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Research Article

NEW STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF NIRMATRELVIR AND RITONAVIR IN BULK AND TABLET DOSAGE FORM

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ABSTRACT:

Nirmatrelvir and Ritonavir are prescribed to treat COVID-19. An cheap HPLC approach was developed and is utilized for validation. KH2PO4 and MeCN are combined in an Agilent C18 150x4.6mm, 5m column at a flow rate of 1 ml/min at a temperature of 30 oC using a 60:40 v/v ratio. Its length was 215.0 nm. Nirmatrelvir had a holding time of 2.243 hours, whereas Ritonavir took 2.815 hours. It was discovered that the %RSD was 0.5% and 0.5%. The Nirmatrelvir regression equation was: y = 58409x + 2794. It was also discovered that ritonavir was y = 59191x + 774.23. Additionally, the LOD and LOQ values were discovered, and all tests passed and met ICH criteria

Keywords: Nirmatrelvir, Ritonavir, Rp HPLC, Validation, Method Development.

INTRODUCTION

Nirmatrelvir and ritonavir are antiviral agents co-formulated under the brand name Paxlovid, developed by Pfizer for the treatment of COVID-19. Nirmatrelvir is a protease inhibitor targeting the SARS-CoV-2 main protease (Mpro), essential for viral replication. Ritonavir, originally developed as an HIV protease inhibitor, functions here primarily to inhibit cytochrome P450 3A4 (CYP3A4), thereby increasing nirmatrelvir's plasma concentration and prolonging its therapeutic effect.[1]

Clinical trials have demonstrated the efficacy of nirmatrelvir-ritonavir in reducing the risk of COVID-19-related hospitalization or death. A pivotal study reported an 89% reduction in such risks among high-risk, non-hospitalized adults treated within five days of symptom onset.[2]

The U.S. Food and Drug Administration (FDA) granted emergency use authorization for Paxlovid in December 2021, followed by full approval in May 2023 for the treatment of mild-to-moderate COVID-19 in adults at high risk for progression to severe disease.[3]

Subsequent studies have reinforced the effectiveness of nirmatrelvir-ritonavir in diverse populations, including vaccinated individuals and those with prior COVID-19 infections. Research indicates significant reductions in hospitalization and mortality rates, underscoring its role in the therapeutic landscape of COVID-19.[4]

However, recent investigations into the use of nirmatrelvir-ritonavir for treating post-acute sequelae of SARS-CoV-2 infection (PASC), commonly known as Long COVID, have not demonstrated significant improvements in symptoms compared to placebo.[5]

It is important to note that nirmatrelvir-ritonavir can interact with various medications, potentially leading to serious side effects. Healthcare providers should thoroughly review a patient's medication regimen to manage potential drug interactions effectively.[6]

In summary, the combination of nirmatrelvir and ritonavir represents a significant advancement in the treatment of COVID-19, offering substantial benefits in reducing severe outcomes in high-risk populations. Ongoing research continues to explore its full therapeutic potential and optimal use in various clinical scenarios.[7]

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Background: Nirmatrelvir is oral protease inhibitor with emergency use authorization for the treatment of mild-to-moderate COVID-19.is chemically known as (1R,2S,5S)-N-[(1S)-1-cyano-2-[(3S)-2-oxopyrrolidin-3-yl]ethyl]-3-[(2S)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido)butanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide[8] and Ritonavir is an HIV protease inhibitoris chemically known as (1,3-thiazol-5-yl)methyl N-[(2S,3S,5S)-3-hydroxy-5-[(2S)-3-methyl-2-{[methyl({[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl})carbamoyl]amino}butanamido]-1,6-diphenylhexan-2-yl]carbamate [9]

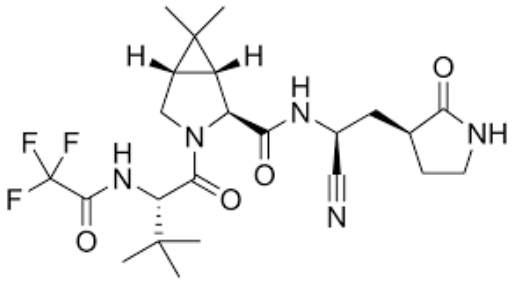

Figure 1: structure of Nirmatrelvir

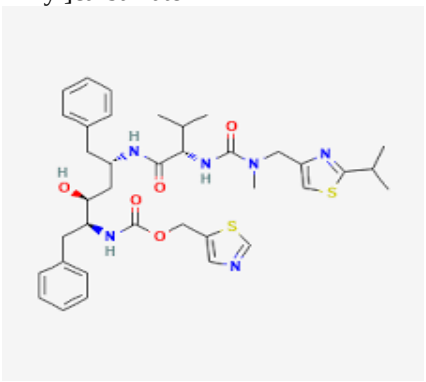

Figure 2: Structure of Ritonavir

Extensive literature research has unearthed a multitude of recorded analytical procedures, including the discovery of more economically efficient ways. Nevertheless, there is currently no documented approach for calculating stability studies. Hence, a reliable and cost-effective approach is suggested for assessing the stability of Nirmatrelvir, Ritonavir, and their medicinal dose form using RP-HPLC. [10-18] must be validated and developed as per ICH guidelines.

Materials and Methods: Nirmatrelvir and Ritonavir pure pharmaceuticals (API) are available as gift samples from Spectrum Pharma Research Solution. The chemicals and buffers used in this estimation were supplied by Rankem, an Indian source.

Instrumentation: The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Objective: In order to ful fill ICH standards, we need to design and test an HPLC technique that can detect Ritonavir and Nirmatrelvir in pharmaceutical formulations at the same time.

Table 1 : Chromatographic Conditions

Mobile phase	Acetonitrile: OPA (40:60 v/v)
Flow rate	1.0 ml/min
Column	Agilent C18 Column, 5 µm, 4.6 x 150 mm
Detector wave length	215 nm
Column temperature	26°C
Injection volume	10µL
Run time	10.0 min

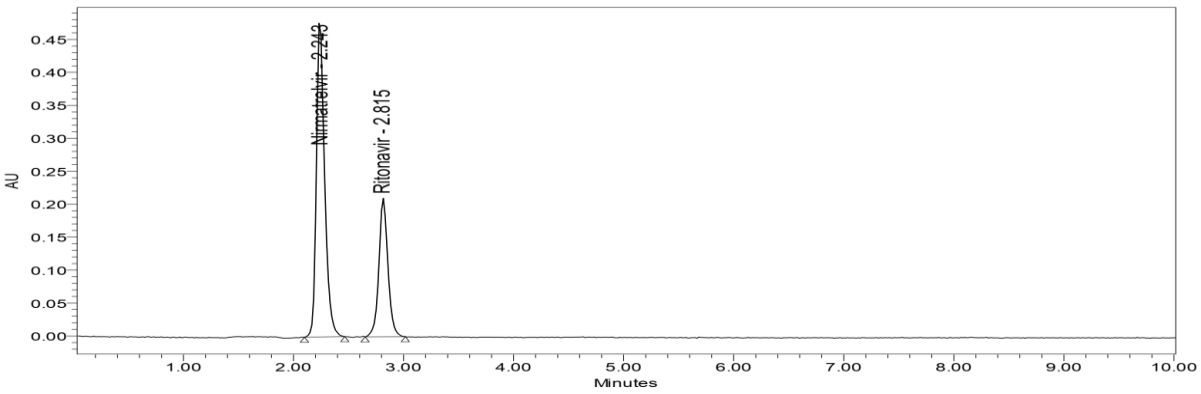

Figure 3: Optimized Chromatogram

Solution of Standard: 7.5 mg of Nirmatrelvir & 5 mg of Ritonavir of API drug was weigh and added in a separate 50ml vf and it was filled 3/4th volume with the Dil. And then it was under sonication for 10-15 min till

the drug dissolves. (Nirmatrelvir- 150µg/ml, Ritonavir- 100µg/ml) from stock 1ml was taken and added in a 10ml vf and madeup till mark with diluent. (Nirmatrelvir- 15µg/ml, Ritonavir- 10µg/ml)

Sample preparation: Ten tabs takes as mean weight of dose determines to similar to 150 mg and 100 mg. Was acquired Subsequently, 20 ml of acetonitrile was introduced, subjected to sonication for 25 minutes, and adjusted to the mark to achieve concentrations of 1100 and 500 µg/ml. The sample was centrifuges till 20minutes. The sediment subsequently taken and filter using 0.45µm filters. (Nirmatrelvir-300µg/ml, Ritonavir-200µg/ml). from it 1 ml solution was added in a 10ml vf and madeup till mark with diluent. (Nirmatrelvir-15µg/ml, Ritonavir- 10µg/ml)

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Nirmatrelvir (15ppm) and Ritonavir (10ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Table 2: System suitability results

S.no	Nirmatrelvir			Ritonavir			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	2.254	4121	1.29	2.805	5264	1.04	3.5
2	2.256	4233	1.29	2.807	5484	1.07	3.6
3	2.257	4246	1.27	2.810	5575	1.06	3.7
4	2.258	4422	1.28	2.813	5241	1.03	3.6
5	2.261	4312	1.27	2.813	5242	1.05	3.7
6	2.261	4295	1.25	2.814	5339	1.03	3.6

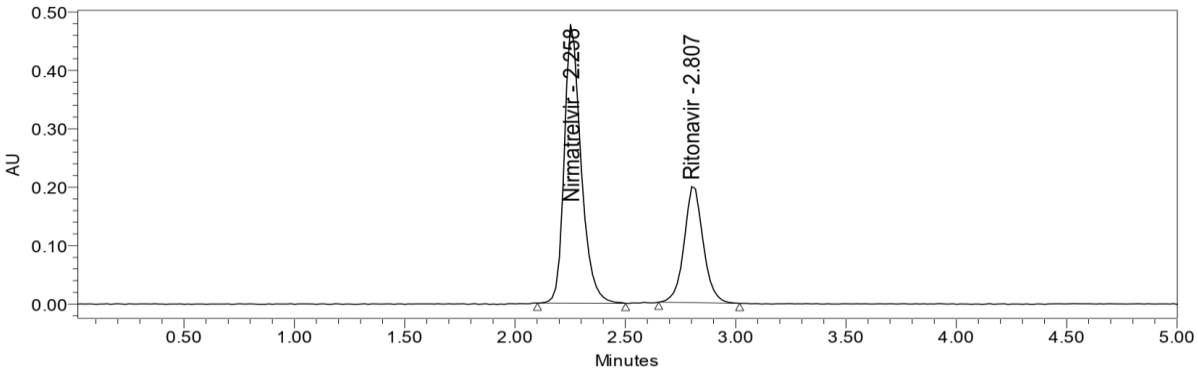

Figure 4: system suitability Chromatogram

Table 3: Specificity data

Sample name	Retention time(mins)	Area	Plate count	Resolution
Nirmatrelvir	2.243	249964	1.31	
Ritonavir	2.815	120306	1.05	4.0

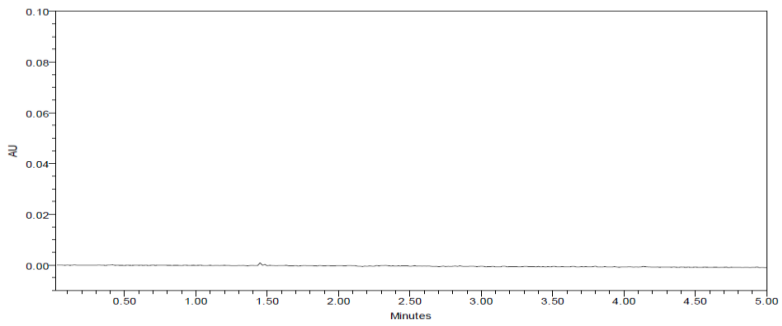

Figure 5: Blank

Linearity:

Calibration data and regression data in table 4 and calibration curve in figure 6, 7.

Table 4: Calibration data of Nirmatrelvir and Ritonavir

	Nirmatrelvir		Ritonavir	
S. no	**Conc (µg/mL)**	**Peak area**	**Conc(µg/mL)**	**Peak area**
1	0	0	0	0
2	3.75	226737	2.5	144621
3	7.5	439995	5	302858
4	11.25	653845	7.5	445501
5	15	881916	10	589965
6	18.75	1105835	12.5	744495
7	22.5	1310944	15	885503
Concentration range	**3.75-22.5**		**2.5-15**	
Regression Equation	**y = 58409x + 2794**		**y = 59191x + 774.23**	
Co-relation	**0.9999**		**0.9999**	
LOD	**0.01**		**0.04**	
LOQ	**0.04**		**0.12**	

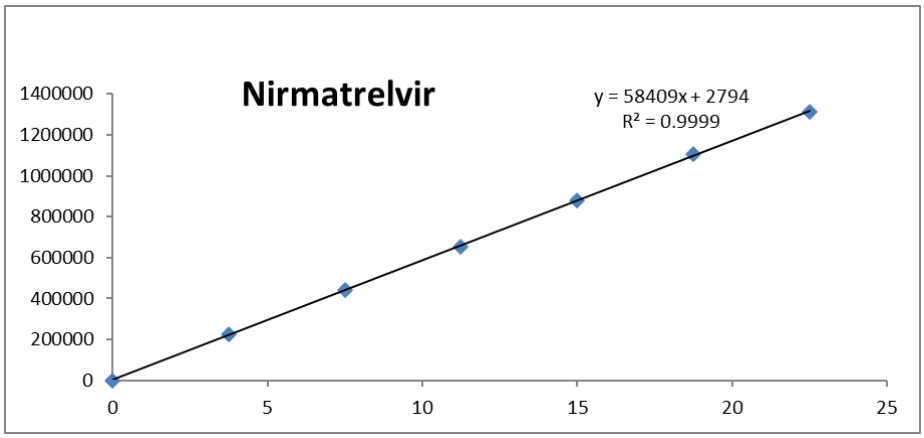

Figure 6: Calibration curve of Nirmatrelvir

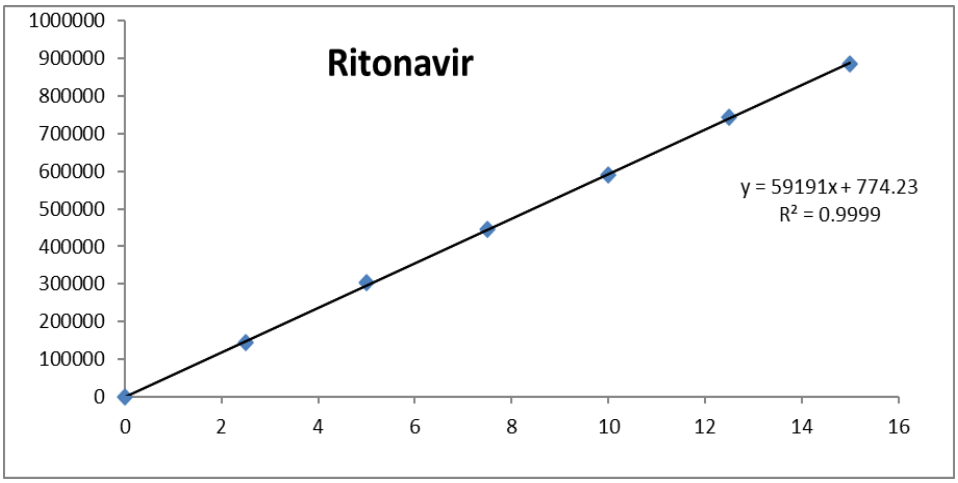

Figure 7: Calibration curve of Ritonavir

Accuracy:

Recovery data shown in table 5

Table 5: recovery data of Nirmatrelvir and Ritonavir

	Nirmatrelvir			Ritonavir		
% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**
50%	7.5	7.42	98.89	5	4.95	99.04
	7.5	7.44	99.26	5	4.96	99.18
	7.5	7.37	98.24	5	4.95	99.09
100%	15	15.09	100.58	10	9.98	99.84
	15	15.08	100.52	10	10.08	100.85
	15	15.05	100.35	10	9.96	99.64
150%	22.5	22.60	100.43	15	14.82	98.78
	22.5	22.31	99.16	15	14.95	99.67
	22.5	22.28	99.01	15	14.80	98.66
% recovery	**99.60**			**99.67**		

System precision was performed and the data was shown in table 6

Table 6: System precision of Nirmatrelvir and Ritonavir

	Nirmatrelvir	**Ritonavir**
1.	883258	596303
2.	885514	594941
3.	879875	596023
4.	878862	596358
5.	880123	589684
6.	873177	590891
Avg	**880135**	**594033**
S.D	**4214.0**	**2970.8**
%RSD	**0.5**	**0.5**

The % RSD for the peak areas of Nirmatrelvir and Ritonavir obtained from six replicate injections of standard solution was within the limit.

Method Precision: The precision of the method was determined by analyzing a sample of Nirmatrelvir and Ritonavir and shown in table 7

Table 7: method Precision

	Nirmatrelvir	**Ritonavir**
1.	879785	593298
2.	876257	589741
3.	874256	590125
4.	872514	587060
5.	876253	587640
6.	872141	586872
Avg	**875201**	**589123**
S.D	**2853.5**	**2463.3**
%RSD	**0.3**	**0.4**

From the above results, the % RSD of method precision study was within the limit for Nirmatrelvir and Ritonavir.

Robustness: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (55B:45A), mobile phase plus (65B:35A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table 8: Robustness data for Nirmatrelvir and Ritonavir.

Condition	%R.S. D of Nirmatrelvir	%R.S. D Ritonavir
F - 0.9ml/min	0.2	0.2
F + 1.1ml/min	0.4	0.5
M.P - 55B:45A	0.4	0.5
M.P + 65B:35A	0.2	0.1
T - 25°C	0.1	0.3
T + 35°C	0.3	0.7

Force Degradation Studies: table 9 shows degradation conditions and the obtained degraded data in table 10 and purity plot chromatogram in figure 8,9

Table 9: degradation conditions

Stress condition	Solvent	Temp(0C)	Exposed time
Acid	2N HCL	600c	30 mins
Base	2N NAOH	600c	30 mins
Oxidation	20% H2O2	600c	30 mins
Thermal	Diluent	1050c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	600c	-

Table 10: degradation data

Type of degradation	Nirmatrelvir			Ritonavir		
	area	%recovered	% degraded	area	%recovered	% degraded
Acid	842149	95.49	4.51	559308	93.97	6.03
Base	846710	96.01	3.99	559710	94.03	5.97
Peroxide	849579	96.34	3.66	562908	94.57	5.43
Thermal	859270	97.43	2.57	569041	95.60	4.40
Uv	865864	98.18	1.82	579732	97.40	2.60
Water	875092	98.18	1.82	591049	99.30	0.70

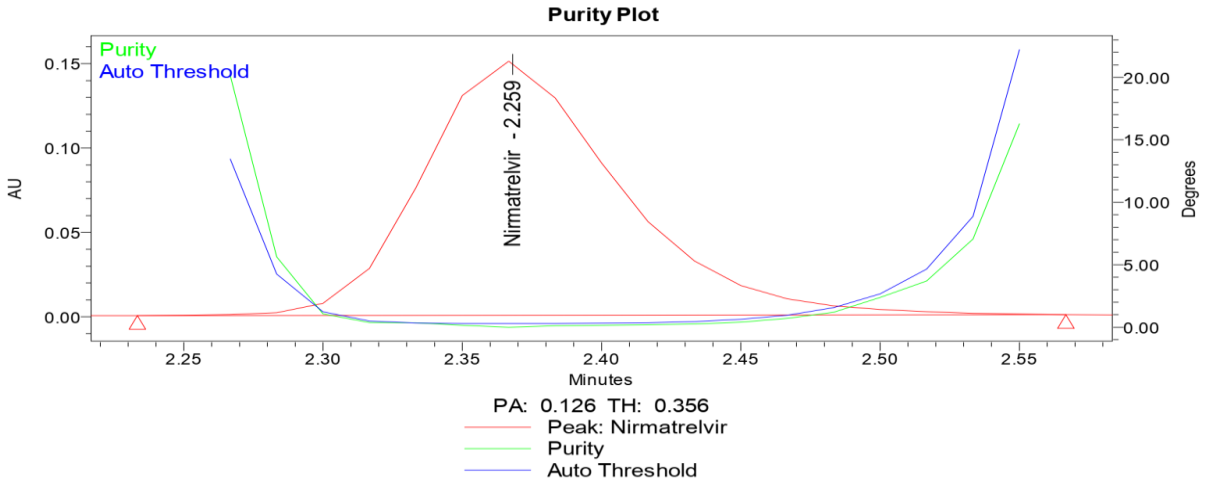

Figure 8: Purity plots for Acid Condition for Nirmatrelvir

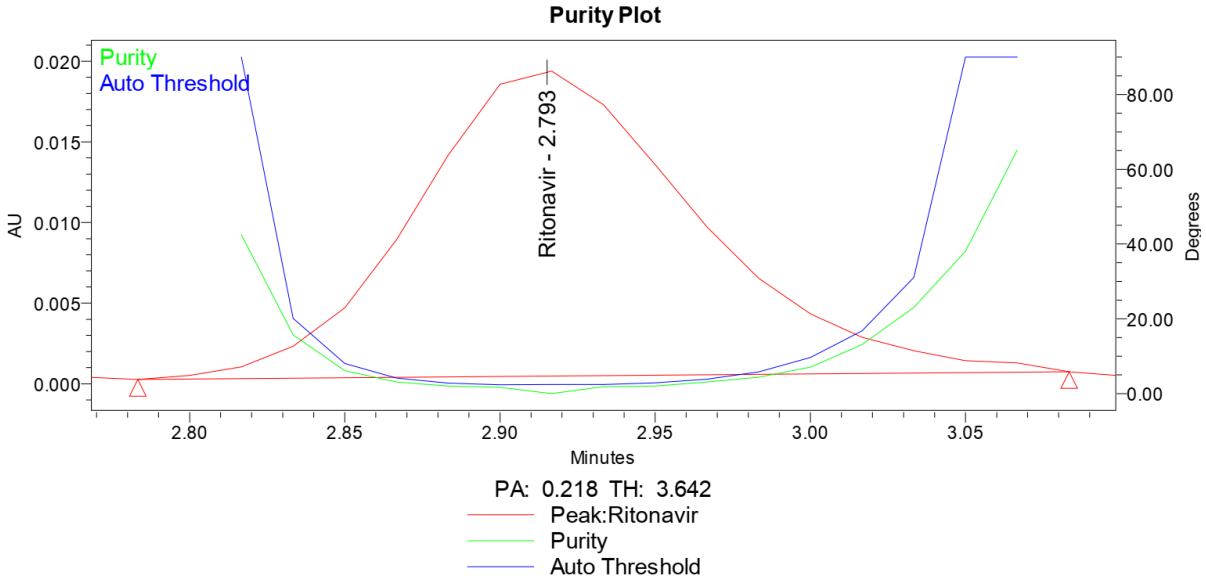

Figure 9: Purity plots for Acid Condition for Ritonavir

Assay: Paxlovid Tablet, bearing the label claim Nirmatrelvir 300mg, Ritonavir 300mg. Assay was performed with the above formulation. Average % Assay for Nirmatrelvir and Ritonavir obtained was 99.24% and 98.97% respectively

Table 11: assay data

	Nirmatrelvir			Ritonavir		
S.no	**Std Area**	**Sample area**	**% Assay**	**Std Area**	**Sample area**	**% Assay**
1	883258	879785	**99.76**	596303	593298	**99.68**
2	885514	876257	**99.36**	594941	589741	**99.08**
3	879875	874256	**99.13**	596023	590125	**99.14**
4	878862	872514	**98.94**	596358	587060	**98.63**
5	880123	876253	**99.36**	589684	587640	**98.73**
6	873177	872141	**98.89**	590891	586872	**98.60**
Avg	880135	875201	**99.24**	594033	589123	**98.97**
Std ev	4214.0	2853.5	**0.32**	2970.8	2463.3	**0.41**
%RSD	0.3	0.3	0.3	0.5	0.3	0.3

Assay was calculated by the formula:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{100}{1} \times \frac{10}{1} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Average Peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of drug working standard taken in mg
P	Assay of drug working standard in % on dried basis
L.C	Label Claim

Figure 10 Formula

CONCLUSION:

The findings of the study will be very helpful in evaluating the quality of reasonably priced drugs that contain Ritonavir and Nirmatrelvir. This might be as a result of the study's straightforward sample preparation method, which required little mobile phase and a brief analytical period. The results of evaluating two medications combined in a single dosage demonstrated that the recently created analytical technique was almost entirely successful.

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