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STABILITY INDICATING DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF NIRMATRELVIR AND RITONAVIR TABLET DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

A robust, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Nirmatrelvir and Ritonavir in pharmaceutical tablet dosage form (Paxlovid). The chromatographic separation was performed on an Agilent C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of 0.01N potassium dihydrogen orthophosphate buffer (pH 3.0) and acetonitrile in the ratio 60:40, at a flow rate of 1.0 mL/min and detection wavelength of 292 nm. The retention times for Nirmatrelvir and Ritonavir were 2.121 min and 2.710 min, respectively. The method demonstrated excellent linearity (r^2 = 0.999), precision (RSD < 2%), accuracy (recovery ~99.3–99.7%), sensitivity (LOD 0.02–0.06 µg/mL; LOQ 0.07–0.17 µg/mL), and robustness under varied conditions. Degradation studies confirmed the method's specificity and stability-indicating capability. The validated method is suitable for routine quality control and stability analysis in pharmaceutical industries.

KEYWORDS: Nirmatrelvir, Ritonavir, Stability-Indicating Method, Pharmaceutical Analysis, Degradation Studies.

INTRODUCTION

Pharmaceutical analysis is a branch of practical chemistry that involves a process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixtureThe wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods (1) are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods.

MATERIALS AND INSTRUMENTS

The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Chemicals and Solvents Used

- Nirmatrelvir and Ritonavir pure drugs (API), Combination Nirmatrelvir and Ritonavirtablets (**Paxlovid**) are received from spectrum lab
- Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate buffer, Ortho-phosphoric acid. Alltheabovechemicals andsolvents are from Rankem

Instruments / Equipment required

- Volumetric flask- Glass, Class-A
- Beakers - Glass
- Measuring cylinder – Glass, Class-A, pH meter
- Volumetric pipettes- Glass, Class-A (graduated)
- Balance - Analytical balance and Micro balance
- Ultrasonicator
- UV-VIS spectrophotometer

Preparation of Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 7.5mg of Nirmatrelvir, 10mg of Ritonavir and transferred to 50ml and 100ml individual volumetric flasks and 3/4th of diluents was added to these flasks and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (150µg/ml of Nirmatrelvir and 100µg/ml Ritonavir).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (15µg/ml of Nirmatrelvir and 10µg/ml Ritonavir).

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the tablet powder weight equivalent to 150mg of Nirmatrelvir and 100mg of Ritonavir was transferred into a 500ml volumetric flask, 50ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. (300µg/ml of Nirmatrelvir and 200µg/ml of Ritonavir).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent.(15µg/ml of Nirmatrelvir and 10µg/ml Ritonavir).

Preparation of buffer

0.1%OPA Buffer: (2.2pH) 1ml of ortho phosphoric acid was diluted to 1000ml with HPLC grade water.

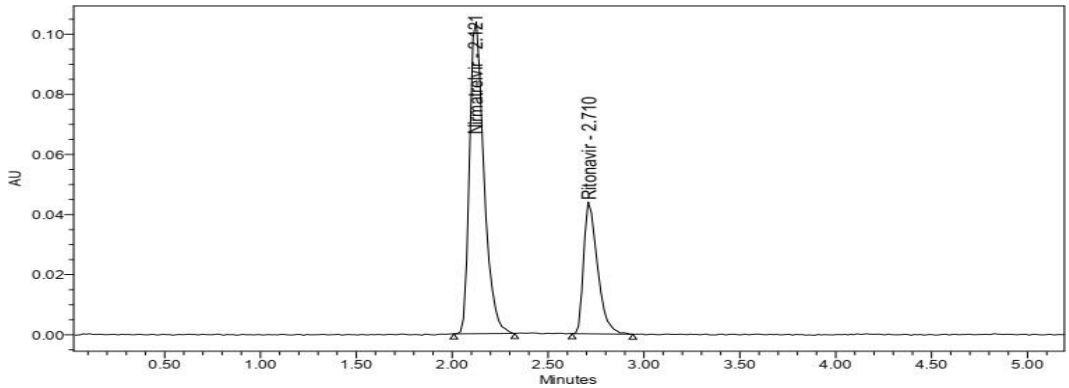
Preparation of Standard stock solutions: Accurately weighed 7.5mg of Nirmatrelvir, 10mg of Ritonavir and transferred to 50ml and 100ml individual volumetric flasks and 3/4th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (150µg/ml of Nirmatrelvir and 100µg/ml Ritonavir)

METHOD DEVELOPMENT

Method development was done by changing the various ratio for mobile phase.

Observation: Nirmatrelvir and Ritonavir were eluted at 2.121min and 2.710 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Column	**Agilent C18 (4.6 x 150mm, 5µm)**
Detector wave length	292nm
Flow rate	1 mL/min
Injection volume	10µL
Column temperature	30 °C
Run time	5 minutes
Diluent	Water and Acetonitrile in the ratio 50:50
Mobile Phase	60% 0.01N kh2po4 (3ph):40% Acetonitrile



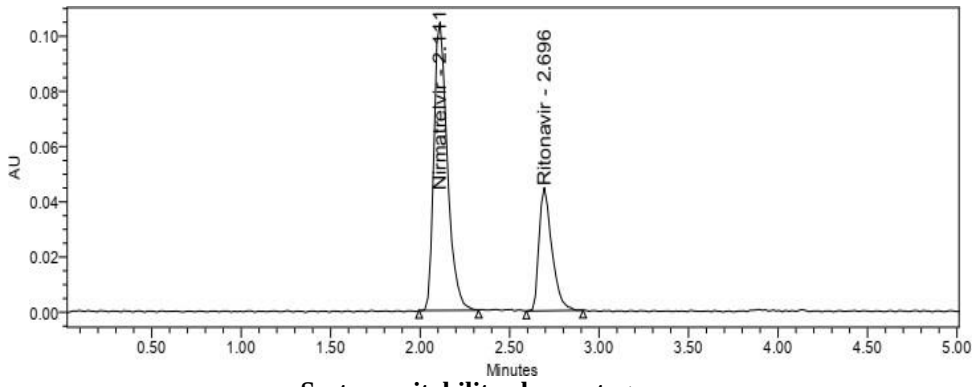
Optimized chromatographic condition

RESULTS AND DISCUSSION

System suitability parameters for Nirmatrelvir and Ritonavir

S.no	**Nirmatrelvir**			**Ritonavir**			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resoluton**
1	2.050	3971	1.35	2.604	7314	1.50	4.4
2	2.096	3655	1.31	2.678	6818	1.55	4.1
3	2.110	3594	1.36	2.696	6178	1.41	4.1
4	2.111	3731	1.37	2.701	6893	1.54	4.3

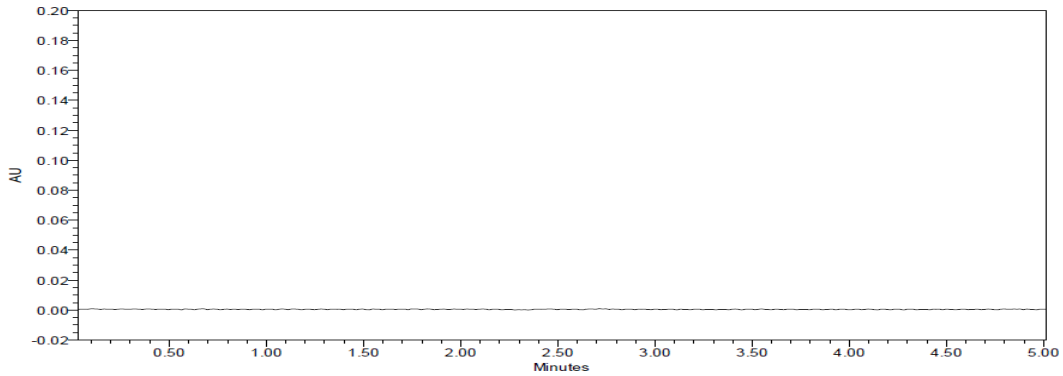
5	2.116	3493	1.34	2.702	6655	1.43	4.2
6	2.117	3380	1.32	2.703	6397	1.45	4.2



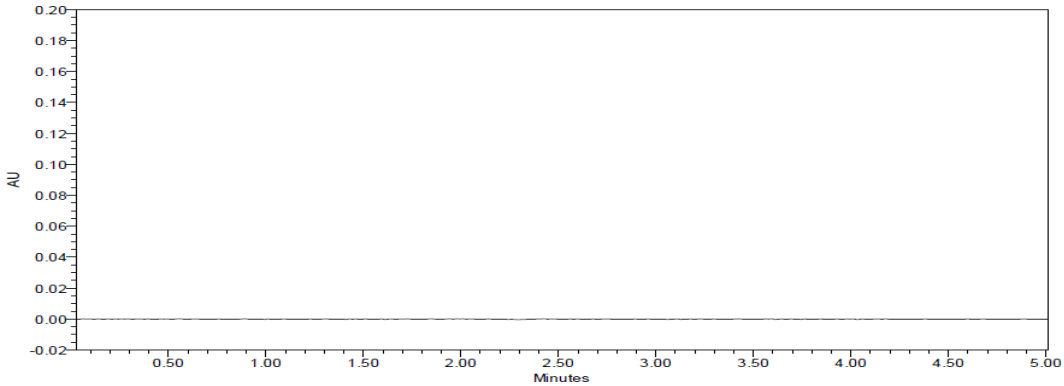
System suitability chromatogram

Discussion: According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

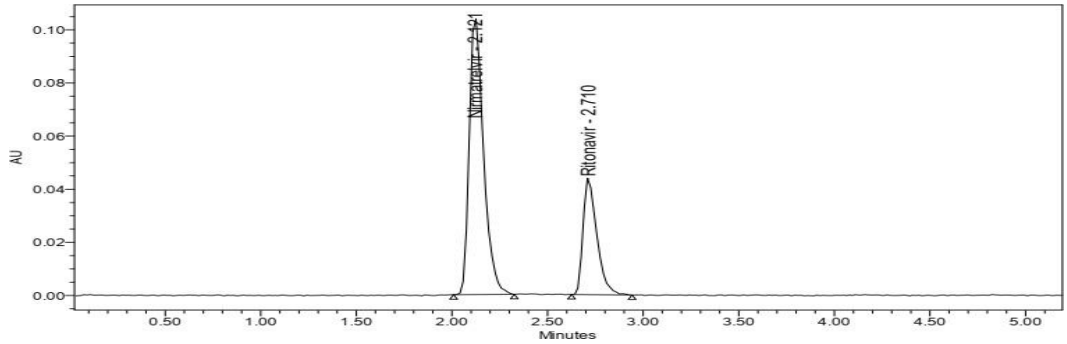
Discussion: Retention times of Nirmatrelvir and Ritonavir were 2.116min and 2.704 min respectively. We did not found and interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.



Chromatogram of blank solution



Chromatogram of placebo

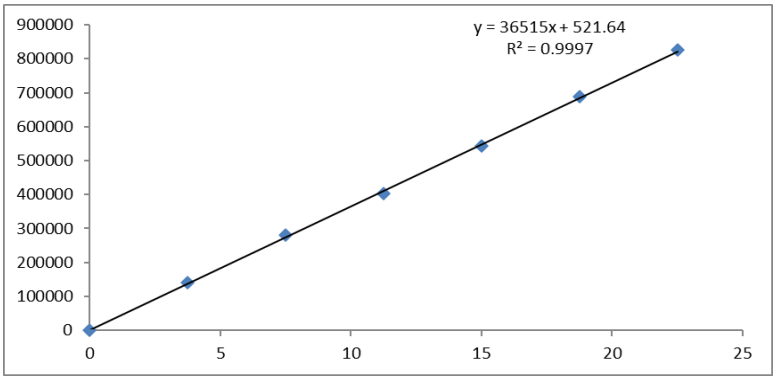


Typical chromatogram

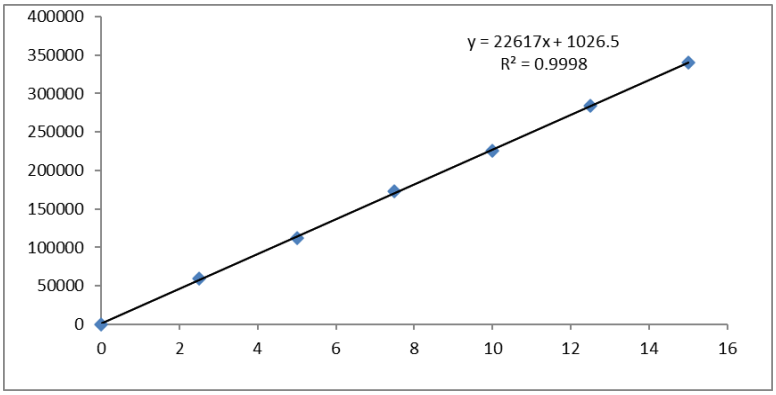
Linearity

Linearity table for Nirmatrelvir and Ritonavir

Nirmatrelvir		Ritonavir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
3.75	140579	2.225	59408
7.5	279815	4.45	112655
11.25	402505	6.675	172738
15	542941	8.9	225396
18.75	687955	11.125	283838
22.5	825387	13.35	340519



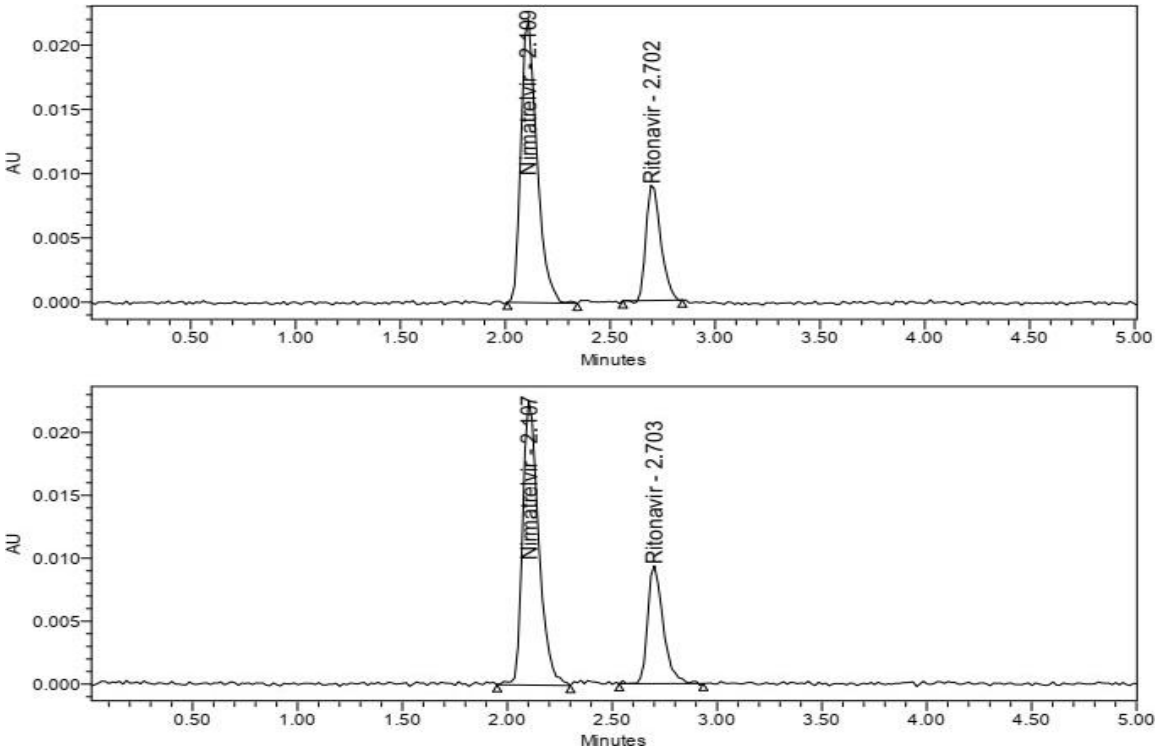
Calibration curve of Nirmatrelvir



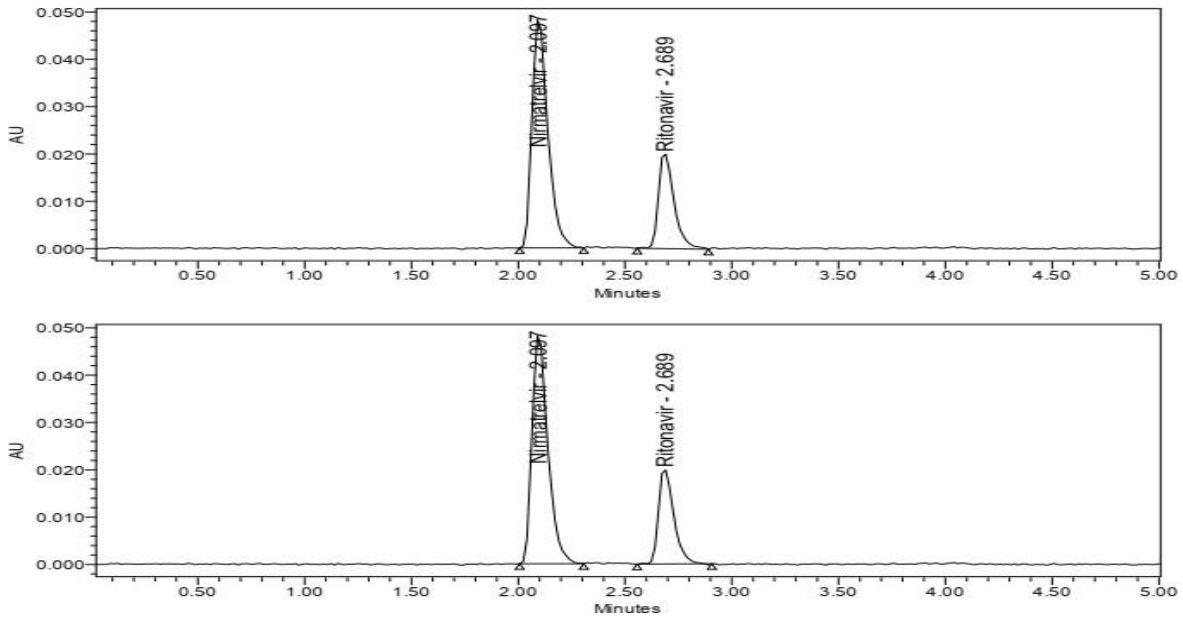
Calibration curve of Ritonavir

Discussion

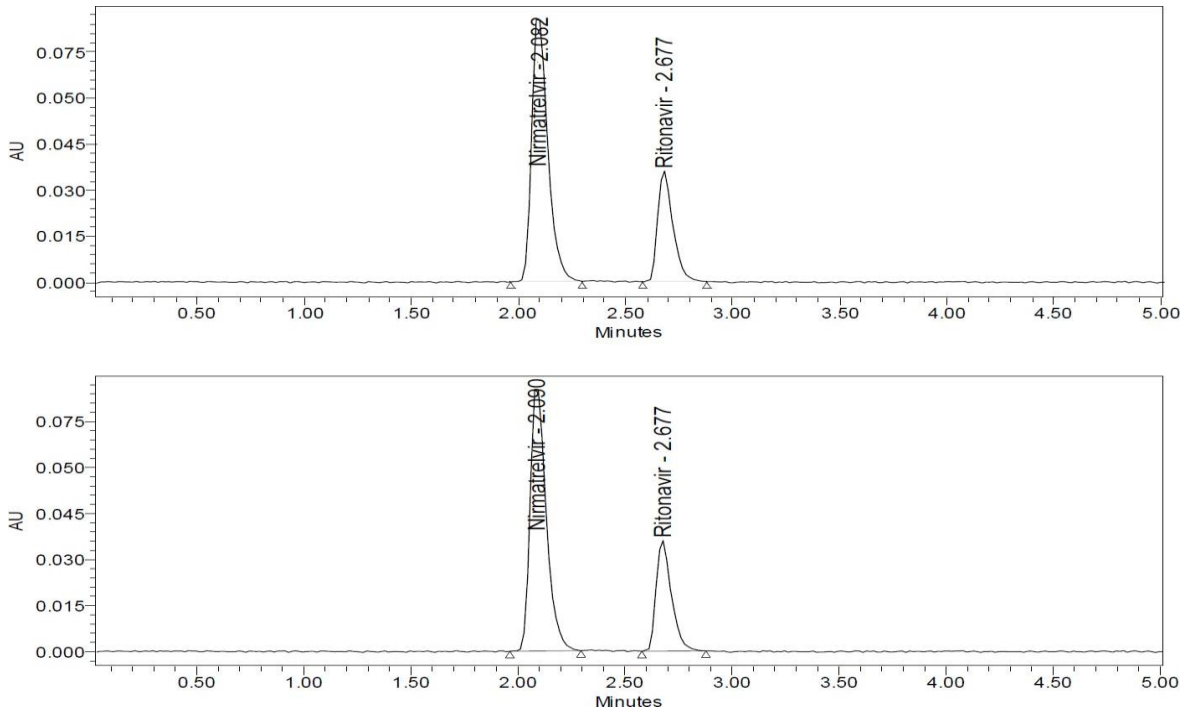
Six linear concentrations of Nirmatrelvir (3.5-22.5 µg/ml) and Ritonavir (2.25- 13.35µg/ml) were injected in a duplicatemanner. Average areas were mentioned above and linearity equations obtained for Nirmatrelvir was y = 36515x + 521.64 and of Ritonavir was y = 22617x + 1026.5 Correlation coefficient obtained was 0.999 for the two drugs.



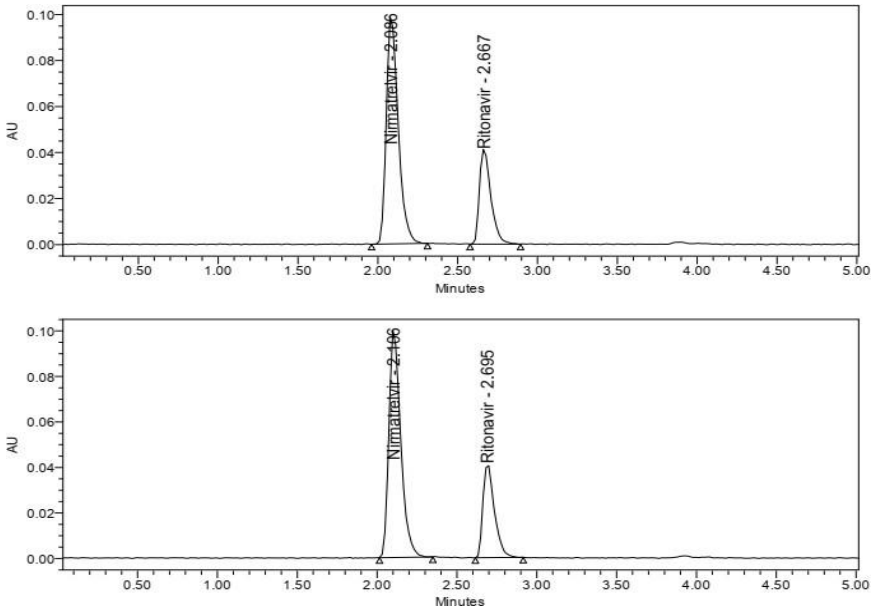
Linearity 25% Chromatogram of Nirmatrelvir and Ritonavir



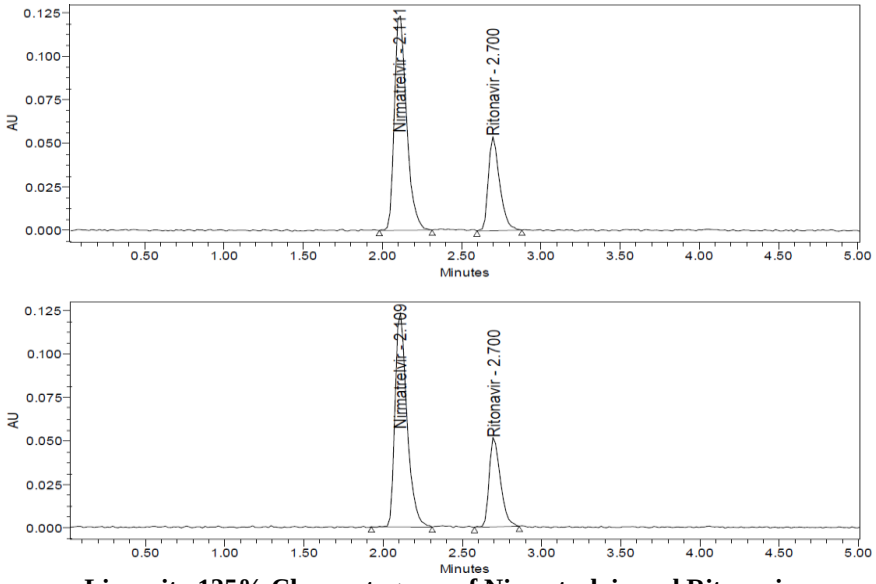
Linearity 50% Chromatogram of Nirmatrelvir and Ritonavir



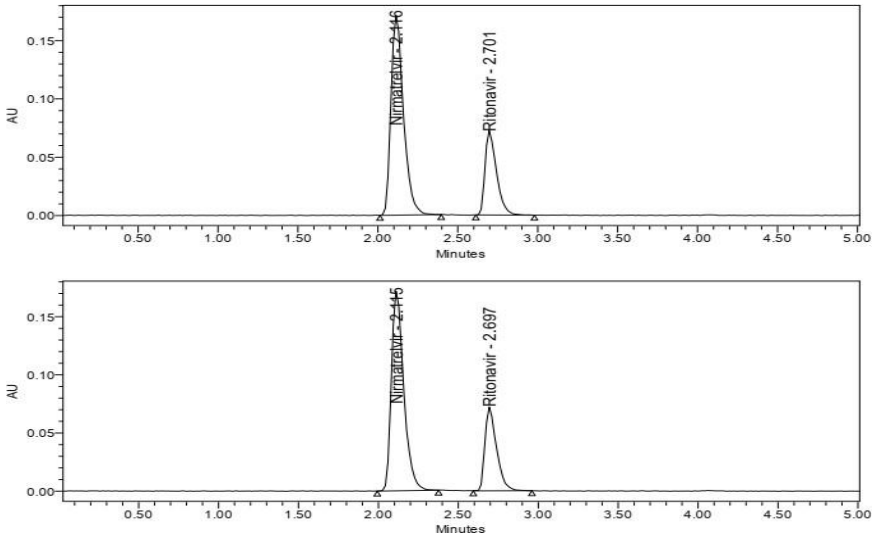
Linearity 75% Chromatogram of Nirmatrelvir and Ritonavir



Linearity 100% Chromatogram of Nirmatrelvir and Ritonavir



Linearity 125% Chromatogram of Nirmatrelvir and Ritonavir



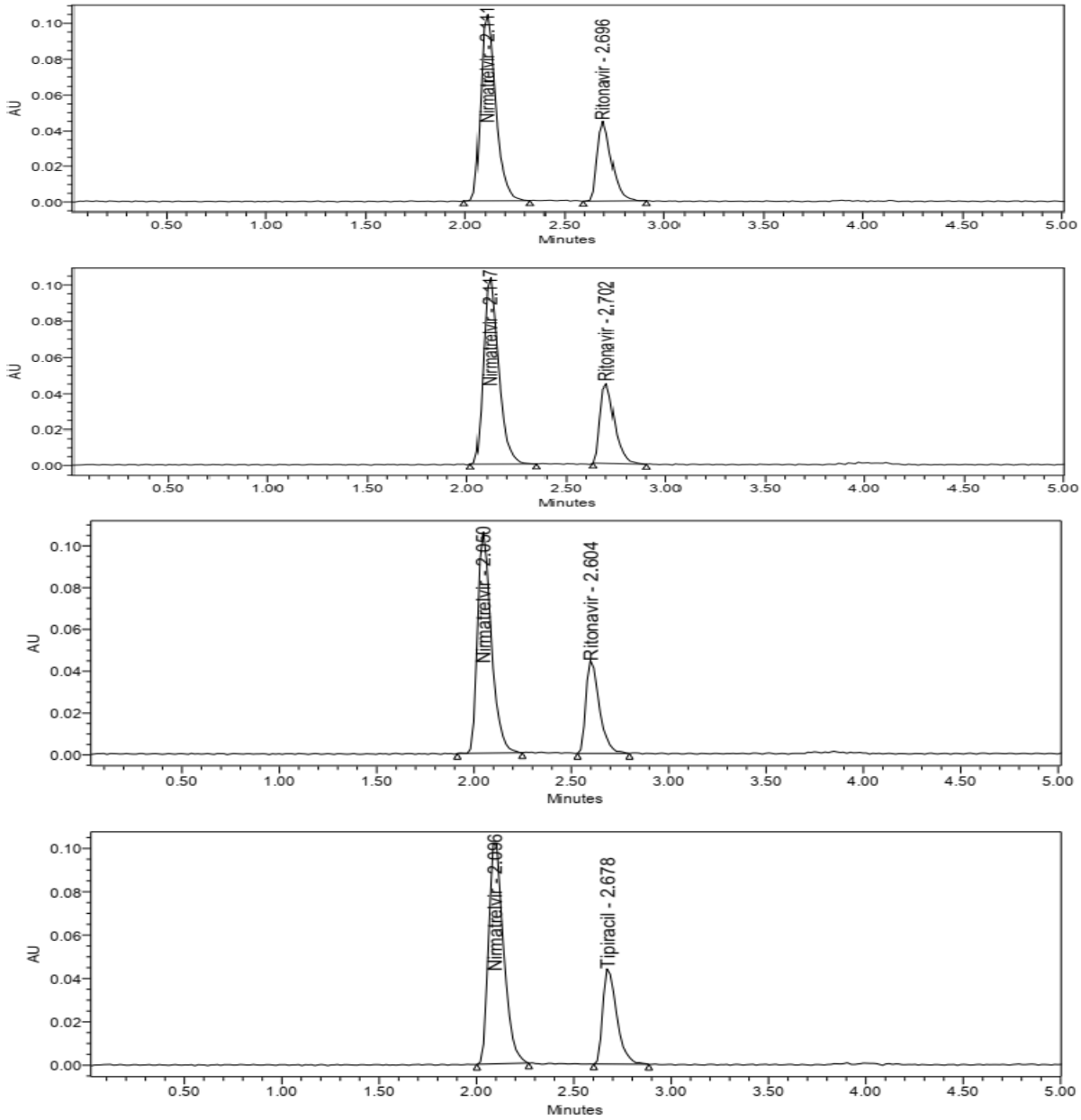
Linearity 150% Chromatogram of Nirmatrelvir and Ritonavir

Precision

System Precision

System precision table of Nirmatrelvir and Ritonavir

S. No	Area of Nirmatrelvir	Area of Ritonavir
1.	540312	222779
2.	546233	222178
3.	546936	223178
4.	544926	221690
5.	556058	219409
6.	546548	222258
Mean	546836	221915
S.D	5132.5	1331.1
%RSD	0.9	0.6



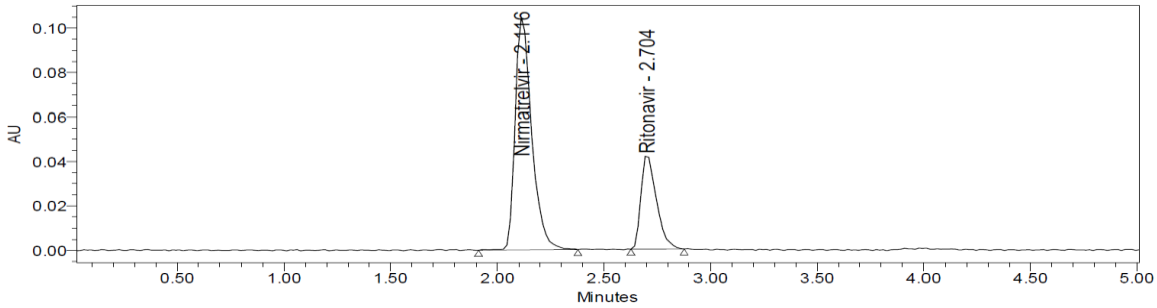
Discussion

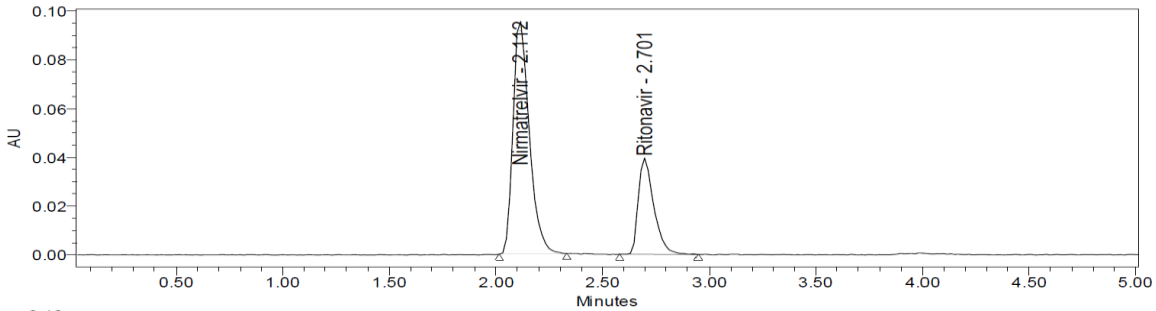
From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.9% and 0.6% respectively for Nirmatrelvir and Ritonavir. As the limit of Precision was less than "2" the system precision was passed in this method.

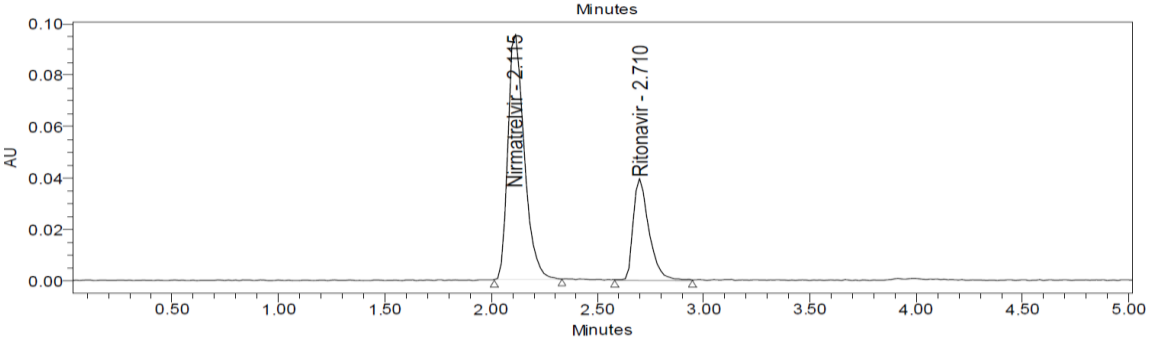
Repeatability

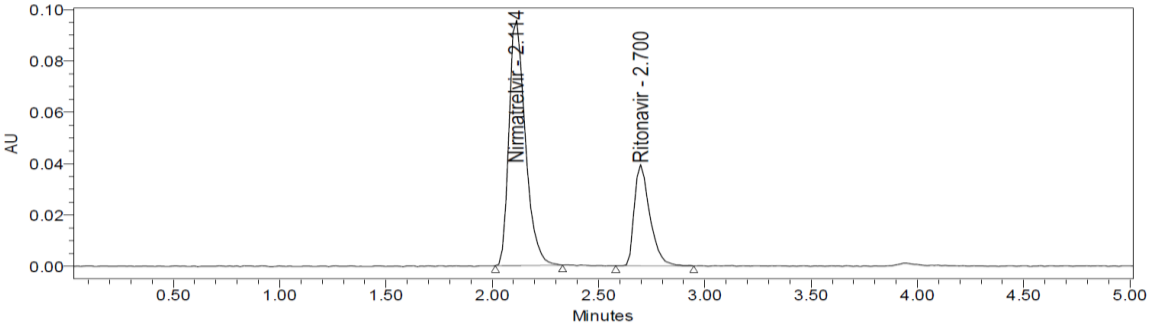
Repeatability table of Nirmatrelvir and Ritonavir

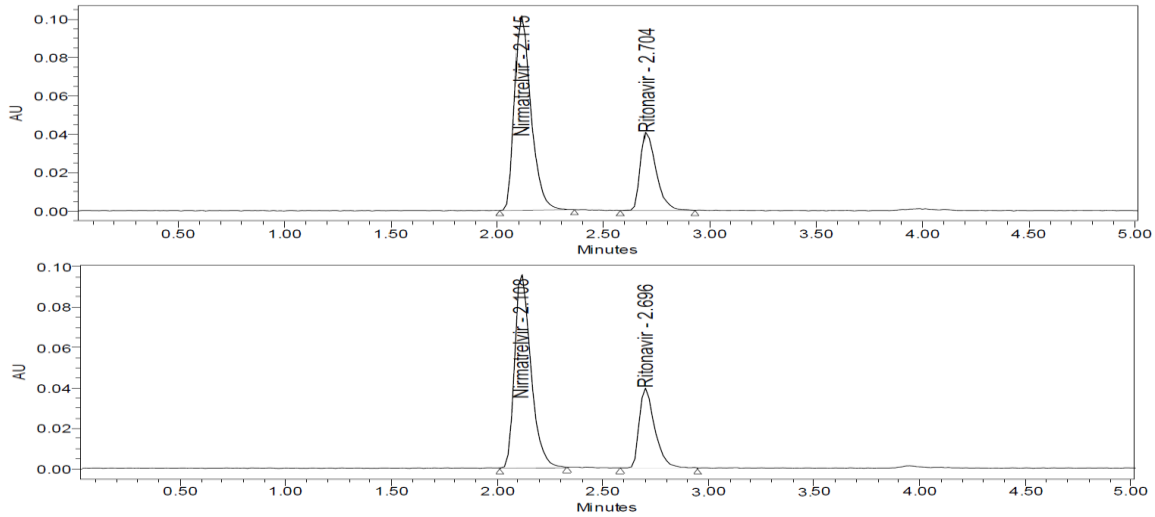
S. No	Area of Nirmatrelvir	Area of Ritonavir
1.	541275	219937
2.	545115	220524
3.	542430	221777
4.	543388	221875
5.	544121	220573
6.	545369	220162
Mean	543616	220808
S.D	1582.0	823.3
%RSD	0.3	0.4


Nirmatrelvir - 2.116
Ritonavir - 2.704
AU
Minutes


Nirmatrelvir - 2.112
Ritonavir - 2.701
AU
Minutes


Nirmatrelvir - 2.115
Ritonavir - 2.710
AU
Minutes


Nirmatrelvir - 2.114
Ritonavir - 2.700
AU
Minutes



Repeatability chromatogram

DISCUSSION

Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.3% and0.4% respectively for Nirmatrelvir and Ritonavir. As the limit of Precision was less than "2" the system precision was passed in this method.

Method precision

S.NO	NAME	RETENTION TIME	AREA
1	BLANK	-	-
2	STANDARD SOLUTION 1	3.05	3393521
3	STANDARD SOLUTION 2	3.05	3376157
4	STANDARD SOLUTION 3	3.05	3372025
5	STANDARD SOLUTION 4	3.05	3373074
6	STANDARD SOLUTION 5	3.05	3384619
7	SAMPLE SOLUTION SET(1-A)	3.05	3253664
8	SAMPLE SOLUTION SET(1-B)	3.05	3405439
9	SAMPLE SOLUTION SET(2-A)	3.05	3409052
10	SAMPLE SOLUTION SET(2-B)	3.05	3309047
11	SAMPLE SOLUTION SET(3-A)	3.05	3329681
12	SAMPLE SOLUTION SET(3-B)	3.05	3275426
13	STD SOLUTION BKT 1	3.05	3285426
14	SAMPLE SOLUTION SET(4-A)	3.05	3254679
15	SAMPLE SOLUTION SET(4-B)	3.05	3256092
16	SAMPLE SOLUTION SET(5-A)	3.05	3378748
17	SAMPLE SOLUTION SET(5-B)	3.05	3345704
18	SAMPLE SOLUTION SET(6-A)	3.05	3249869
19	SAMPLE SOLUTION SET(6-B)	3.05	3265739
20	STD SOLUTION BKT 1	3.05	3385670

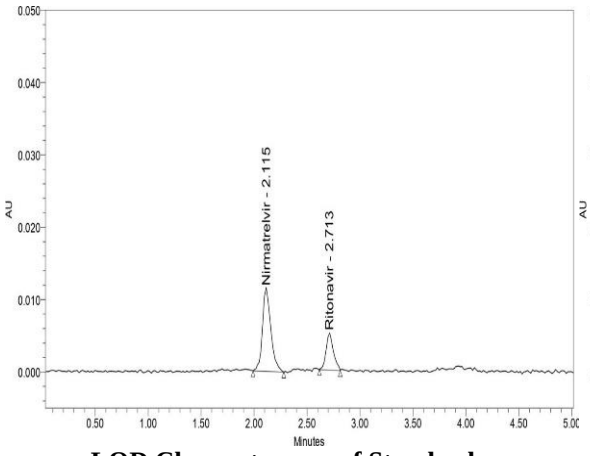
DISCUSSION

The prescribed method is precise and performed method is within the limit.

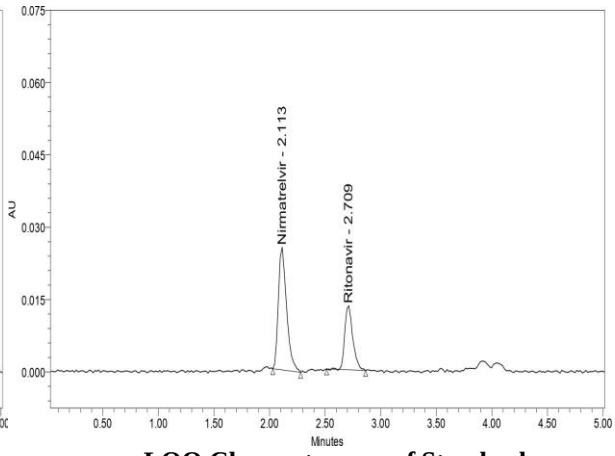
Sensitivity

Sensitivity table of Nirmatrelvir and Ritonavir

Molecule	LOD	LOQ
Nirmatrelvir	0.02	0.07
Ritonavir	0.06	0.17



LOD Chromatogram of Standard



LOQ Chromatogram of Standard

ROBUSTNESS

Robustness data for Nirmatrelvir and Ritonavir.

S.no	Condition	%RSD of Nirmatrelvir	% RSD of Ritonavir
1	Flow rate (-) 1.1ml/min	0.5	0.6
2	Flow rate (+) 1.3ml/min	0.6	0.1
3	Mobile phase (-) 35B:65A	0.9	0.2
4	Mobile phase (+) 45B:55A	0.8	0.6
5	Temperature (-) 25°C	0.8	0.8
6	Temperature (+) 35°C	0.5	0.4

DISCUSSION

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (40B:60A), mobile phase plus (50B:50A), 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.

Assay: bearing the label claim(Paxlovid)Nirmatrelvir, Ritonavir. Assay was performed with the above formulation. Average % Assay for Nirmatrelvir and Ritonavir obtained was 99.21 and 99.30% Respectively.

Assay Data of Nirmatrelvir

S.no	Standard Area	Sample area	% Assay
1	540312	541275	98.79
2	546233	545115	99.49
3	546936	542430	99.00
4	544926	543388	99.17
5	556058	544121	99.30
6	546548	545369	99.53
Avg	546836	543616	99.21
Stdev	5132.5	1582.0	0.29
%RSD	0.9	0.3	0.29

Assay Data of Ritonavir

S.no	Standard Area	Sample area	% Assay
1	222779	219937	98.91
2	222178	220524	99.17
3	223178	221777	99.74
4	221690	221875	99.78
5	219409	220573	99.20
6	222258	220162	99.01
Avg	221915	220808	99.30
Stdev	1331.1	823.3	0.37
%RSD	0.6	0.4	0.4

DEGRADATION

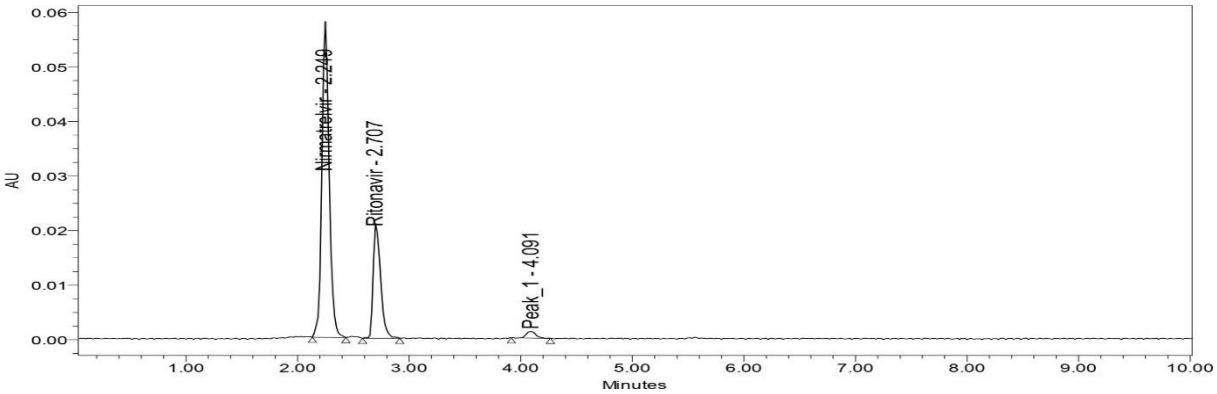
Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Degradation Data of Nirmatrelvir

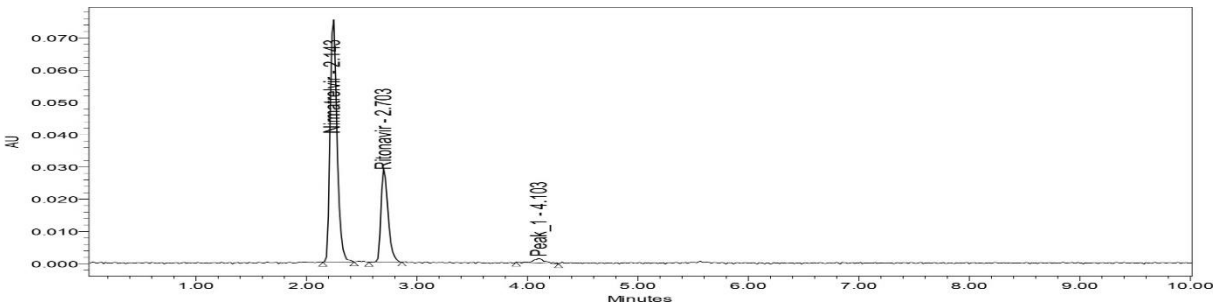
S.NO	Degradation Condition	Area	% Drug Degraded	% drug degreded
1	Acid	520279	94.95	5.05
2	Alkali	525619	95.93	4.07
3	Oxidation	529619	96.66	3.34
4	Thermal	529161	96.57	3.43
5	UV	539316	98.43	1.57
6	Water	542096	98.43	1.57

Degradation Data of Ritonavir

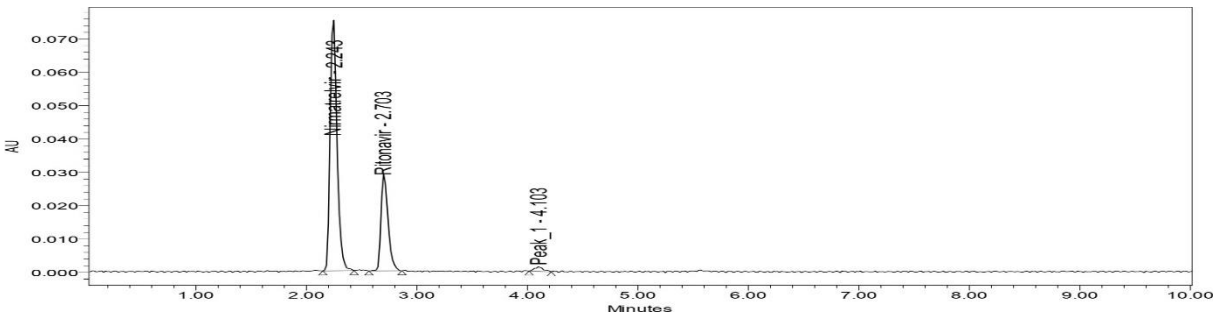
S.NO	Degradation Condition	Area	% Drug Degraded	% drug degreded
1	Acid	211284	95.02	4.98
2	Alkali	213868	96.18	3.82
3	Oxidation	214868	96.63	3.37
4	Thermal	215967	97.12	2.88
5	UV	217916	98.00	2.00
6	Water	220336	99.09	0.91



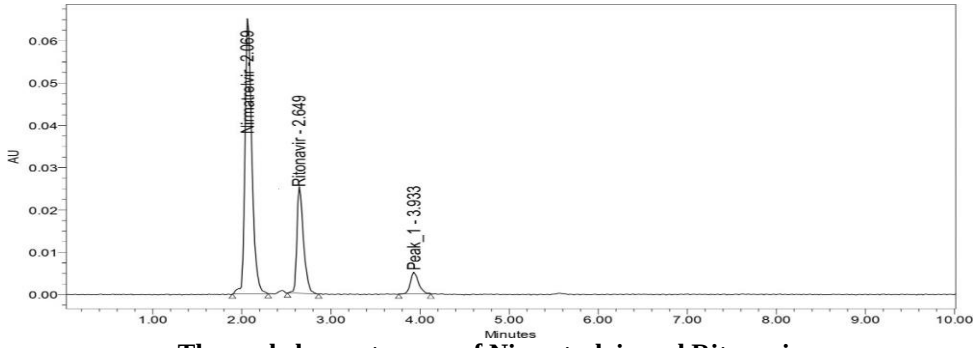
Acid chromatogram of Nirmatrelvir and Ritonavir



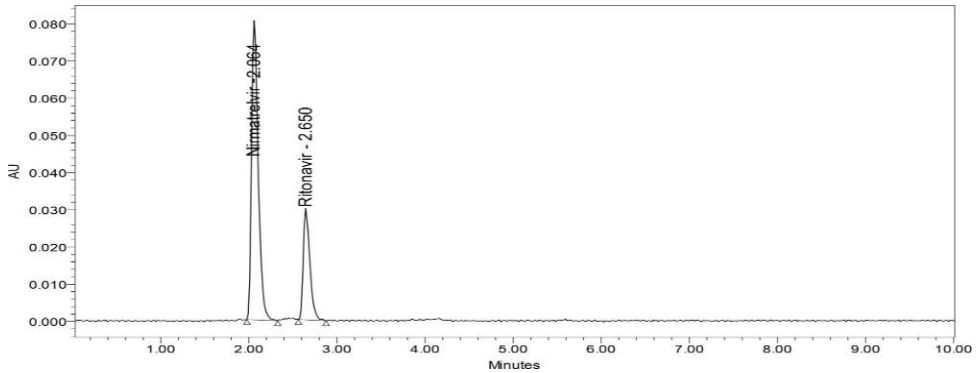
Base chromatogram of Nirmatrelvir and Ritonavir



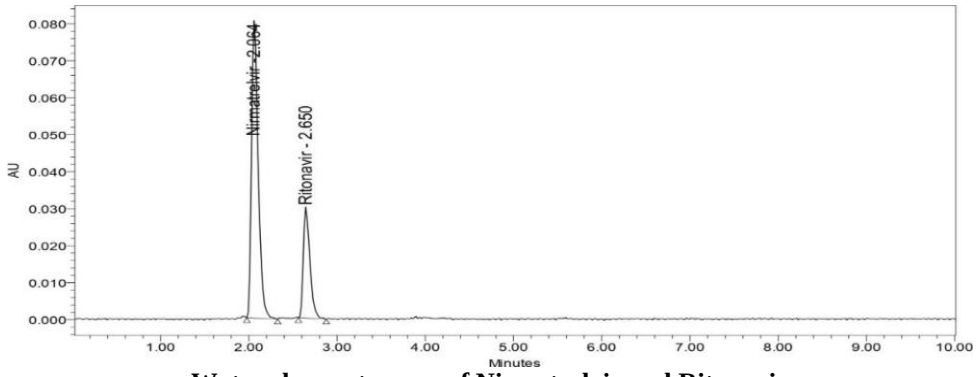
Peroxide chromatogram of Nirmatrelvir and Ritonavir



Thermal chromatogram of Nirmatrelvir and Ritonavir



UV chromatogram of Nirmatrelvir and Ritonavir



Water chromatogram of Nirmatrelvir and Ritonavir

Parameters	**Nirmatrelvir**		**Ritonavir**	**LIMIT**
Linearity Range(µg/ml)	3.75-22.5 µg/ml		2.5-15 µg/ml	
Regression coefficient	0.999		0.999	
Slope(m)	36515		22617	R< 1
Intercept(c)	521.64		1026.5	
Regression equation (Y=mx+c)	y = 36515.x +521.64		y = 22617x +1026.5.	
Assay (% mean assay)	99.21%		99.30%	90-110%
Specificity	Specific		Specific	No interference of any peak
System precision % RSD	0.9		0.6	NMT 2.0%
Method precision %RSD	0.3		0.4	NMT 2.0%
Accuracy % recovery	99.33%		99.71%	98-102%
LOD	0.02		0.06	NMT 3
LOQ	0.07		0.17	NMT 10
Robustness	FM	0.5	0.6	%RSD NMT 2.0
	FP	0.6	0.1	
	MM	0.9	0.2	
	MP	0.8	0.6	
	TM	0.8	0.8	
	TP	0.5	0.4	

DISCUSSION

Regarding the Ph adjustment in mobile phase for the acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N Acid solution there will be no change in retention time.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Nirmatrelvir and Ritonavirin Tablet dosage form. Retention time of Nirmatrelvir and Ritonavirwere found to be 2.121min and 2.710 min. %RSD of the Nirmatrelvir and Ritonavirwere and found to be 0.9and 0.6respectively. %Recovery was obtained as 99.33%and 99.71%for Nirmatrelvir and Ritonavir respectively. LOD, LOQ values obtained from regression equations of Nirmatrelvir and Ritonavir were 0.02, 0.07 and 0.06, 0.17 respectively. Regression equation of Nirmatrelvir is y = 36515.x +521.64, and of y = 22617x+1026.5. Ritonavir. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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