ritonavir, is indicated for the treatment of mild-to-moderate coronavirus disease 2019 (COVID-19) in adults who are at high risk for progression to severe COVID-19, including hospitalization or death [2]. In Europe, this therapeutic indication is approved under conditional marketing authorization. The IUPAC Name of Nirmatrelvir is 5 (1R, 2S, 5S)-N-[(1S)-1-cyano-2-[(3S)-2-oxo pyrrolidin-3-yl] ethyl]-3-[(2S)-3, 3-dimethyl-2-[(2, 2, 2-trifluoro acetyl) amino] butanoyl]-6, 6-dimethyl-3-azabi cyclo [3.1.0] hexane-2-carboxamide [3]. The Chemical Structure of Nirmatrelvir is shown in follows

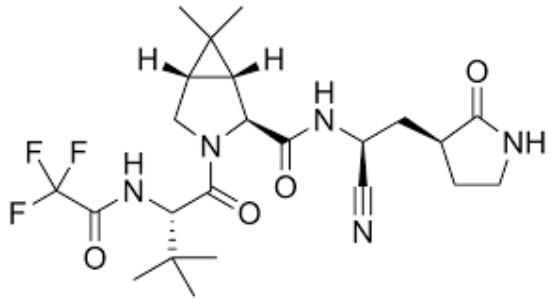

Fig-1: Chemical Structure of Nirmatrelvir

Ritonavir is an antiretroviral protease inhibitor that is widely used in combination with other protease inhibitors in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) [4]. Ritonavir can cause transient and usually asymptomatic elevations in serum aminotransferase levels and, rarely, can lead to clinically apparent acute liver injury. In HBV or HCV coinfected patients, highly active antiretroviral therapy with ritonavir may result of an exacerbation of the underlying chronic hepatitis B or C [5]. Ritonavir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. The IUPAC Name and the Chemical Structure of Ritonavir is follows 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-diphenyl hexan-2-yl] carbamate [6].

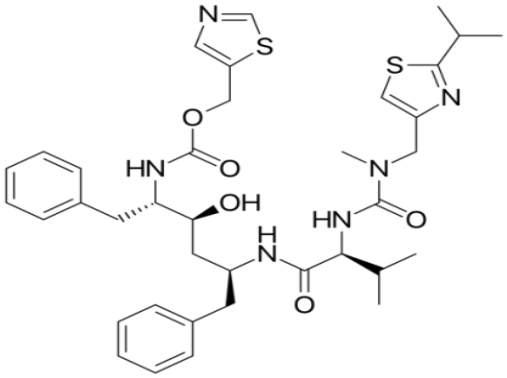

Fig-2: Chemical Structure of Ritonavir

MATERIALS AND METHODS:

Table-1: Instruments Used

S.No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S.No	Chemical	Brand Names
1	Nirmatrelvir	Synpharma Research Lab, Hyderabad
2	Ritonavir	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Nirmatrelvir and Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [7-10].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 28:72 (pH-3.8) v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry (C18) (150mm x 4.6mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow [11].

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.8):

Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of Mobile Phase:

Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration [12-14].

Diluent Preparation:

Procedure:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent [15].

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity Study of Drug:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent [16].

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Nirmatrelvir and Ritonavir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.2ml of the sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

The mean and percentage relative standard deviation were calculated from the peak areas [17].

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Preparation of Drug Solutions for Linearity:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Nirmatrelvir & 10ppm of Ritonavir):
Pipette out 0.1ml of Nirmatrelvir and 0.1ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (15ppm of Nirmatrelvir & 20ppm of Ritonavir):
Pipette out 0.15ml of Nirmatrelvir and 0.2ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Nirmatrelvir & 30ppm of Ritonavir):
Pipette out 0.2ml of Nirmatrelvir and 0.3ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent [18].

Preparation of Level – IV (25ppm of Nirmatrelvir & 40ppm of Ritonavir):
Pipette out 0.25ml of Nirmatrelvir and 0.4ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (30ppm of Nirmatrelvir & 50ppm of Ritonavir):
Pipette out 0.3ml of Nirmatrelvir and 0.5ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:
Inject each level into the chromatographic system and measure the peak area.
Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient [19-20].

Precision

Repeatability

Preparation of Nirmatrelvir and Ritonavir Product Solution for Precision:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [21].

Intermediate Precision:
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2:
The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits [22].

Accuracy:

For preparation of 50% Standard stock solution:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.1ml of the above Nirmatrelvir and 0.15ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of Nirmatrelvir and 0.45ml of Ritonavir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents [23].

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for

Nirmatrelvir and Ritonavir and calculate the individual recovery and mean recovery values [24].

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded [25].

Effect of Variation of Mobile Phase Organic Composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 33:64, 23:77 instead (28:72), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION:

Analytical Method Development:
Optimized Chromatographic Conditions:
Mobile phase ratio: Methanol: Phosphate Buffer (pH-3.8) (28:72v/v)
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Column temperature: Ambient
Wavelength: 252nm
Flow rate: 1.0ml/min
Injection volume : 20µl
Run time: 8minutes

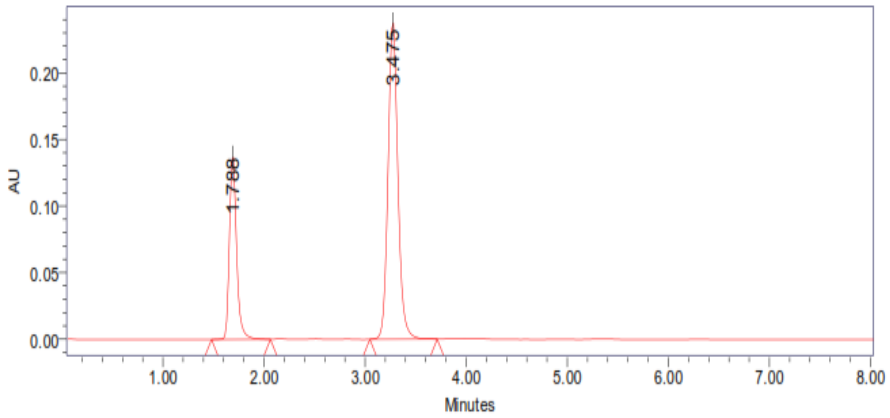

Fig-3: Optimized Chromatogram (Standard)

Analytical Method Validation
Specificity
The ICH documents define specificity as the ability to assess unequivocally the analysis in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. The analytical method was tested for specificity to measure accurately quantities of Nirmatrelvir and Ritonavir in drug product [26].

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of Standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of simple}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Observation: The % purity of Nirmatrelvir and Ritonavir in pharmaceutical dosage form was found to be 100.154%.

Linearity:

Table-3: Chromatographic Data For Linearity Study For Nirmatrelvir:

Concentration µg/ml	Average Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584

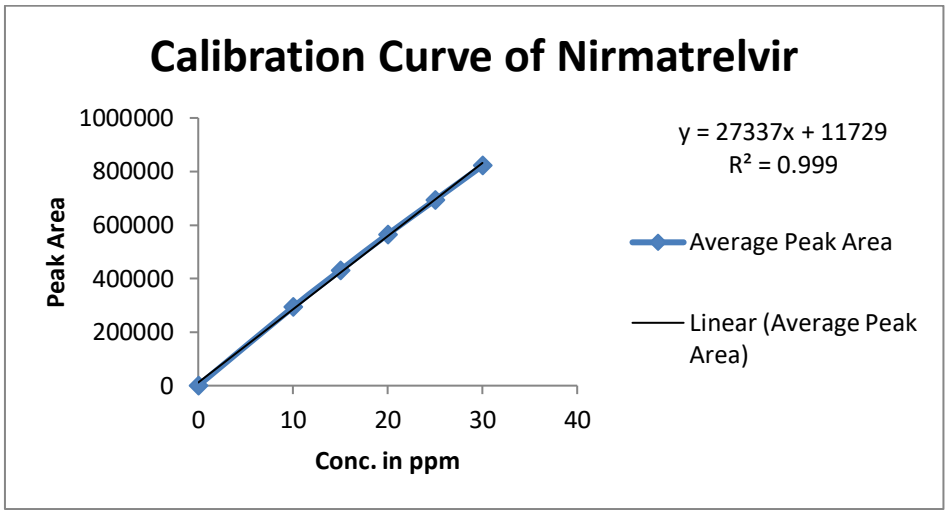

Fig-4: Chromatogram Showing Linearity Level

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Nirmatrelvir is a straight line.

Y = mx + c
Slope (m) = 27337
Intercept (c) = 11729
Correlation Coefficient (r) = 0.999

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 11729. These values meet the validation Criteria [27].

Table-4: Chromatographic Data For Linearity Study For Ritonavir:

Concentration µg/ml	Average Peak Area
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985

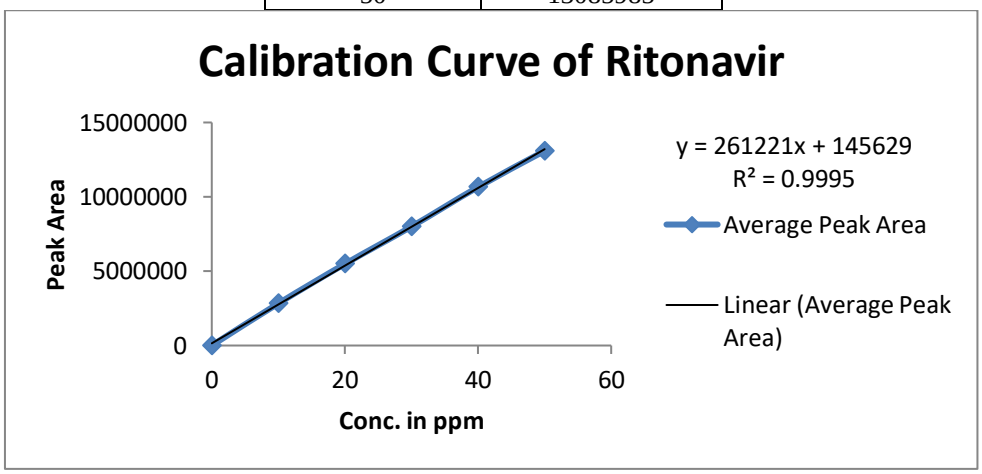

Fig-5: Chromatogram Showing Linearity Level

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Ritonavir is a straight line.

Y = mx + c
Slope (m) = 26122
Intercept (c) = 14562
Correlation Coefficient (r) = 0.9994

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 14562. These values meet the validation criteria.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [28].

Repeatability: Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-5: Results of Repeatability for Nirmatrelvir

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nirmatrelvir	1.792	548698	7458	7569	1.10
2	Nirmatrelvir	1.791	548955	7485	7546	1.10
3	Nirmatrelvir	1.790	548745	7469	7592	1.09
4	Nirmatrelvir	1.790	549856	7463	7519	1.10
5	Nirmatrelvir	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std. Dev			1202.217			
%RSD			0.2191554			

Table-6: Results of Repeatability for Ritonavir:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ritonavir	3.435	7768958	43659	8659	1.12
2	Ritonavir	3.428	7765984	43856	8647	1.13
3	Ritonavir	3.419	7785469	43658	8675	1.12
4	Ritonavir	3.414	7785498	43549	8652	1.12
5	Ritonavir	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std. Dev			9539.236			
%RSD			0.122689			

Intermediate Precision:

Day 1:

Table-7: Results of Intermediate Precision day1 for Nirmatrelvir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nirmatrelvir	1.787	556985	75986	7695	1.11
2	Nirmatrelvir	1.789	558649	75986	7642	1.12
3	Nirmatrelvir	1.789	557847	75689	7683	1.12
Mean			557827			
Std. Dev.			832.1803			
% RSD			0.149183			

Table-8: Results of Intermediate Precision day1 for Ritonavir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ritonavir	3.482	7856982	44586	8758	1.13
2	Ritonavir	3.477	7845285	44758	8769	1.14
3	Ritonavir	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std. Dev.			6187.659			
% RSD			0.078801			

Day 2:

Table-9: Results of Intermediate Precision Day 2 for Nirmatrelvir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nirmatrelvir	1.790	536598	7365	7459	1.08
2	Nirmatrelvir	1.789	534875	7358	7436	1.07
3	Nirmatrelvir	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std. Dev.			1049.608			
% RSD			0.196045			

Table-10: Results of Intermediate Precision Day 2 for Ritonavir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ritonavir	3.474	7698521	42568	8582	1.11
2	Ritonavir	3.473	7685985	42698	8546	1.10
3	Ritonavir	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std. Dev.			27487.83			
% RSD			0.358064			

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated [29].

Table-11: The Accuracy Results for Nirmatrelvir

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

Table-12: The Accuracy Results for Ritonavir

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

Limit of Detection for Nirmatrelvir and Ritonavir

The detection limit of an individual analytical procedure is the lowest Amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Nirmatrelvir = 0.86µg/ml

Ritonavir = 1.28µg/ml

Quantitation Limit For Nirmatrelvir and Ritonavir

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Nirmatrelvir = 2.58µg/ml

Ritonavir = 3.84µg/ml

Robustness: The robustness was performed for the Flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Nirmatrelvir and Ritonavir. The method is robust only in less Flow condition, and the method is robust even with a change in the mobile phase ±5%. The Standard and samples of Nirmatrelvir and Ritonavir were injected by changing the conditions of chromatography. There was no significant change in parameters like resolution, tailing factor, asymmetric factor, and Plate count [30].

Table-13: Results for Robustness -Nirmatrelvir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase (about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table-14: Results for Robustness-Ritonavir

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase (about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

Stability Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient [31].

Table-15: Results of Forced Degradation Studies of Nirmatrelvir

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	545265	0	100%	100%
2	Acidic	389537.316	28.56	71.44	100%
3	Basic	501899.409	7.97	92.03	100%
4	Oxidative	176393.227	32.35	67.65	100%
5	Thermal	117831.766	21.61	78.39	100%
6	Photolytic	212489.770	38.97	61.03	100%

Table-16: Results of Forced Degradation Studies of Ritonavir

S.No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	7768545	0	100%	100%
2	Acidic	5706773.157	26.54	73.46	100%
3	Basic	7093458.439	8.69	91.31	100%
4	Oxidative	5424774.973	30.17	69.83	100%
5	Thermal	1545163.600	19.89	80.11	100%
6	Photolytic	2522446.561	32.47	67.53	100%

SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 252 nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Symmetry (C18) (150mm x 4.6mm, 5µm) Column because it was giving good peak. An ambient temperature was found to be suitable for the nature of drug solution.

The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Run time was selected to be 8 min because analyze gave peak around 1.788, 3.475 ± 0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102% was linear and precise over the same range. Both system and method precision were found to be accurate and well within range. The analytical method was found linearity over the range 10-30mg/ml of Nirmatrelvir and 10-50mg/ml of Ritonavir of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory. Stability study correspondingly confirmed the specificity of the method. As a part of peak purity study, peak threshold was found to be higher than angle and no flag for both the analytes was observed. Degradation study revealed that Nirmatrelvir and Ritonavir were degraded in oxidative and photolytic condition only.

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