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QUANTITATIVE ESTIMATION OF NIRMATRELVIR AND RITONAVIR MALEATE IN TABLET DOSAGE FORMS BY RP-HPLC METHOD

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ABSTRACT
A Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and optimized for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablet dosage forms. The method was carefully developed by studying various chromatographic parameters to ensure optimal separation and accurate quantification of both drugs. The maximum absorbance for both drugs was observed at 220 nm, with excellent peak purity. A Sunfire C18 column (4.6×250 mm, 5 µm) was used to achieve good peak resolution. The flow rate was fixed at 0.9 mL/min, and the mobile phase was optimized as a mixture of Water and Acetonitrile (60:40% v/v), providing symmetrical peaks and satisfactory separation. The method demonstrated high precision, with recovery values ranging from 98.0-102% and both system and method precision found to be accurate within acceptable limits. The total run time for the analysis was 6 minutes, with Nirmatrelvir and Ritonavir eluting at 3.0 and 3.8 ± 0.02 minutes, respectively. This RP-HPLC method provides a reliable, accurate, and efficient approach for the quantitative analysis of Nirmatrelvir and Ritonavir in tablet dosage forms, suitable for routine quality control and regulatory compliance.

KEYWORDS: Nirmatrelvir and Ritonavir, RP-HPLC, Validation, Accuracy.

INTRODUCTION
Nirmatrelvir is an inhibitor of a cysteine residue in the 3C-like protease (3CLPRO) of SARS-CoV-2. This cysteine is responsible to the activity of the 3CLPRO of SARS-CoV-2 and potentially other members of the coronavirus family. The 3CLPRO, also known as the main protease or nonstructural protein 5, is responsible for cleaving polyproteins 1a and 1ab. These polyproteins contain the 3CLPRO itself, a papain-like (PL) cysteine protease, and 14 other nonstructural proteins. Without the activity of the 3CLPRO, nonstructural proteins (including proteases) cannot be released to perform their functions, inhibiting viral replication.

Ritonavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.

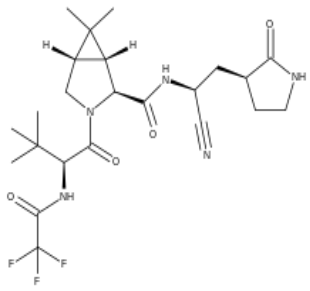

Fig. 1: Chemical Structure of Nimatrelvir.

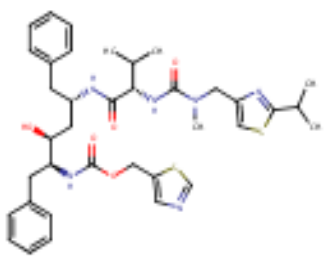

Fig. 2: Chemical Structure of Ritanovir.

MATERIALS AND METHODS

Table 1: Instruments used.

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, PDA 996 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	Sura labs
2	Ritonavir	Sura labs
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck
5	Triethylamine	Merck

HPLC METHOD DEVELOPMENT

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60 v/v respectively.

VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) were mixed and degassed in digital ultra sonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

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 0.15ml of the Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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 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 0.15ml of the Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution

Take average weight of Tablet and crush in a mortar 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 0.3ml of the Sample stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure

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 (5ppm of Nirmatrelvir & 10ppm of Ritonavir)
Pipette out 0.05ml 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 (10ppm of Nirmatrelvir & 20ppm of Ritonavir)
Pipette out 0.1ml 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 (15ppm of Nirmatrelvir & 30ppm of Ritonavir)
Pipette out 0.15ml of Nirmatrelvir and 0.3ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (20ppm of Nirmatrelvir & 40ppm of Ritonavir)
Pipette out 0.2ml 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 (25ppm of Nirmatrelvir & 50ppm of Ritonavir)
Pipette out 0.25ml 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.

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 0.15ml of the 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.

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.

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 0.075ml of the 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 0.15ml of the 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 0.225ml of the Nirmatrelvir and 0.45ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure
Inject the Three replicate injections of individual concentrations (50%, 100%, 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.

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 0.15ml of the Nirmatrelvir and 0.45ml 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 0.8ml/min and 1.0ml/min instead of 0.9ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile and Water was taken in the ratio and 35:65, 45:55 instead (40:60), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	:	Acetonitrile: Water (40:60v/v)
Column	:	Sunfire C18 (4.6×250mm) 5µ
Column temperature	:	35ºC
Wavelength	:	220nm
Flow rate	:	0.9ml/min
Injection volume	:	10µl
Run time	:	6minutes

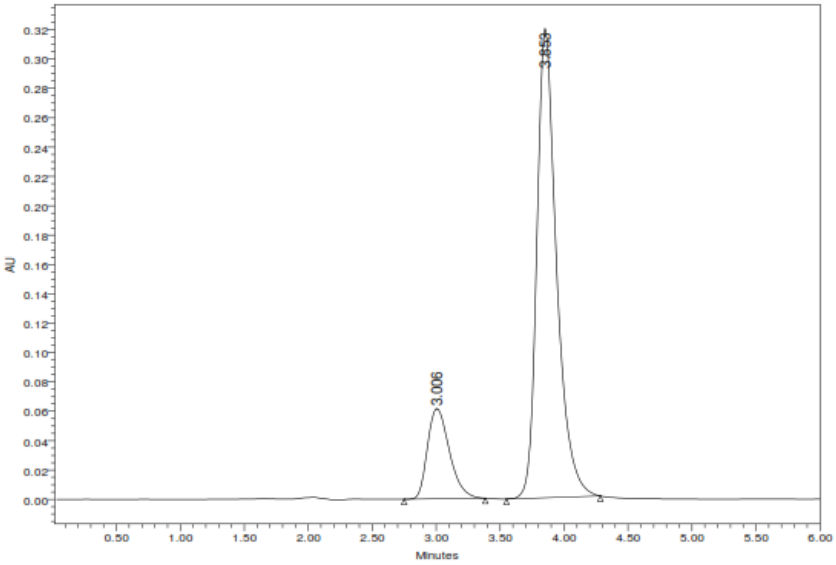

Fig. No. 3: Optimized Chromatogram (Standard).

Table No. 3: Optimized Chromatogram (Standard).

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nirmatrelvir	3.006	731322	61677	1.2	8574
2	Ritonavir	3.853	3421257	319786	1.1	9664

Optimized Chromatogram (Sample)

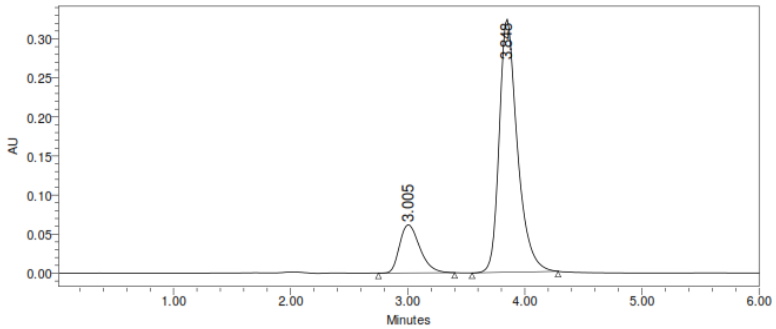

Fig. No. 4: Optimized Chromatogram (Sample).

Table No. 4: Optimized Chromatogram (Sample).

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nirmatrelvir	3.005	658995	61772	1.1	7442
2	Ritonavir	3.848	3096188	324054	1.2	7331

VALIDATION

SPECIFICITY

Table No. 5: Peak results for assay standard of Nirmatrelvir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nirmatrelvir	3.008	658263	61335	7462	1.2
2	Nirmatrelvir	3.009	658264	61947	8264	1.1
3	Nirmatrelvir	3.008	653426	61049	6627	1.2
4	Nirmatrelvir	3.010	653058	61141	7264	1.1
5	Nirmatrelvir	3.006	657393	61735	6645	1.1
Mean			656080.8			
Std. Dev.			2618.946			
% RSD			0.39918			

Acceptance criteria

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table No. 6: Peak results for assay standard of Ritonavir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ritonavir	3.857	3028176	381011	9583	1.1
2	Ritonavir	3.859	3018373	381645	8927	1.2
3	Ritonavir	3.857	3018462	381663	8465	1.1
4	Ritonavir	3.861	3081711	381746	9222	1.2
5	Ritonavir	3.853	3075143	381193	8462	1.1
Mean			3044373			
Std. Dev.			31427.07			
% RSD			1.0323			

Table No. 7: Peak results for Assay sample of Nirmatrelvir.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nirmatrelvir	3.008	651712	61173	1.2	8563
2	Nirmatrelvir	3.005	657635	61936	1.1	7462
3	Nirmatrelvir	3.007	658917	61196	1.1	9264

Table No. 08: Peak results for Assay sample of Ritonavir.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Ritonavir	3.854	3029472	361938	1.1	6476
2	Ritonavir	3.853	3017462	361746	1.1	7264
3	Ritonavir	3.855	3028171	371864	1.2	6545

The % purity of Nirmatrelvir and Ritonavir in pharmaceutical dosage form was found to be 100.5%

LINEARITY

Table No. 09: Chromatographic data for linearity study for Nirmatrelvir.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	230247
66.6	10	462332
100	15	659905
133.3	20	892989
166.6	25	1101075

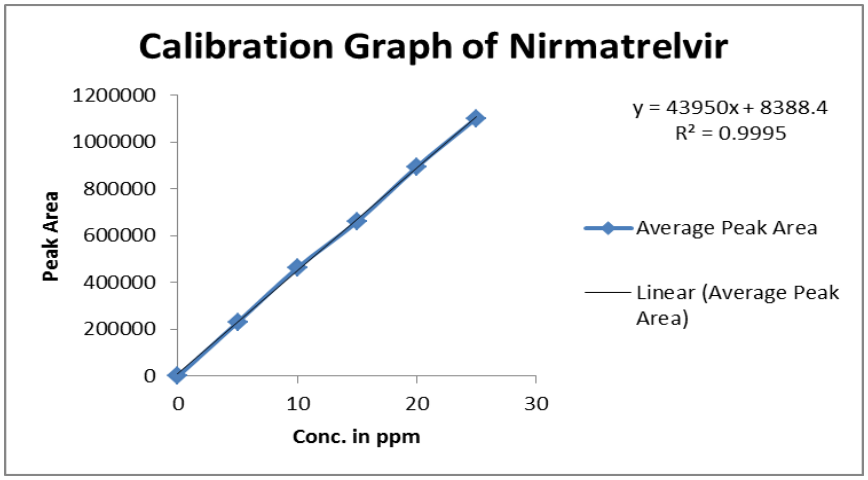

Fig. No. 05: Chromatogram showing linearity level.

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 8388. These values meet the validation criteria.

Table No. 10: Chromatographic data for linearity study for Ritonavir.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	10	1215225
66.6	20	2135937
100	30	3020839
133.3	40	4078841
166.6	50	5058145

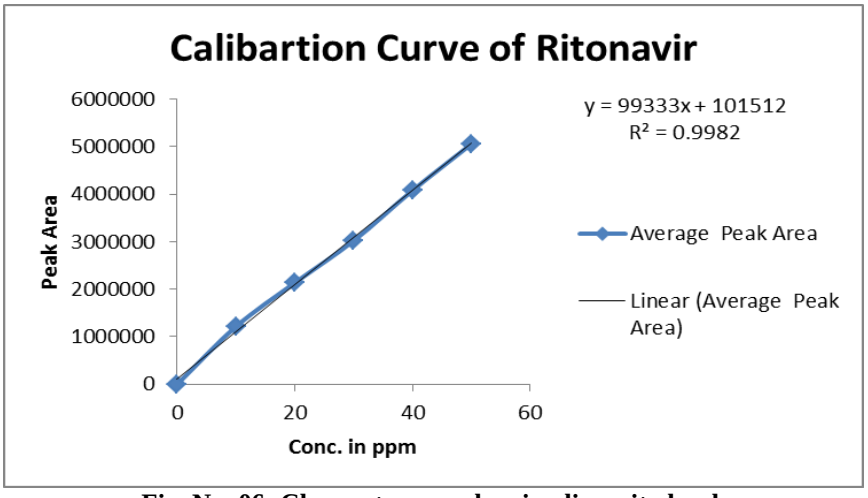

Fig. No. 06: Chromatogram showing linearity level.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 10151. 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.

REPEATABILITY

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

Table 11: Results of repeatability for Nirmatrelvir.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Nirmatrelvir	3.003	654426	61521	8474	1.1
2	Nirmatrelvir	3.005	659862	61937	8262	1.2
3	Nirmatrelvir	3.007	650837	62018	8117	1.1
4	Nirmatrelvir	3.008	651433	61893	7917	1.2
5	Nirmatrelvir	3.005	652752	61867	8011	1.1
Mean			653862			
Std. dev			3626.323			
%RSD			0.554601			

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table No. 12: Results of repeatability for Ritonavir.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Ritonavir	3.851	3028371	381736	6881	1.1
2	Ritonavir	3.852	3009188	380138	9363	1.2
3	Ritonavir	3.854	3067464	386615	7844	1.1
4	Ritonavir	3.853	3076611	380183	9746	1.2
5	Ritonavir	3.851	3011912	379471	7883	1.2
Mean			3038709			
Std. dev			31463.69			
%RSD			1.035429			

Acceptance criteria

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Day 1

Table No. 13: Results of Intermediate precision day1 for Nirmatrelvir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Nirmatrelvir	3.007	658911	60173	9141	1.1
2	Nirmatrelvir	3.005	650383	61936	9662	1.2
3	Nirmatrelvir	3.005	658813	60383	9746	1.1
4	Nirmatrelvir	3.005	651138	60774	7746	1.1
5	Nirmatrelvir	3.005	659937	61947	8264	1.2
6	Nirmatrelvir	3.010	653715	61893	7836	1.1
Mean			655482.8			
Std. Dev.			4258.945			
% RSD			0.649742			

Acceptance criteria

%RSD of Six different sample solutions should not more than 2

Table No. 14: Results of Intermediate precision day1 for Ritonavir.

S. No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Ritonavir	3.851	3021731	369771	8564	1.1
2	Ritonavir	3.848	3019183	372746	9227	1.1
3	Ritonavir	3.848	3029847	371866	7565	1.2
4	Ritonavir	3.850	3028471	369017	7726	1.1
5	Ritonavir	3.849	3088641	376453	6746	1.2
6	Ritonavir	3.860	3056633	386621	5977	1.1

Mean			3040751			
Std. Dev.			26990.09			
% RSD			0.887613			

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2.

Day 2

Table No. 15: 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	3.006	648822	61847	6983	1.1
2	Nirmatrelvir	3.008	640863	59882	7728	1.2
3	Nirmatrelvir	3.008	643382	60774	9576	1.1
4	Nirmatrelvir	3.007	641884	58928	8275	1.2
5	Nirmatrelvir	3.007	647822	61483	9837	1.1
6	Nirmatrelvir	3.005	649181	60928	8744	1.2
Mean			645325.7			
Std. Dev.			3711.009			
% RSD			0.57506			

Acceptance criteria

%RSD of Six different sample solutions should not more than 2 Table:

Table No. 16: 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	3.853	3075833	389911	7039	1.1
2	Ritonavir	3.857	3029583	379019	9857	1.2
3	Ritonavir	3.854	3021991	381875	7881	1.1
4	Ritonavir	3.855	3022485	391099	7902	1.2
5	Ritonavir	3.854	3085833	389222	9285	1.1
6	Ritonavir	3.853	3019482	391184	8955	1.2
Mean			3042535			
Std. Dev.			30022.42			
% RSD			0.986757			

Acceptance criteria

- %RSD of Six different sample solutions should not more than 2

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

Table No. 17: Results of Accuracy for concentration-50%.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nirmatrelvir	3.006	335352	31861	1.1	8573
2	Nirmatrelvir	3.022	336153	39371	1.1	5891
3	Nirmatrelvir	3.006	330183	37857	1.2	6573
4	Ritonavir	3.855	1593716	179472	1.1	9164
5	Ritonavir	3.877	1583631	178947	1.2	8264
6	Ritonavir	3.854	1579482	176534	1.1	7248

Table No. 18: Results of Accuracy for concentration-150%.

S. No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Nirmatrelvir	3.004	974626	89388	1.1	8462
2	Nirmatrelvir	3.006	975411	89749	1.2	9771
3	Nirmatrelvir	3.008	970815	88937	1.2	8947
4	Ritonavir	3.847	4598264	436613	1.1	7917
5	Ritonavir	3.851	4589462	439282	1.1	9364
6	Ritonavir	3.853	4501948	437167	1.2	8462

Table No. 19: The accuracy results for Nirmatrelvir.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	331938	7.5	7.3	99.88	100.166
100%	658274	15	14.7	98.89	
150%	970963	22.5	22.2	101	

Acceptance Criteria

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table No. 20: The accuracy results for Ritonavir.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	7.5	7.49	99.7%	99%
100%	420697.7	15	14.9	99%	
150%	631550.7	22.5	22.48	99%	

Acceptance Criteria: The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

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 quantitated as an exact value.

$$LOD = 3.3 \times \sigma / s$$

Nirmatrelvir
=3.3×9373/43950 =0.7µg/ml

Ritonavir
=3.3×5548/9933 =1.8µg/ml

QUANTITATION LIMIT

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

$$LOQ = 10 \times \sigma / S$$

Nirmatrelvir
=10×9373/43950 =2.1µg/ml
Ritonavir
=10×5548/9933 =5.5µg/ml
Robustness

Table No. 21: Results for Robustness –Nirmatrelvir.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

Table No. 22: Results for Robustness-Ritonavir.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

Acceptance criteria: The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

SUMMARY AND CONCLUSION

Summary

A Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablet dosage forms. The method was optimized by evaluating several chromatographic parameters to ensure optimal separation and accurate quantification. The maximum absorbance for both drugs was observed at 220 nm, and a Sunfire C18 column (4.6×250 mm, 5 µm) was used for efficient peak resolution. The mobile phase consisted of a mixture of Water and Acetonitrile (60:40% v/v), and the flow rate was fixed at 0.9 mL/min. The injection volume was 10 µL, and the temperature was maintained at 35 ºC for improved drug stability. The analysis was completed in 6

minutes, with Nirmatrelvir and Ritonavir eluting at 3.0 and 3.8 ± 0.02 minutes, respectively. The method demonstrated excellent linearity in the concentration range of 5-25 µg/mL for Nirmatrelvir and 10-50 µg/mL for Ritonavir, with recovery values between 98.0-102%, indicating high accuracy and precision. Additionally, the method passed robustness and ruggedness tests, ensuring its reliability for routine quality control applications.

CONCLUSION

The developed RP-HPLC method for the simultaneous estimation of Nirmatrelvir and Ritonavir is reliable, accurate, and efficient. The method is characterized by excellent linearity, precision, and recovery, making it suitable for routine quantitative analysis of these drugs in tablet dosage forms. The optimized chromatographic conditions, including the choice of mobile phase, flow rate, and column, resulted in good separation and peak symmetry. With a short analysis time of 6 minutes, this method is not only time-efficient but also meets the stringent requirements for regulatory compliance and quality control in pharmaceutical laboratories. The method's robustness and ruggedness further confirm its applicability for consistent and reproducible analysis in routine quality assessments.

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