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Research

Method development and validation for simultaneous estimation of nirmatrelvir and ritonavir by using rp hplc

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Abstract

For the simultaneous estimate of nimatrelvir and ritonavir in a pharmaceutical dose form, a straightforward, precise, and accurate approach was created. Using a typical Agilent C18 column (150 x 4.6 mm, 5), the chromatogram was conducted. The mobile phase was pumped through the column at a flow rate of 0.8 ml/min, comprising a 60:40 ratio of Acetonitrile to Buffer 0.01N KH2PO4 (2.2 pH). This approach employed 0.01N KH2PO4 as a buffer. The 30-degree Celsius mark was kept constant. The chosen optimum wavelength was 265 nm. Ritonavir and Nirmatrelvir were shown to have retention times of 2.816 and 2.241 minutes, respectively. It was discovered that Ritonavir and Nirmatrelvir had percentage RSDs of 0.6% and 0.4%, respectively. 99.15% and 99.77% of the patients recovered from nirmatrelvir and ritanavir, respectively. Regression models for Nirmatrelvir and Ritonavir yielded LOD and LOQ values of 0.21 and 0.16 and 0.65 and 0.48, respectively. Nirmatrelvir's regression equation is y = 16802x + 995.5, while Ritonavir's is y = 16802x + 995.5. The devised method was found to be straightforward and cost-effective due to the reduction of both the retention times and run time. Industries can use this approach for routine quality control testing.

Keywords: Nirmatrelvir, Ritonavir, RP-HPLC

INTRODUCTION

A drug's quality plays a critical role in guaranteeing both its efficacy and safety. For pharmaceutical and chemical formulations to be safe and effective for customers, quality assurance and control are crucial. Thus, determining whether pure drug compounds and their medicinal dosage forms are appropriate for patient usage requires careful analysis. The caliber of the techniques utilized to produce the data determines the caliber of the analytical data.(1). The statutory certification of pharmaceuticals and their formulations with regulatory agencies is therefore dependent upon the development of robust and durable analytical procedures.

In general, a drug's potency and contaminants can be successfully monitored and controlled to assure both

quality and safety. The drug's potency is determined by the assay, and its safety is determined by the impurities. Pharmaceutical product assaying is essential to guaranteeing the medication's effectiveness in patients.

Depending on the drug's nature and qualities, developing new procedures for it presents a range of obstacles. It is crucial to attain selectivity, speed, affordability, ease of use, sensitivity, repeatability, and precision in the outcomes. Researchers have the chance to tackle these issues and develop fresh approaches to analysis that the chemical and pharmaceutical industries can use. The physical phenomena brought about by chemical reactions are studied using a variety of physicochemical techniques. These techniques include chromatographic (column, paper, thin layer, gas-liquid, and high-performance liquid chromatography), photometric (photocolorimetry and spectrophotometry including UV-visible, IR Spectroscopy, and nepheloturbidimetry), and optical (refractometry, polarimetry, emission, and fluorescence). Methods such as paramagnetic resonance (PMR) and nuclear magnetic resonance (NMR) are also becoming more and more common. Gas chromatography and mass spectroscopy (MS) together provide a potent instrument. Chemical methods based on complex formation, acid-base, precipitation, and redox reactions include gravimetric and volumetric approaches. Pharmaceutical analysis also makes use of complexometry and non-aqueous media titrations. New approaches to quality control are always needed as the number of novel medications keeps rising.

High performance liquid chromatography (hplc)

One analytical method for separating ions or molecules dispersed in a solvent is liquid chromatography. It entails varying the amount of time that the sample solution is exposed to a second solid or liquid phase because of variations in adsorption, ion exchange, partitioning, or size. By measuring the length of time it takes for the solutes to travel through a column, these discrepancies allow the components of the mixture to be separated.

Analytical method validation (16-19)

The ICH Guidelines define method validation as "the process of establishing documented evidence that provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics."

A main component assay necessitates a different methodology and set of acceptance standards than a trace impurity assay. A last technique might be used in several locations worldwide. Certain elements of the HPLC process may be necessary due to variances in HPLC instrumentation, laboratory equipment, and reagent supplies, as well as in staff backgrounds and skill levels. Furthermore, approach techniques may need to be flexible in order to generate alternative formulations of the same medicine with varied strengths or physical shapes.

The method validation research encompasses system suitability, linearity, precision, accuracy, specificity, robustness, limit of detection, limit of quantification, and stability of samples, reagents, and equipment.

Scope and objective

It was discovered via the reference of numerous literature reviews that a variety of analytical techniques, including UV-Visible spectroscopy, HPLC, and RP-HPLC, were employed in the estimate of Nirmatrelvir and Ritonavir, both separately and even in the combination dose form.

Many efforts are being made to create an easy-to-use and widely acknowledged technique for quickly estimating nimatrelvir and ritonavir in tablet and bulk form. The most widely accepted type of analysis is chromatographic analysis. As a result, a novel RP-HPLC technique was created, verified, and degradation analyses needed to be carried out.

MATERIALS AND METHODS

Materials

Combination Nirmatrelvir and Ritonavir dose form: distilled water, acetonitrile, phosphate buffer, methanol, potassium dihydrogen ortho phosphate buffer, ortho-phosphoric acid. Nirmatrelvir and Ritonavir pure medicines (API). The solvents and chemicals listed above are all Rankem products.

Instruments

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- Waters HPLC System series with Binary pumps, Photo Diode array detector and manual sampler integrated with empower software
- Lab India UV double beam spectrophotometer with UVwin5 software was used for measuring absorbances of Nirmatrelvir and Ritonavir solutions.

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 buffer

Buffer: 0.01N Potassium dihydrogen Ortho phosphate

1.36 grams of potassium dihydrogen orthophosphate were precisely weighed and poured to a 1000 ml volumetric flask along with approximately 900 ml of milli-Q water, which was then degassed to sonicate and used to make up the volume with water. One milliliter of triethylamine was then added, and the pH was adjusted to 3.5 using a solution of dilute orthophosphoric acid.

Preparation of Standard stock solutions

Accurately weighed 7.5mg of Nirmatrelvir,5mg of Ritonavir was then transferred to fifty milliliter flasks, to which 3/4 of the diluents were added, and the mixture was sonicated for ten minutes. Diluents were added to the flask, which was then marked "Standard stock solution." (100µg/ml Ritonavir and 150µg/ml Nirmatrelvir)

Preparing a 100% solution, or standard working solutions

Each stock solution was pipetted out to yield 1 milliliter, which was then put into a 10 milliliter volumetric flask and diluted. Ritonavir (10µg/ml) with Nirmatrelvir (15µg/ml)

Preparation of Sample stock solutions

Ten tablets were taken, and the average amount of each tablet was determined to be 150 mg and 100 mg. Was captured After adding 20ml of acetonitrile and sonicating it for 25 minutes, the mixture was adjusted to produce 1100 and 500µg/ml. It spent 20 minutes in a centrifuge. Subsequently, the supernatant was gathered and filtered through Millipore, Milford, PVDF 0.45µm filters (containing 300µg/ml of Nirmatrelvir and 200µg/ml of Ritonavir). Sample working solutions (100% solution) were prepared by adding diluent (15µg/ml of Nirmatrelvir and 10µg/ml of Ritonavir) to 0.5 ml of filtered sample stock solution that had been transferred to a 10 ml volumetric flask.

Validation

System suitability parameters

By making standard solutions of Ritonavir (10 ppm) and Nirmatrelvir (15 ppm), and injecting the solutions six times, the system appropriateness parameters such as peak tailing, resolution, and USP plate count were ascertained. It is recommended that the percentage RSD for the results of six standard injections not exceed 2%.

Specificity

Verifying that the optimized procedure is free of interference. Interfering peaks in blank and placebo at these medications' retention times should not be observed using this method. It was said that this approach was specific.

Precision

Preparation of Sample stock solutions

Ten tablets were taken, and the average amount of each tablet was determined to be 150 mg and 100 mg. Was captured After adding 20ml of acetonitrile and sonicating it for 25 minutes, the mixture was adjusted to produce 1100 and 500µg/ml. It spent 20 minutes in a centrifuge. Next, the supernatant was gathered and filtered through Millipore, Milford, PVDF 0.45 µm filters (containing 200µg/ml of Ritonavir and 300µg/ml of Nirmatrelvir). Example working solution preparation (100 percent solution): After filtering, 0.5 ml of the sample stock solution was added to a 10 ml volumetric flask and diluted. Nirmatrelvir (15µg/ml) and Ritonavir (10µg/ml). In order to measure precision, sample solutions of 15 ng of nimatrelvir and 10 ppm of ritanavir were prepared. The solutions were then injected six times, and the percentage RSD for the area of the six standard injection results should not exceed 2%.

Linearity

Standard stock solution preparation: Weighed precisely 7.5 mg of Nirmatrelvir and 5 mg of Ritonavir, the samples were transferred to 50 ml flasks, filled with 3/4 th of diluent, and sonicated for 10 minutes. Diluents were added to the flask, which was then marked "Standard stock solution." (100µg/ml Ritonavir and 150µg/ml Nirmatrelvir)

25% Standard solution: Two standard stock solutions were pipetted out to a total of 10 milliliters, or 0.25 milliliters each. (3.75µg of Nirmatrelvir and 2.5µg of Ritonavir per milliliter)
50% Standard solution: two standard stock solutions, each containing 0.5 ml, were
75% Standard solution: Two standard stock solutions were pipetted out, yielding a total of 10 milliliters (0.75) each. (7.5µg/ml of Ritonavir and 11.25µg/ml of Nirmatrelvir)
100% Standard solution: Two standard stock solutions were pipetted out into 1.0 ml each, for a total of 10 ml. Nirmatrelvir (15µg/ml) with Ritonavir (10µg/ml)
125% Standard solution: Two standard stock solutions were pipetted out into 1.25 ml each, for a total of 10 ml. Nirmatrelvir (18.75µg/ml) with Ritonavir (12.5µg/ml)
150% Standard Solution: Two standard stock solutions were pipetted out to yield 1.5 ml each, which was then increased to 10 ml (22.5 µg/ml of Nirmatrelvir and 15 µg/ml of Ritonavir).

Accuracy
Preparation of Sample stock solutions: Ten tablets were taken, and the average amount of each tablet was determined to be 150 mg and 100 mg. Was captured After adding 20ml of acetonitrile and sonicating it for 25 minutes, the mixture was adjusted to produce 1100 and 500µg/ml. It spent 20 minutes in a centrifuge. Next, the supernatant was gathered and filtered through Millipore, Milford, PVDF 0.45 µm filters (containing 300µg/ml of Nirmatrelvir and 200µg/ml of Ritonavir).
Preparation of Standard working solutions (100% solution): Each stock solution was pipetted out to yield 1 milliliter, which was then put into a 10 milliliter volumetric flask and diluted. Nirmatrelvir (15µg/ml) with Ritonavir (10µg/ml)
Making the 50% Spiked Solution: Using a 10 ml volumetric flask, 0.5 ml of the sample stock solution was added. Next, 1.0 ml of each standard stock solution was pipetted out, and the remaining amount was adjusted with diluent.
The 100% spiked solution was prepared as follows: 1.0 ml of the sample stock solution was pipetted out of a 10 ml volumetric flask, and diluent was added to make up the difference.
Preparation of 150% Spiked Solution: A 10 ml volumetric flask was filled with 1.5 ml of the sample stock solution, 1.0 ml of each standard stock solution was pipetted out, and the remaining volume was adjusted with diluent.
Acceptance Criteria: Every level's recovery percentage should range from 98.0 to 102.

Robustness
Modest, purposeful adjustments are made to the temperature, mobile phase ratio, and flow rate; these changes do not noticeably alter the outcome and fall within the acceptable range specified by the ICH guidelines.
Samples were injected in duplicate under robustness settings that included Flow minus (0.7 ml/min), Flow plus (0.9 ml/min), mobile phase minus, mobile phase plus, temperature minus (25 °C), and temperature plus (35 °C). All of the system suitability parameters passed with little to no impact. %RSD was not over the upper bound.
LOD sample Preparation: Two standard stock solutions were pipetted out, weighed down to a volume of 10 ml each, and then made up with diluents. From the aforementioned solutions, 10 ml volumetric flasks containing 0.1 ml of each of the solutions for Nirmatrelvir and Ritonavir were prepared using the same diluents.
LOQ instance Preparation: Two standard stock solutions were pipetted out onto separate 10 ml volumetric flasks, and 0.25 ml of each was made up with diluent. From the aforementioned solutions, 0.3 ml of Ritonavir and Nirmatrelvir solutions, respectively, were put into 10 ml volumetric flasks and diluted with the same solution.

Degradation studies
Oxidation
Each of the two stock solutions—1 milliliter of Nirmatrelvir and Ritonavir—was mixed with one milliliter of 20% hydrogen peroxide (H2O2). The solutions were maintained at 600°C for thirty minutes. In order to evaluate the stability of the sample, the HPLC investigation involved diluting the sample solution to obtain 15µg/ml and 10µg/ml solutions. After injecting 10µl into the system, the chromatograms were recorded.

Acid Degradation Studies
1 milliliter of 2N hydrochloric acid was added to 1 milliliter of stock solution Nirmatrelvir and Ritonavir, and the mixture was refluxed for 30 minutes at 600 degrees Celsius. The resulting solution was diluted to get solutions that were 15µg/ml and 10µg/ml. After injecting 10µl of these solutions into the system, the chromatograms were recorded in order to evaluate the sample's stability.

Alkali Degradation Studies
After adding 1 ml of 2N sodium hydroxide to 1 ml of the stock solution containing Ritonavir and Nirmatrelvir, the mixture was refluxed for 30 minutes at 600 degrees Celsius. After diluting the sample solution to yield 15µg/ml and 10µg/ml

solutions, 10µl of the mixture was injected into the system, and chromatograms were collected to evaluate the sample's stability.

Studies on Dry Heat Degradation

For one hour, the usual medication solution was kept at 105°C. In order to evaluate the stability of the sample for the HPLC analysis, the final solution was diluted to 15µg/ml and 10µg/ml. 10µl was then injected into the system, and the chromatograms were recorded.

Photo Stability studies

By subjecting the 312.5µg/ml Nirmatrelvir and 125µg/ml Ritonavir solution to UV light and holding the beaker in a UV chamber for a day or 200-Watt hours/m2, the photochemical stability of the medication was also investigated. In order to evaluate the stability of the sample for the HPLC investigation, the resultant solution was diluted to create 15µg/ml and 10µg/ml solutions. 10µl was then injected into the system, and the chromatograms were recorded.

Studies on Neutral Degradation

In order to study stress testing in neutral conditions, the medication was refluxed in water for one hour at 60º. The final solution was diluted to 15µg/ml and 10µg/ml for the HPLC analysis. 10µl of the mixture was then injected into the system, and the chromatograms were recorded to evaluate the sample's stability.

RESULTS AND DISCUSSION

Optimized wavelength selected was 215nm.

Method development

The development of the method involved adjusting buffers, mobile phase ratios, and other variables.

Trial 1:

Chromatographic conditions:

Mobile phase **:** Acetonitrile: Water (60:40 v/v)
Flow rate **: 1** ml/min
Column **:** Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length **:** 215nm
Column temperature **:** 30°C
Injection volume **:** 10.0µL
Run time **:** 10.0 min
Diluent **:** Water and Acetonitrile in the ratio 50:50 v/v
Results : In this trail only one peak was eluted, so, further trial is carried out.

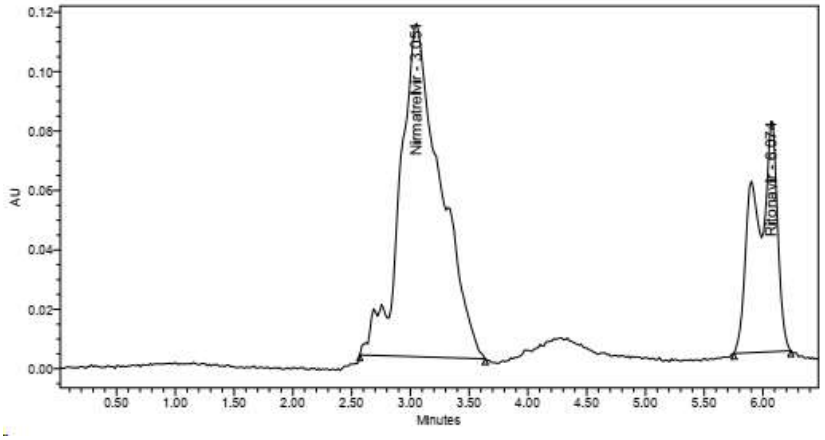


Fig 1: Trial chromatogram 1

Chromatographic conditions

Mobile phase **:** Water**:** Methanol (50:50)
Flow rate **: 1** ml/min
Column **:** Agilent C18 (4.6 x 250mm, 5µm)

Detector wave length **:** 215nm
Column temperature **:** 30°C
Injection volume **:** 10µL
Run time **:** 5 min
Diluent **:** Water and Acetonitrile in the ratio (50:50)
Results : Both peaks were eluted but both peaks were eluted with broad shape and peak splitting is observed. so, Further trial is carried out.

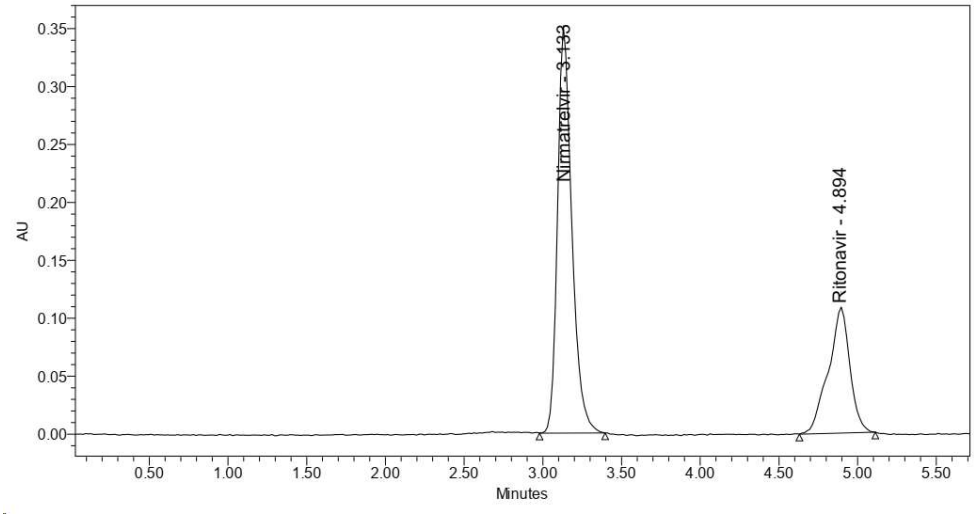


Fig 2: Trial chromatogram 2

Trial 3:
Chromatographic conditions
Mobile phase **:** 0.1%OPA**:** Methanol(50:50)
Flow rate **: 1** ml/min
Column **:** AgilentC18 (4.6 x 250mm, 5µm)
Detector wave length **:** 215nm
Column temperature **:** 30°C
Injection volume **:** 10µL
Run time **:** 5 min
Diluent **:** Water and Acetonitrile in the ratio (50:50)
Results : Both peaks were eluted but Ritonavir peak shape isnot good so, Further trial is carried out.

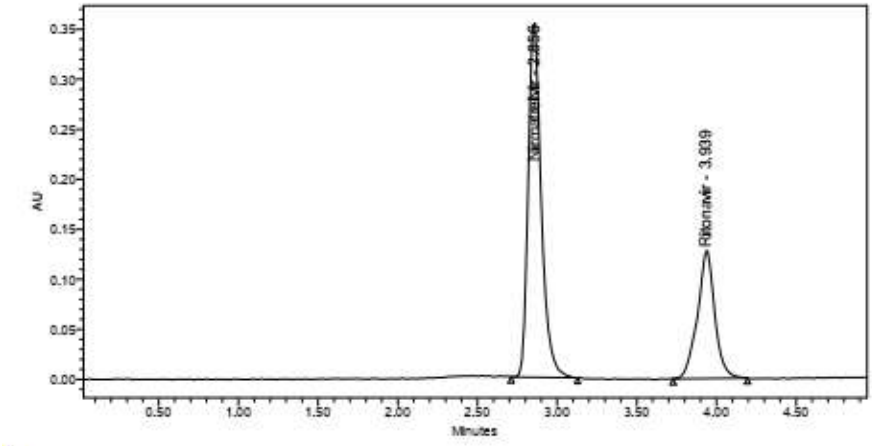


Fig 3: Trial chromatogram 3

Trial 4:

Chromatographic conditions

Mobile phase **:** 0.1%OPA: Acetonitrile (50:50)
Flow rate **: 1** ml/min
Column **:** Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length **:** 215nm
Column temperature **:** 30°C
Injection volume **:** 10µL
Run time **: 5** min
Diluent **:** Water and Acetonitrile in the ratio 50:50
Results **:** Ritonavir's peak retention period is longer than that of Nirmatrelvir's, although both peaks are eluted. Thus, more trails are conducted.

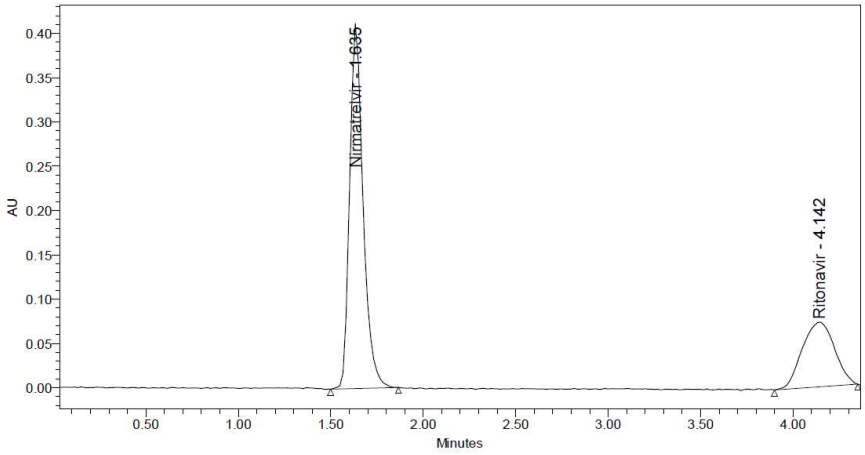


Fig 4: Trial chromatogram 4

Optimized method

Chromatographic conditions

Mobile phase **:** 0.01N Kh2po4: Acetonitrile (50:50)
Flow rate **:** 1ml/min
Column **:** Agilent C8 (4.6 x 150mm, 5µm)
Detector wave length **:** 215nm
Column temperature **:** 30°C
Injection volume **:** 10µL
Run time **: 10**min
Diluent **:** Water and Acetonitrile in the ratio 50:50
Results **:** Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

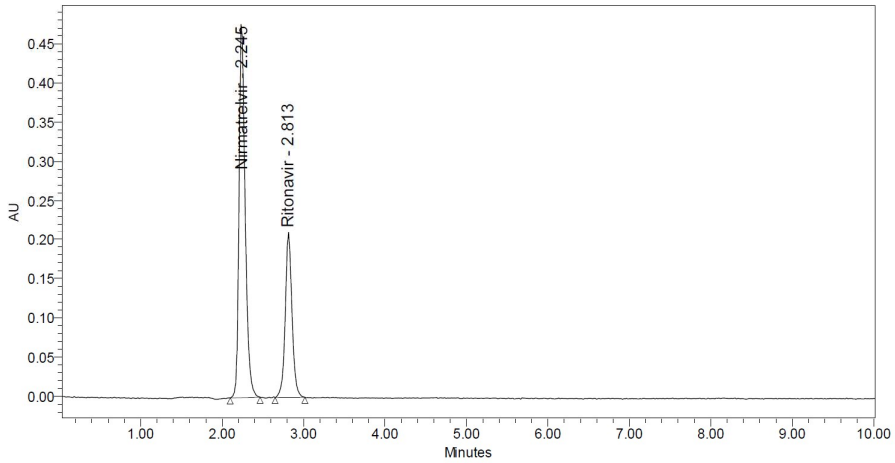


Fig 5: Optimized Chromatogram

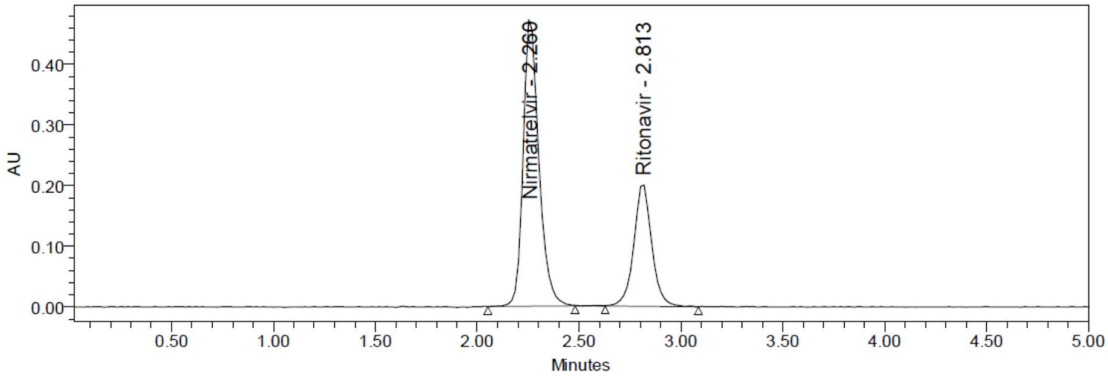
Ritonavir and nimatrelvir were eluted with high resolution at 2.241 and 2.816 minutes, respectively. The plate count and tailing factor were highly satisfactory, leading to the optimization and validation of this approach.

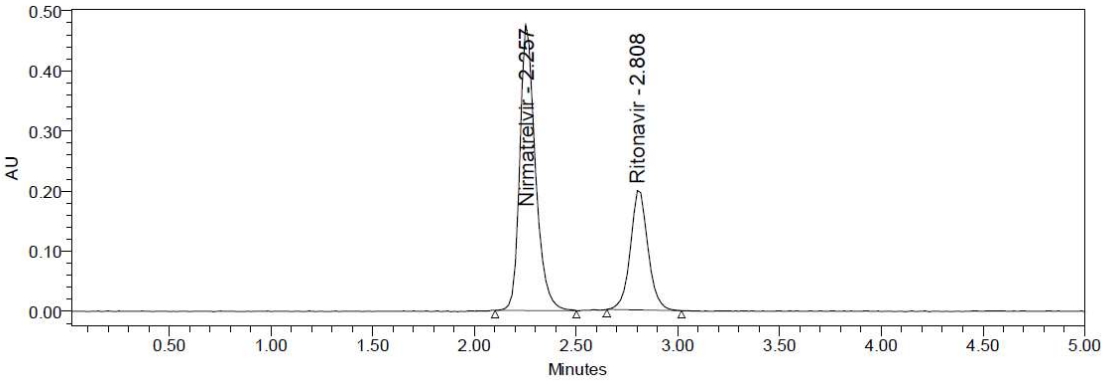
System suitability

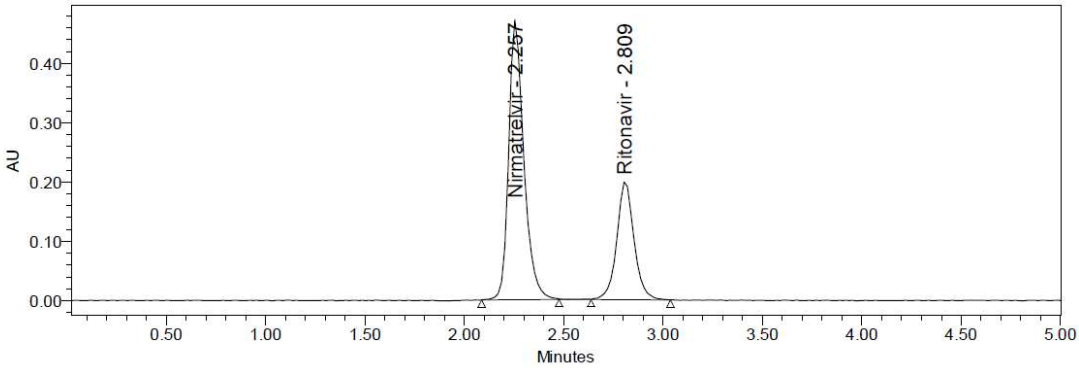
According to ICH criteria, all of the system suitability parameters were satisfactory and within the range.

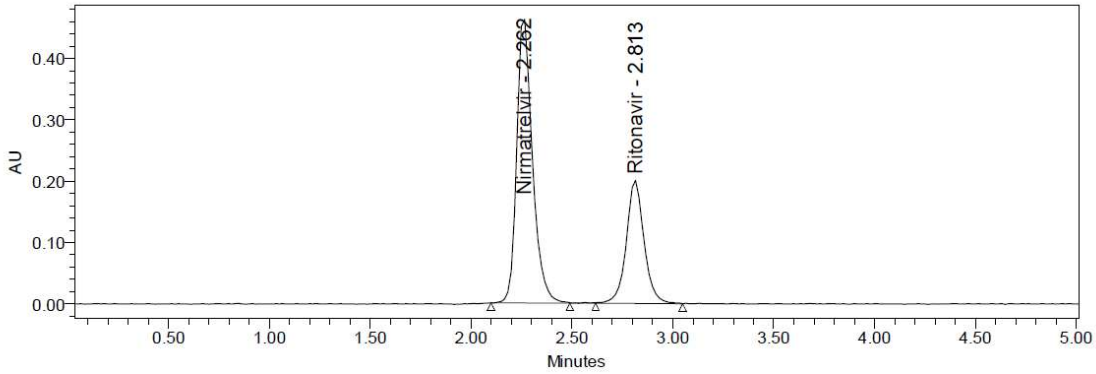
Table 1: System suitability parameters for Nirmatrelvir and Ritonavir

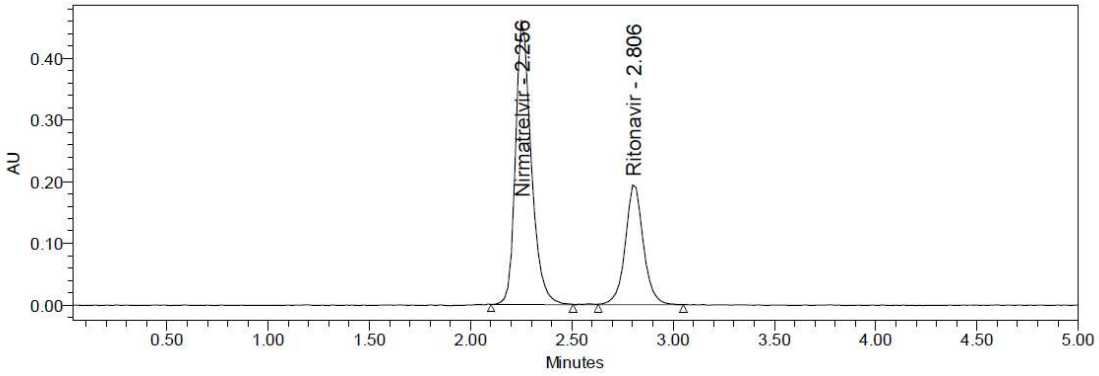
S no	Nirmatrelvir			Ritonavir			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	2.256	4131	1.29	2.806	5264	1.04	3.5
2	2.257	4355	1.29	2.808	5484	1.07	3.6
3	2.257	4284	1.27	2.809	5575	1.06	3.7
4	2.258	4410	1.28	2.812	5241	1.03	3.6
5	2.260	4330	1.27	2.813	5242	1.05	3.7
6	2.262	4285	1.25	2.813	5339	1.03	3.6




Nirmatrelvir - 2.257
Ritonavir - 2.808
AU
Minutes


Nirmatrelvir - 2.257
Ritonavir - 2.809
AU
Minutes


Nirmatrelvir - 2.262
Ritonavir - 2.813
AU
Minutes


Nirmatrelvir - 2.256
Ritonavir - 2.806
AU
Minutes

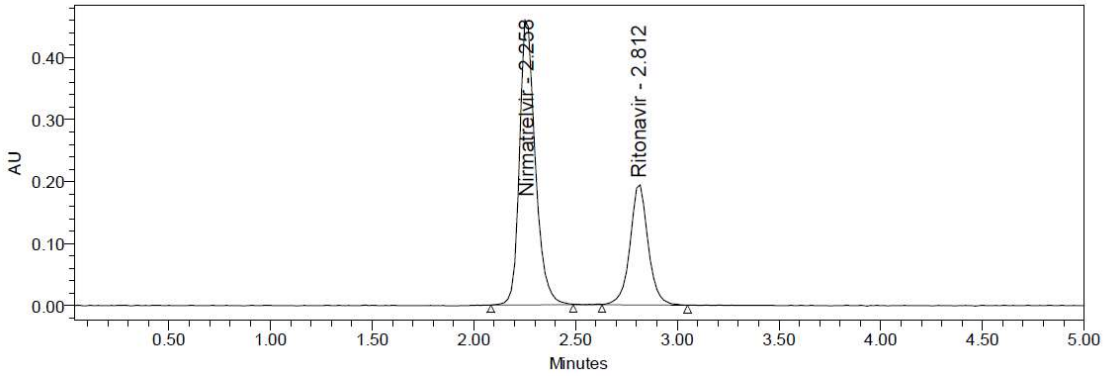


Fig 6: System suitability Chromatogram

The ICH criteria state that a plate count of more than 2000, a tailing factor of less than 2, and a resolution of more than 2 are required. Every system-appropriate parameter passed and remained within the range.

Validation
Specificity

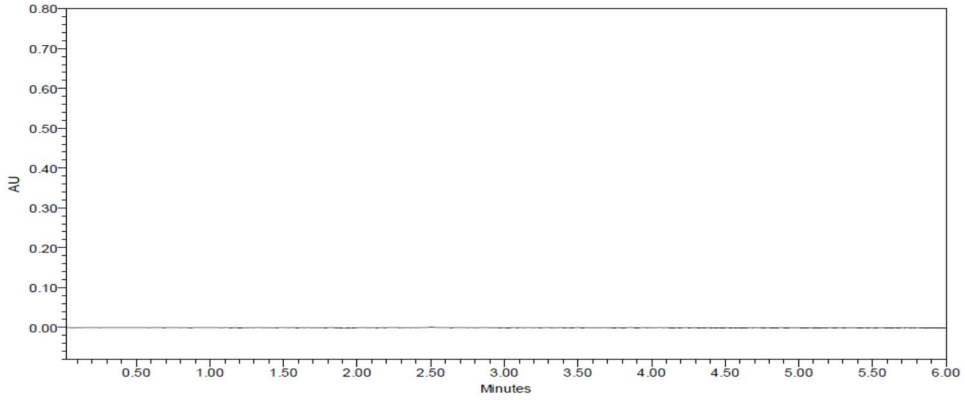


Fig 7: Chromatogramof blank

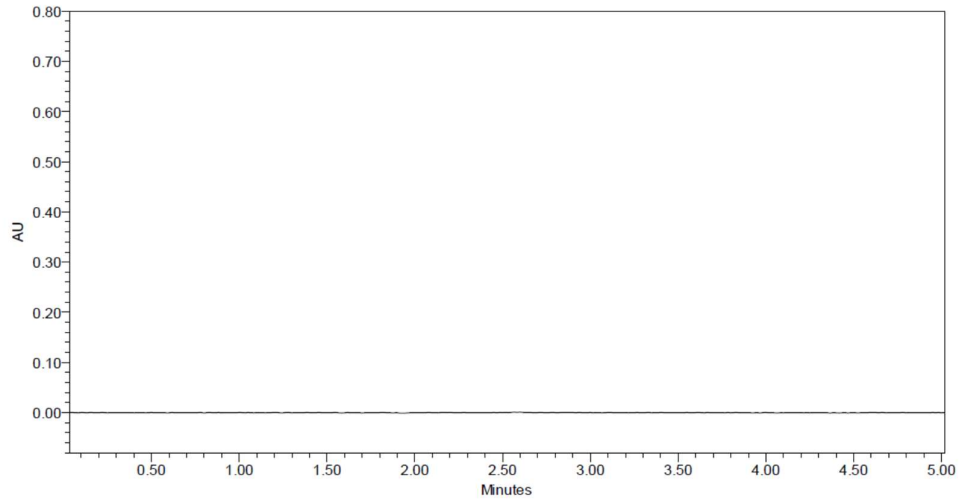


Fig 8: Chromatogramof placebo

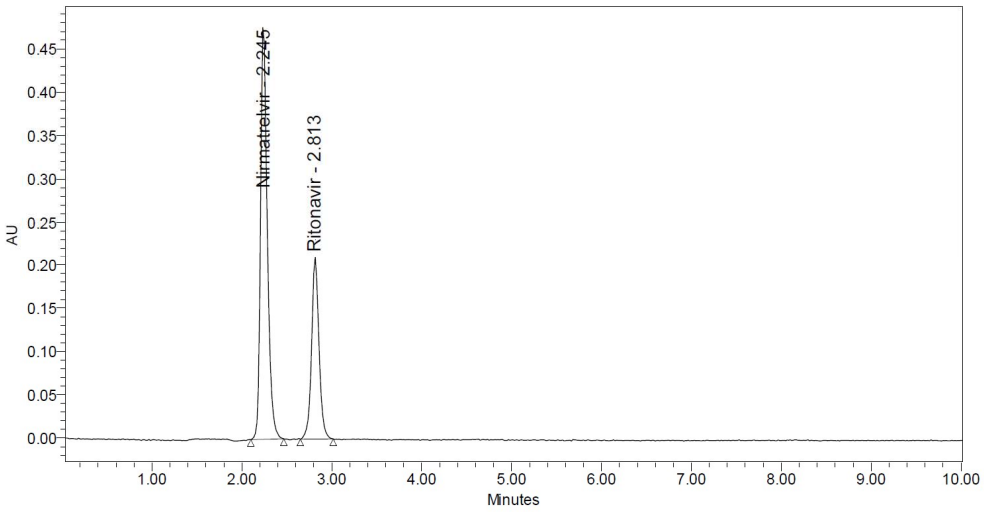


Fig 9: Typical Chromatogram

Ritonavir and Nirmatrelvir had retention periods of 2.812 and 2.258 minutes, respectively. Using this strategy, we were unable to find any interfering peaks in the blank or placebo at the retention times of these medications. It was said that this approach was specific.

Linearity

Table 2: Linearity table for Nirmatrelvirand Ritonavir

Nirmatrelvir		Ritonavir	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
3.75	63530	2.5	30323
7.5	130051	5	59595
11.25	186426	7.5	87814
15	252346	10	122107
18.75	318423	12.5	147962
22.5	378385	15	175627

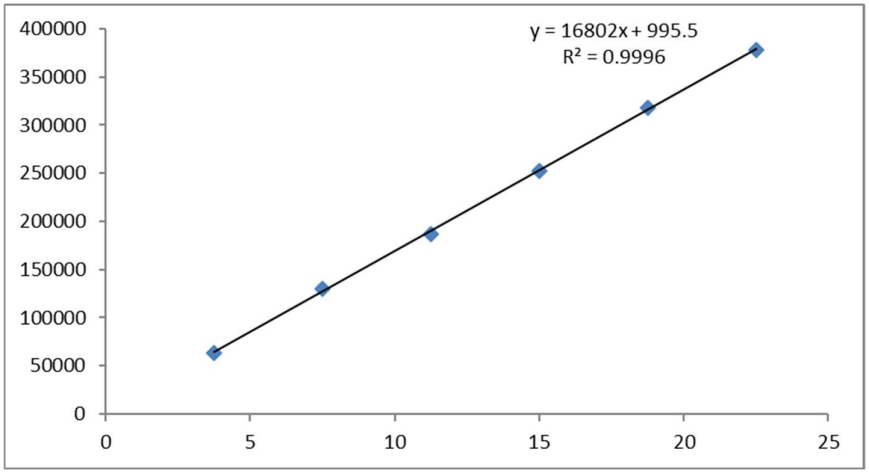


Fig 10: CalibrationcurveofNirmatrelvir

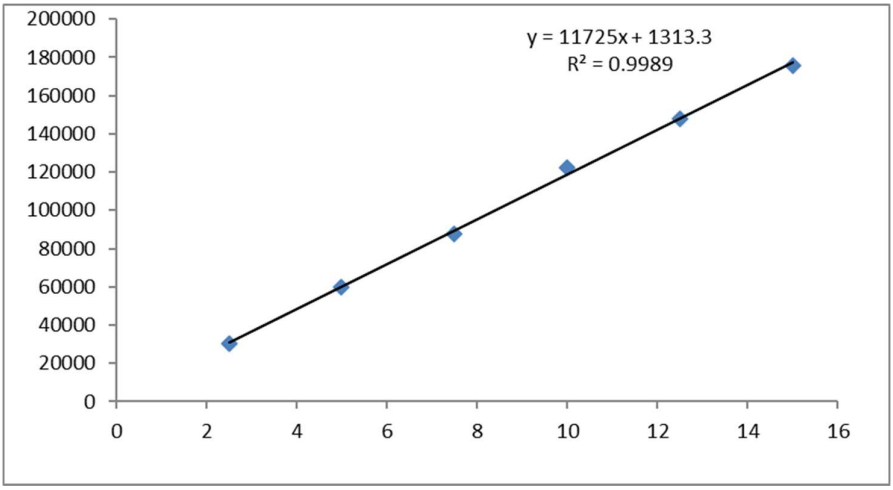


Fig 11: CalibrationcurveofRitonavir

Six linear dosages of Ritonavir (2.5-15µg/ml) and Nirmatrelvir (3.75-22.5µg/ml) were injected in duplicate. The aforementioned average areas were used to derive the linearity equations for Nirmatrelvir (y= 16802x + 995.5) and Ritonavir (y= 11725x + 1313.3).The obtained correlation coefficient was 0.999.

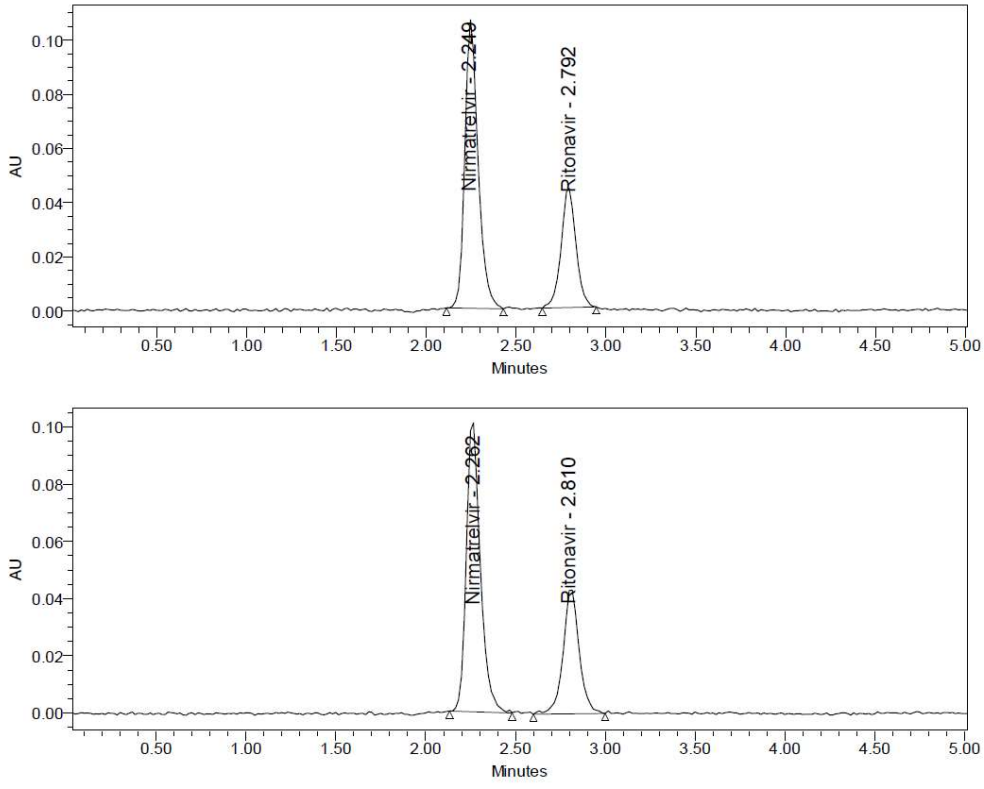


Fig 12: Linearity 25% Chromatogramof Nirmatrelvir and Ritonavir

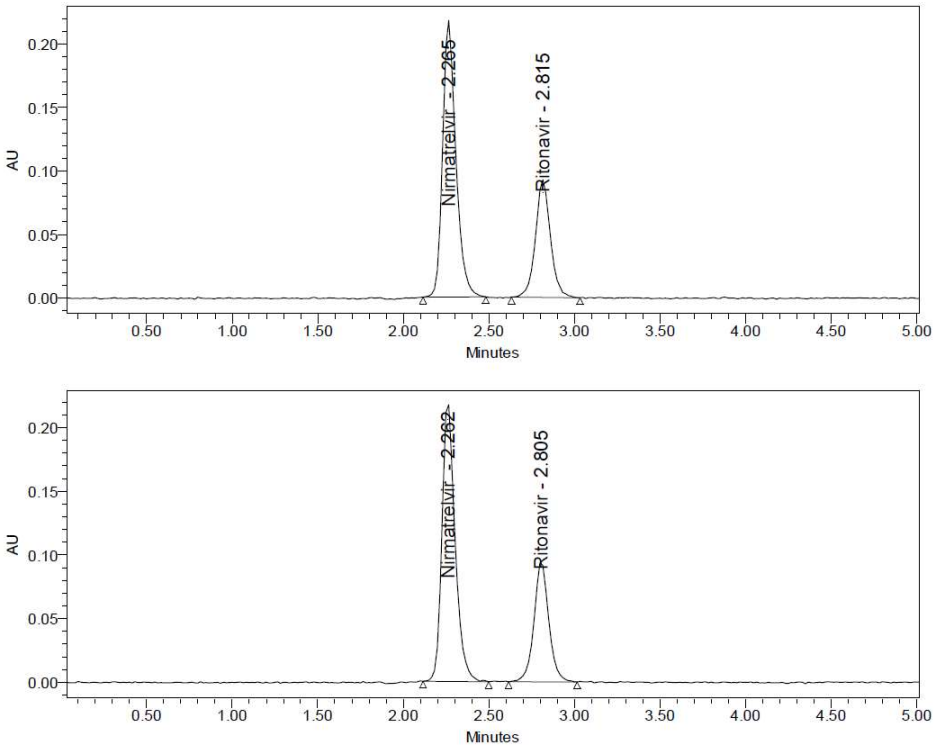


Fig 13: Linearity 50% Chromatogramof Nirmatrelvir and Ritonavir

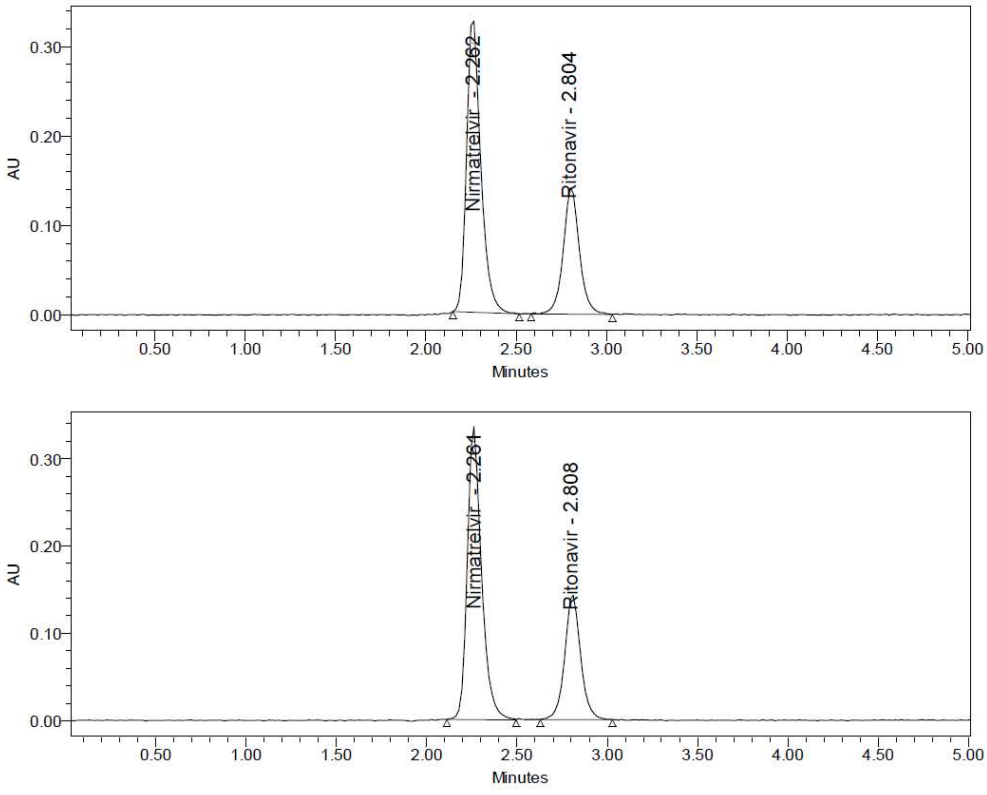


Fig 14: Linearity 75% Chromatogramof Nirmatrelvir and Ritonavir

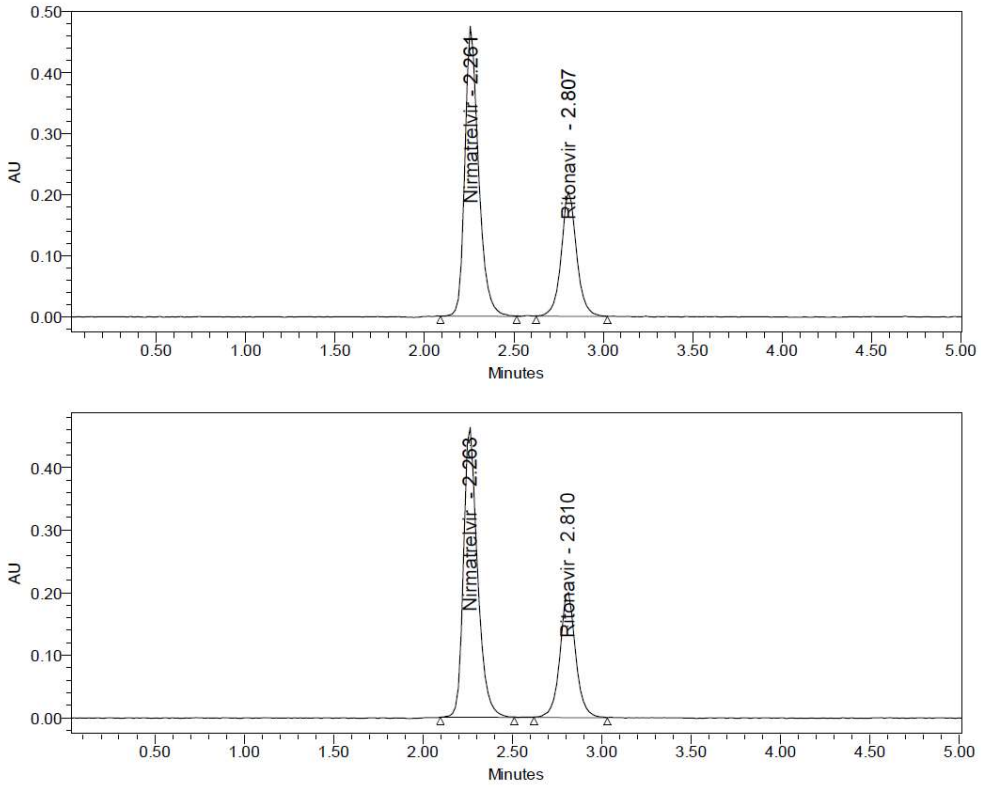


Fig 15: Linearity 100% ChromatogramofNirmatrelvir and Ritonavir

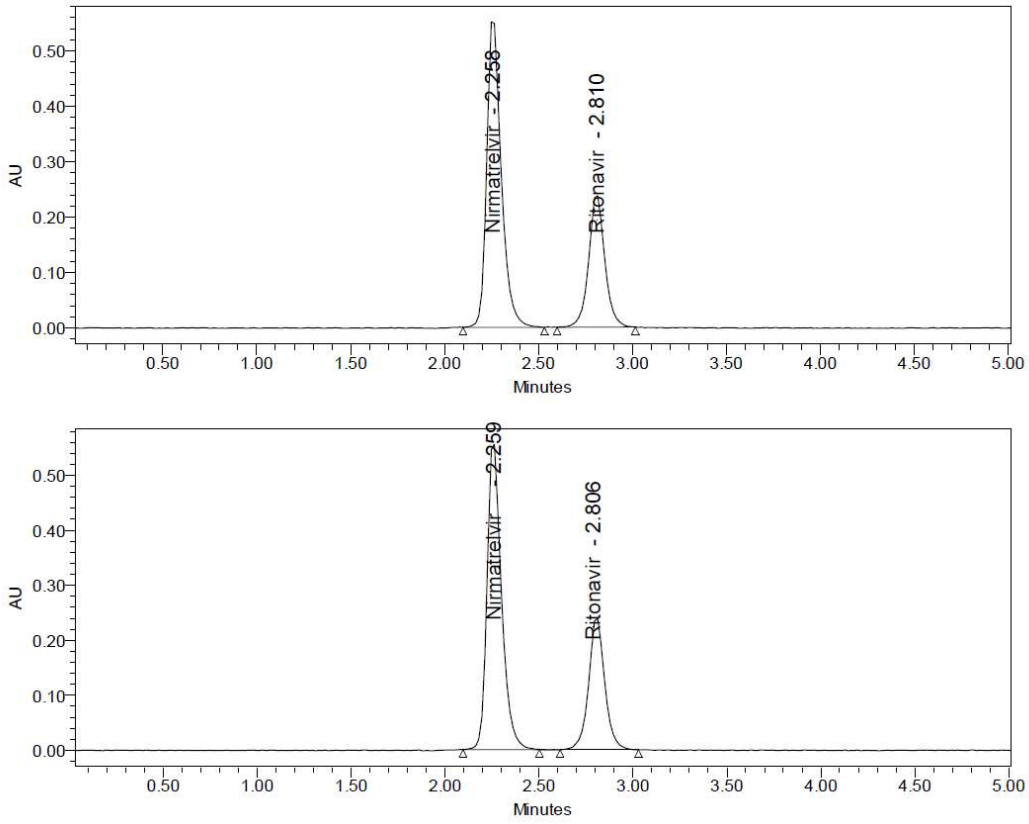


Fig 16: Linearity 125% ChromatogramofNirmatrelvir and Ritonavir

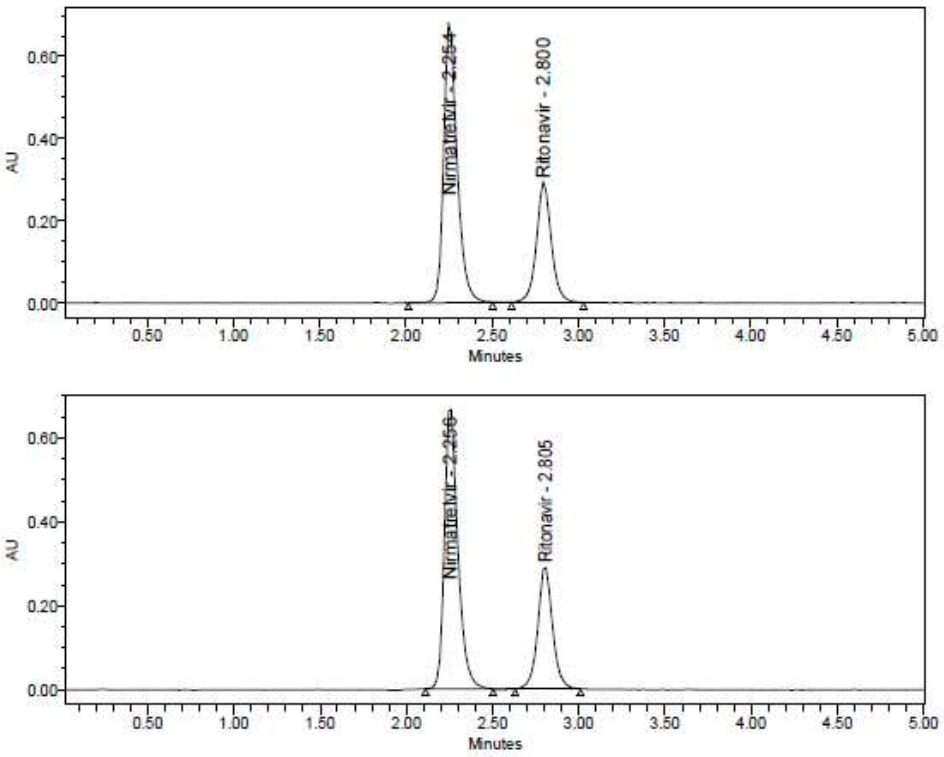
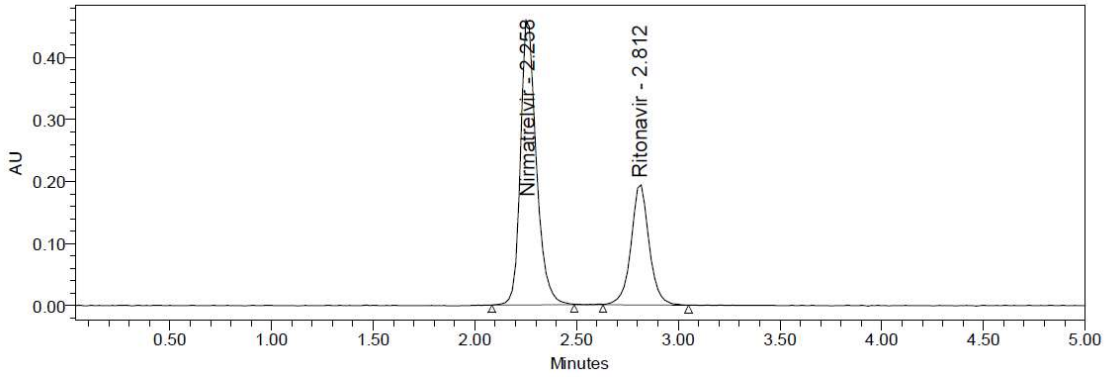


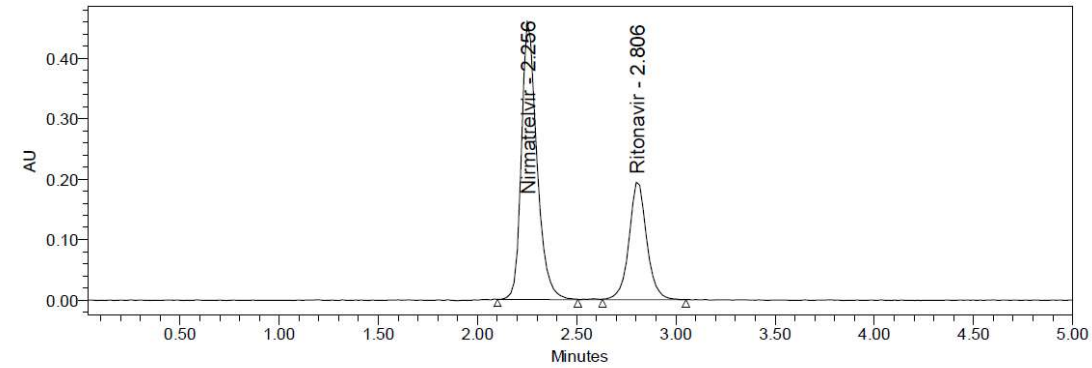
Fig 17: Linearity 150% Chromatogramof Nirmatrelvir and Ritonavir

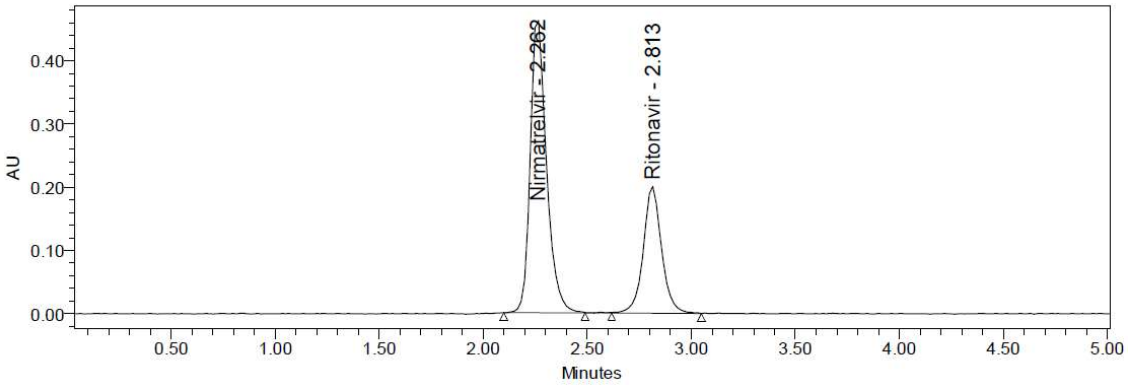
Precision
System Precision

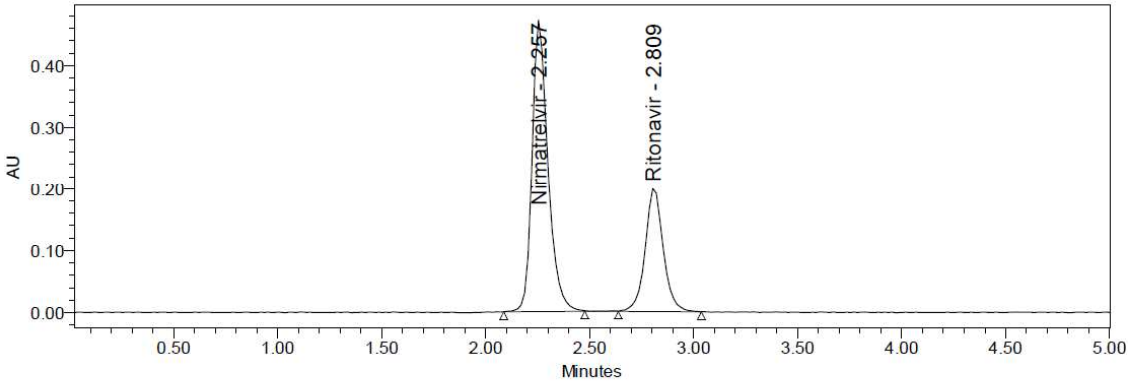
Table 3: System precision table of Nirmatrelvir and Ritonavir

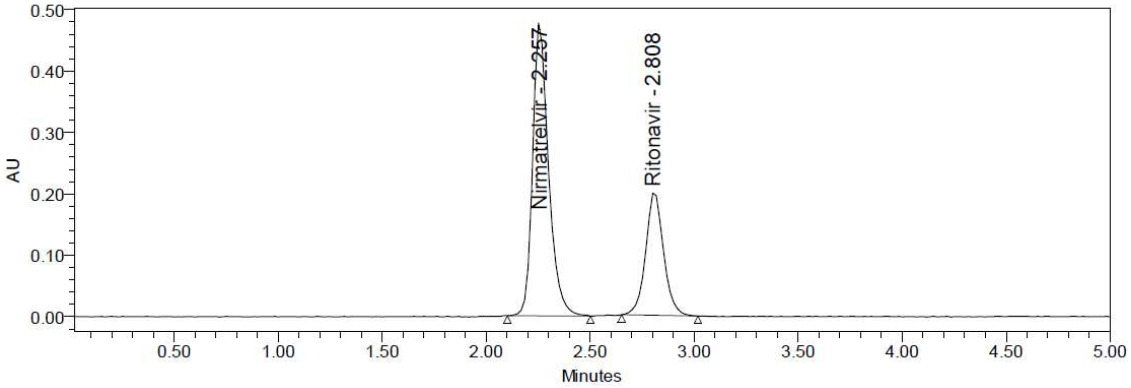
S. No	Area of Nirmatrelvir	Area of Ritonavir
1.	252676	119169
2.	252661	119165
3.	252638	118338
4.	252495	119901
5.	254357	118480
6.	254772	118065
Mean	253267	118853
S.D	1016.0	681.3
%RSD	0.4	0.6




Nirmatrelvir - 2.256
Ritonavir - 2.806
AU
Minutes


Nirmatrelvir - 2.262
Ritonavir - 2.813
AU
Minutes


Nirmatrelvir - 2.257
Ritonavir - 2.809
AU
Minutes


Nirmatrelvir - 2.257
Ritonavir - 2.808
AU
Minutes

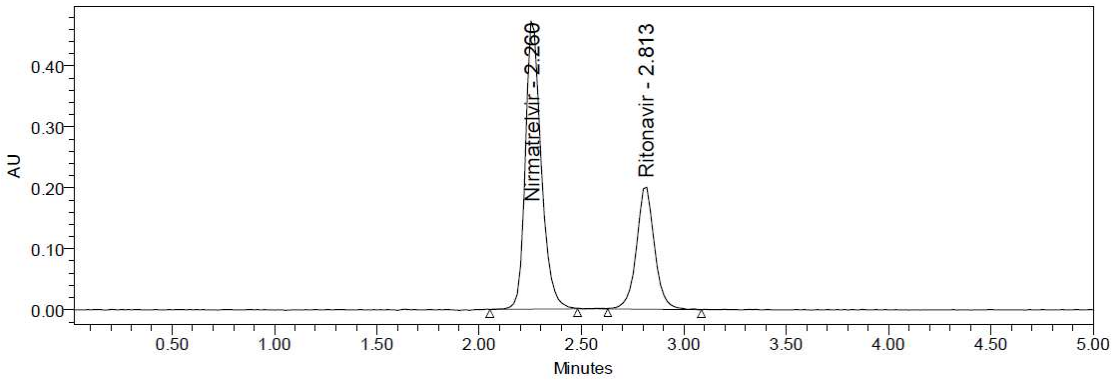


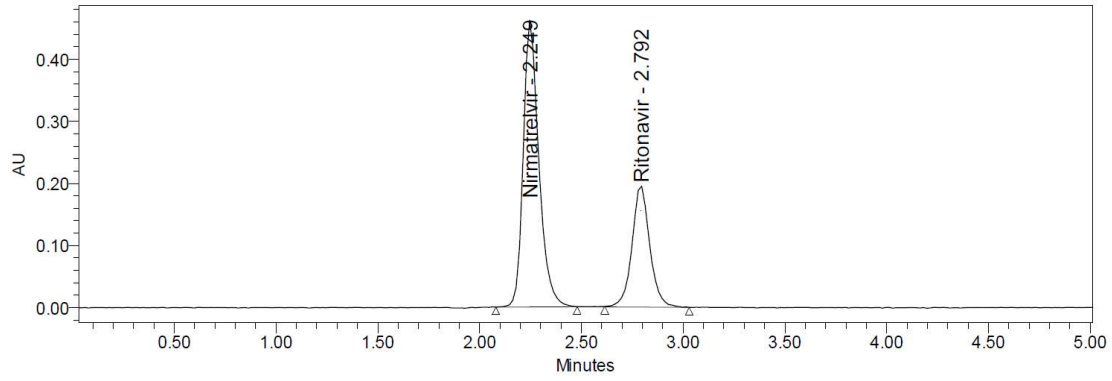
Fig 18: System precision chromatogram

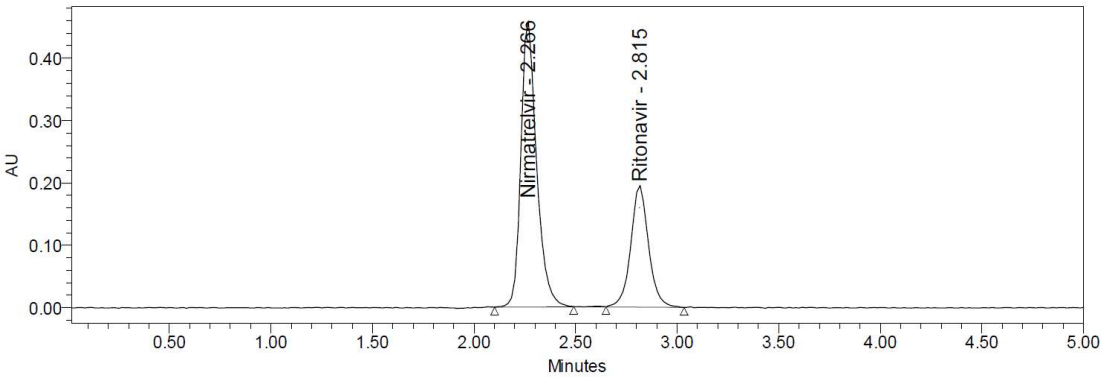
Six injections were made from a single volumetric flask containing the working standard solution, and the areas that were obtained were as previously described. For two medications, the average area, standard deviation, and percentage RSD were computed. RSD values for Ritonavir and Nirmatrelvir were found to be 0.6% and 0.4%, respectively. The system precision was passed using this method because the precision limit was less than "2".

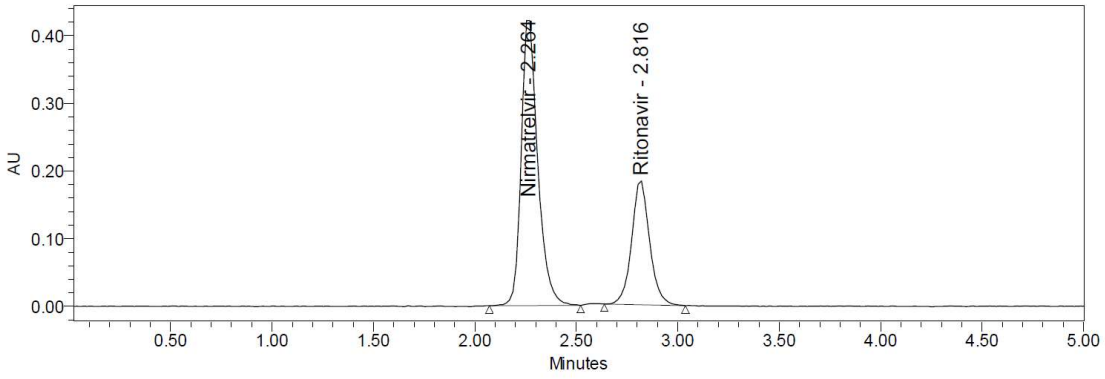
Repeatability

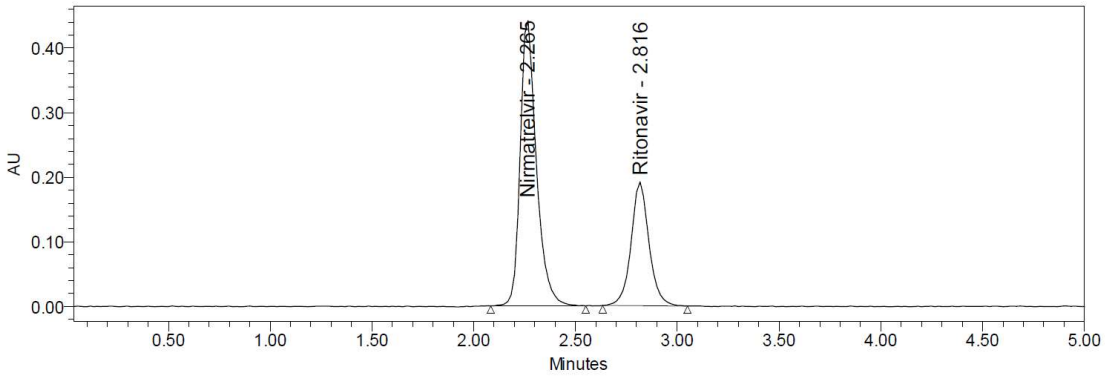
Table 4: Repeatability table of Nirmatrelvir and Ritonavir

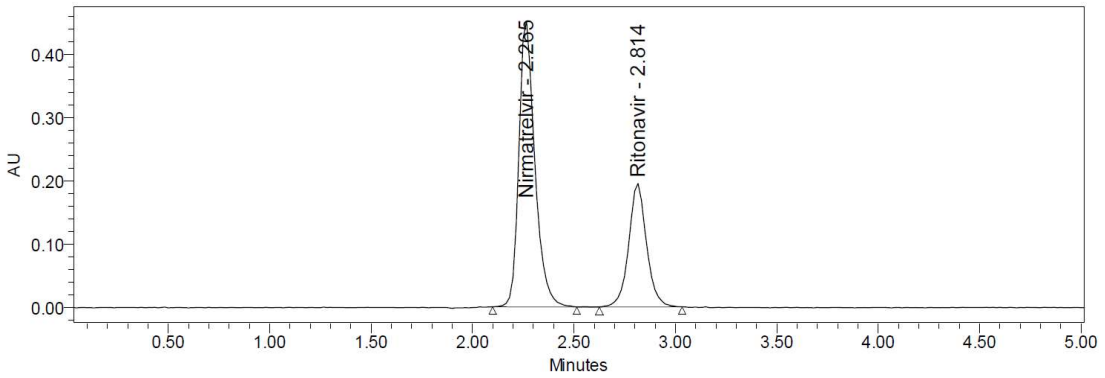
S. No	Area of Nirmatrelvir	Area of Ritonavir
1.	251414	118516
2.	252509	118393
3.	252274	118673
4.	256473	118782
5.	253323	118796
6.	252751	118832
Mean	253124	118665
S.D	1756.1	176.1
%RSD	0.7	0.1




Nirmatrelvir - 2.266
Ritonavir - 2.815
AU
Minutes


Nirmatrelvir - 2.264
Ritonavir - 2.816
AU
Minutes


Nirmatrelvir - 2.265
Ritonavir - 2.816
AU
Minutes


Nirmatrelvir - 2.265
Ritonavir - 2.814
AU
Minutes

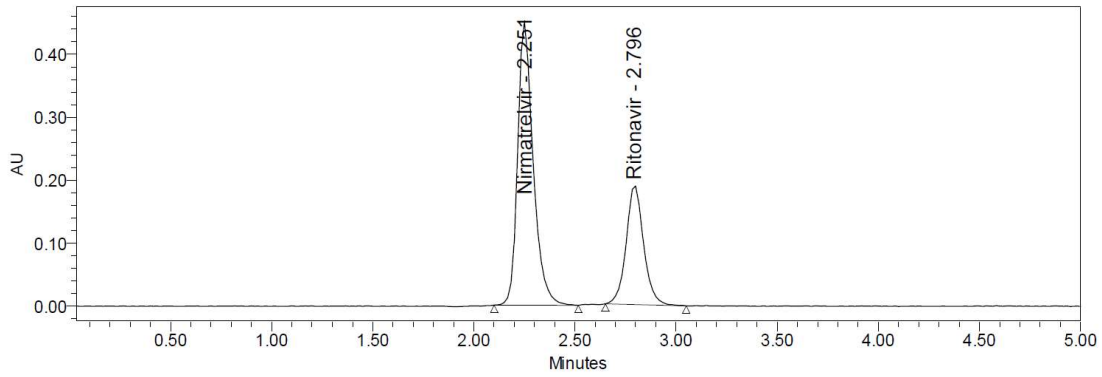


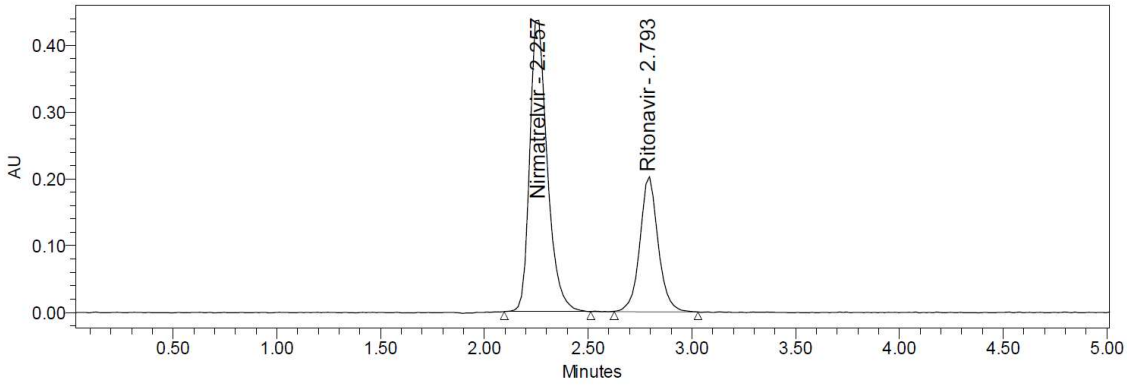
Fig 19: Repeatability chromatogram

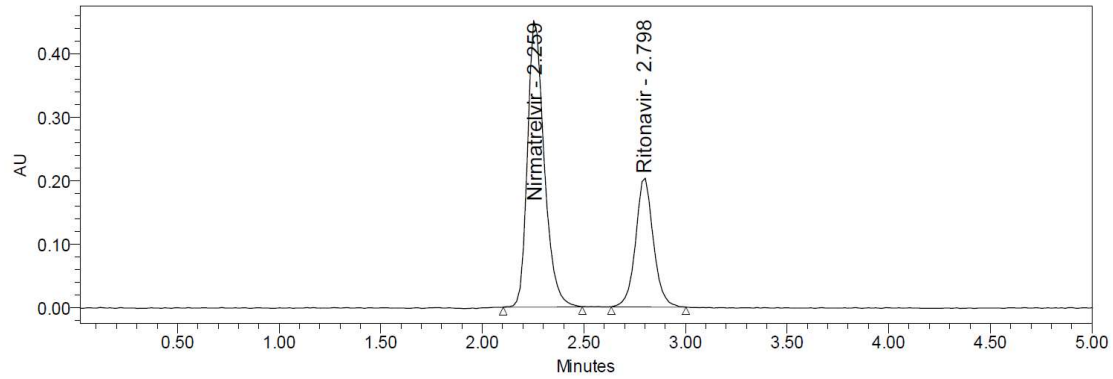
Six working sample solutions with the same concentrations were prepared after multiple sampling from a sample stock solution. Each injection from a working sample solution was provided, and the areas that were obtained are listed in the above table. After calculating the average area, standard deviation, and percentage RSD for two medications, it was found that Ritonavir and Nirmatrelvir had respective values of 0.7% and 0.1%. The system precision was passed using this method because the precision limit was less than "2".

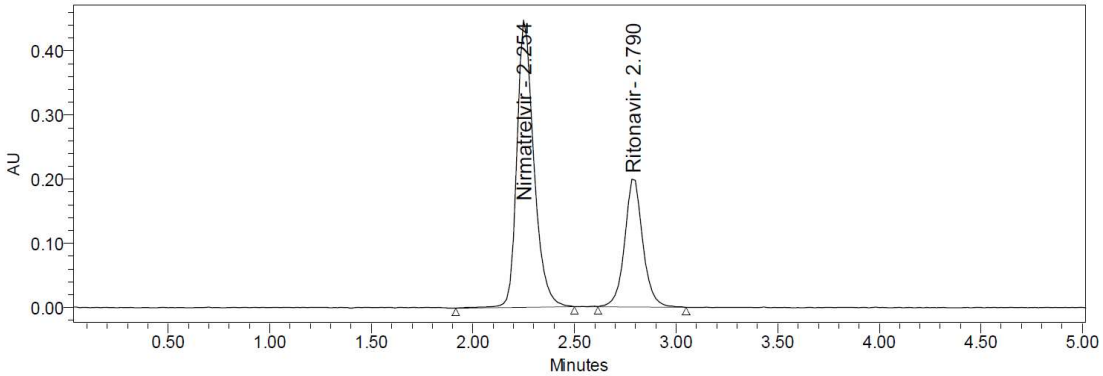
Intermediate precision (Day_ Day Precision)

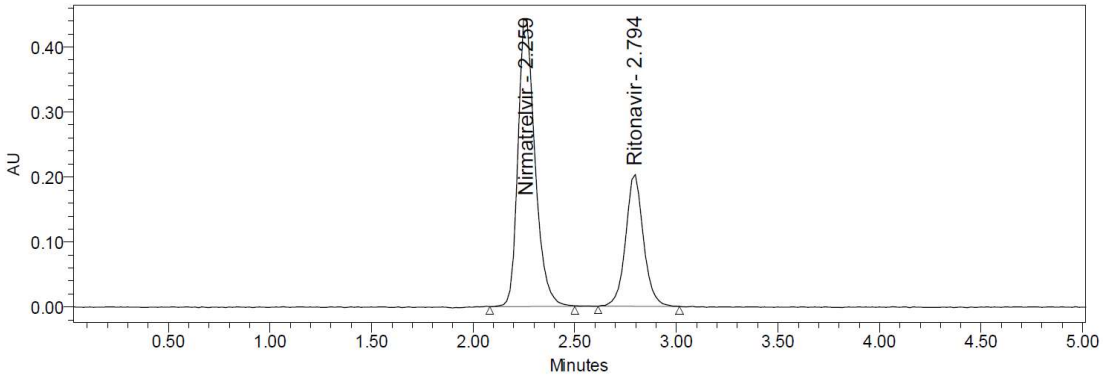
Table 5: Intermediate precision table of Nirmatrelvir and Ritonavir

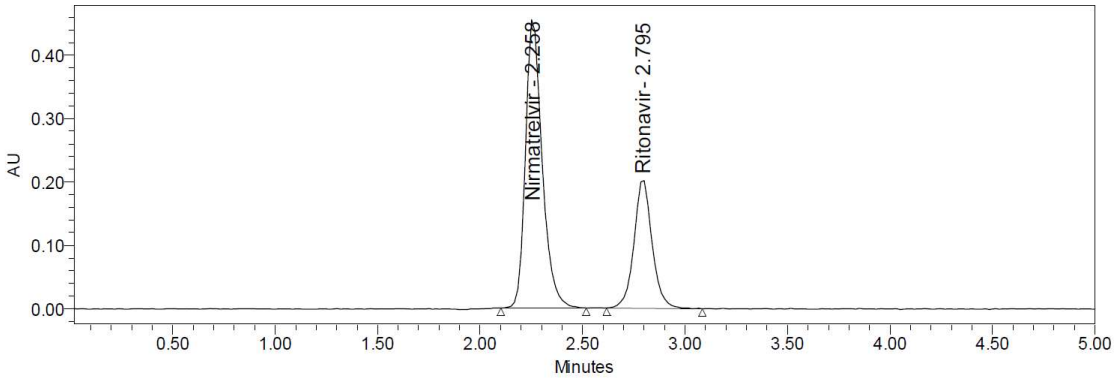
S. No	Area of Nirmatrelvir	Area of Ritonavir
1.	248551	110678
2.	249426	110488
3.	246627	110077
4.	246743	110417
5.	243234	111001
6.	248538	110191
Mean	247187	110475
S.D	2228.7	334.8
%RSD	0.9	0.3




Nirmatrelvir - 2.259
Ritonavir - 2.798
AU
Minutes


Nirmatrelvir - 2.254
Ritonavir - 2.790
AU
Minutes


Nirmatrelvir - 2.259
Ritonavir - 2.794
AU
Minutes


Nirmatrelvir - 2.258
Ritonavir - 2.795
AU
Minutes

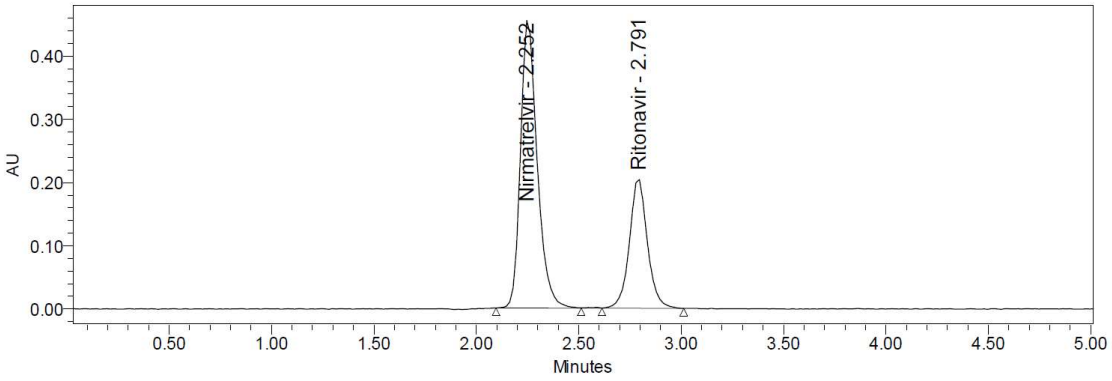


Fig 20: Inter Day precision Chromatogram

The next day of the sample preparation, each injection from each working sample solution was administered, and the areas that were acquired were listed in the above table. Multiple sampling from a sample stock solution was carried out, and six working sample solutions of the same concentrations were prepared. After calculating the average area, standard deviation, and percentage RSD for two medications, it was found that nimatrelvir and ritonavir had respective values of 0.9% and 0.3%. The system precision was passed using this method because the precision limit was less than "2".

Accuracy

Table 6: Accuracy table of Nirmatrelvir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	7.5	7.38	98.35	99.15%
	7.5	7.45	99.39	
	7.5	7.47	99.55	
100%	15	14.85	98.99	
	15	14.91	99.42	
	15	14.71	98.06	
150%	22.5	22.40	99.55	
	22.5	22.44	99.75	
	22.5	22.35	99.33	

Table 7: Accuracy table of Ritonavir

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	5.0	99.6	99.77%
	5	5.0	99.6	
	5	5.0	99.8	
100%	10	9.9	99.5	
	10	10.0	99.9	
	10	10.0	100.1	
150%	15	15.0	100.0	
	15	14.9	99.4	
	15	15.0	100.1	

The conventional addition method was used to prepare three levels of accuracy samples. For every accuracy level, three injections were administered, and the mean percentage recovery for nizamatetravir and ritanavir was found to be 99.15% and 99.77%, respectively.

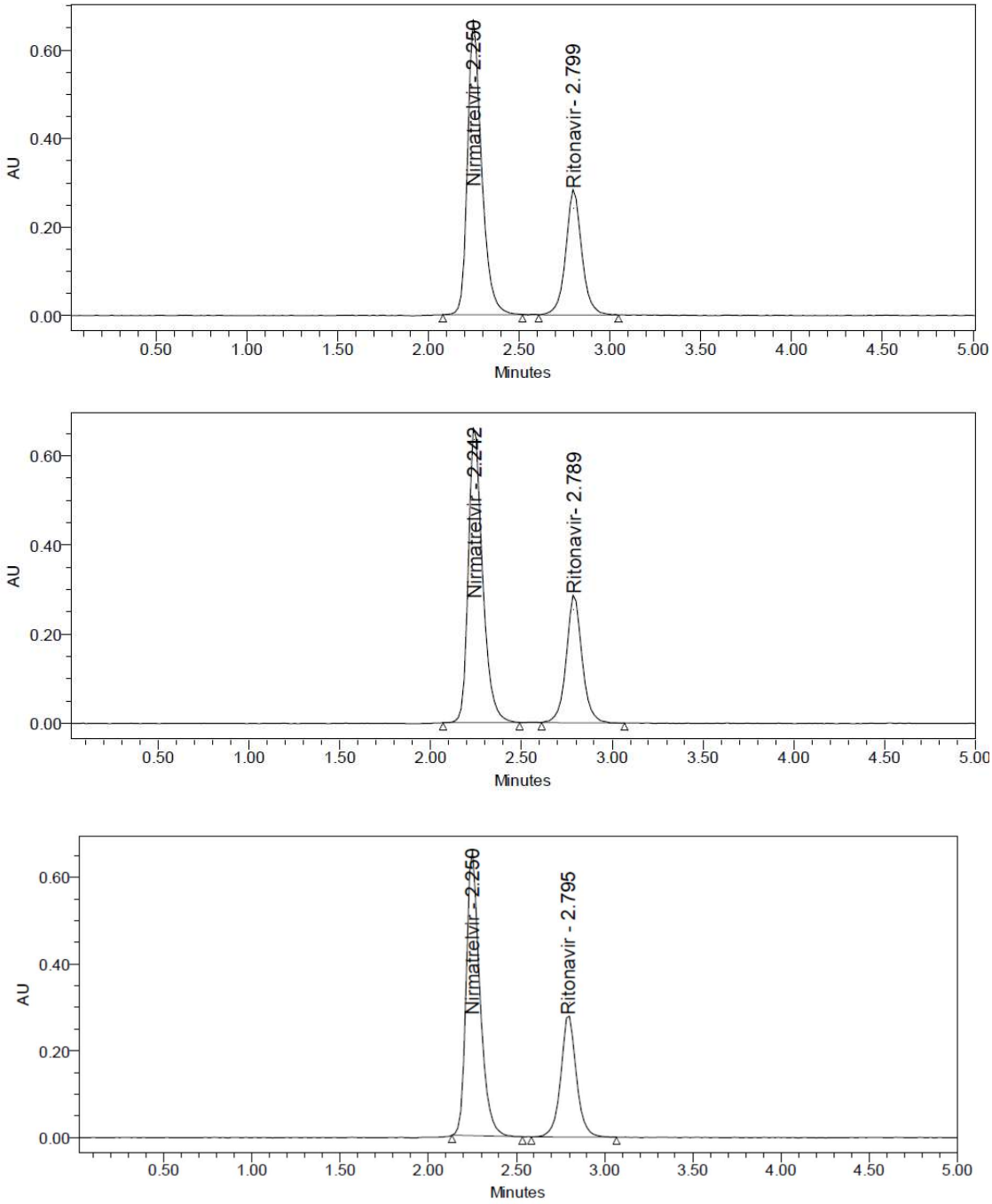


Fig 21: Accuracy 50% Chromatogram of Nirmatrelvir and Ritonavir

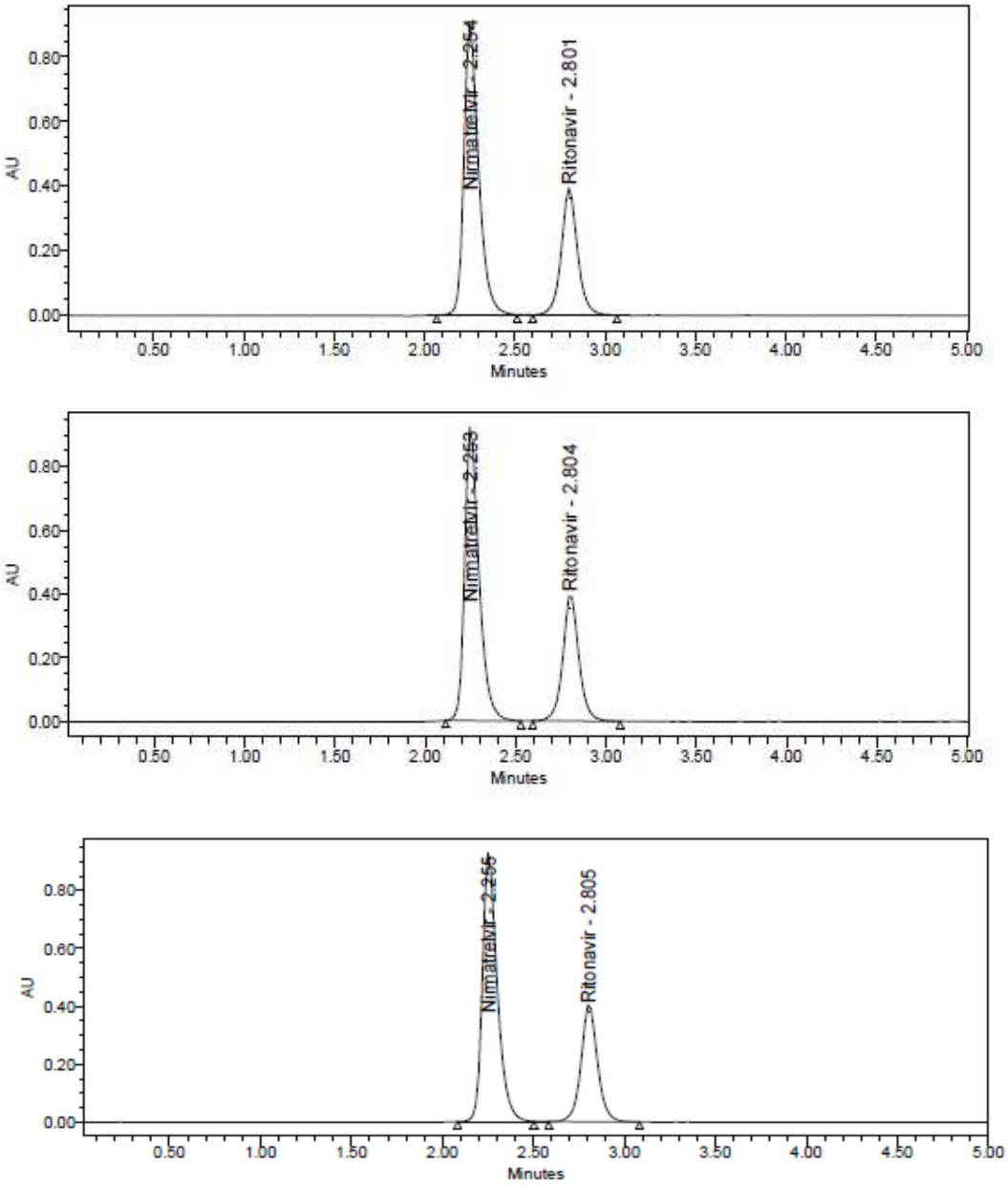


Fig 22: Accuracy 100% Chromatogramof Nirmatrelvir and Ritonavir

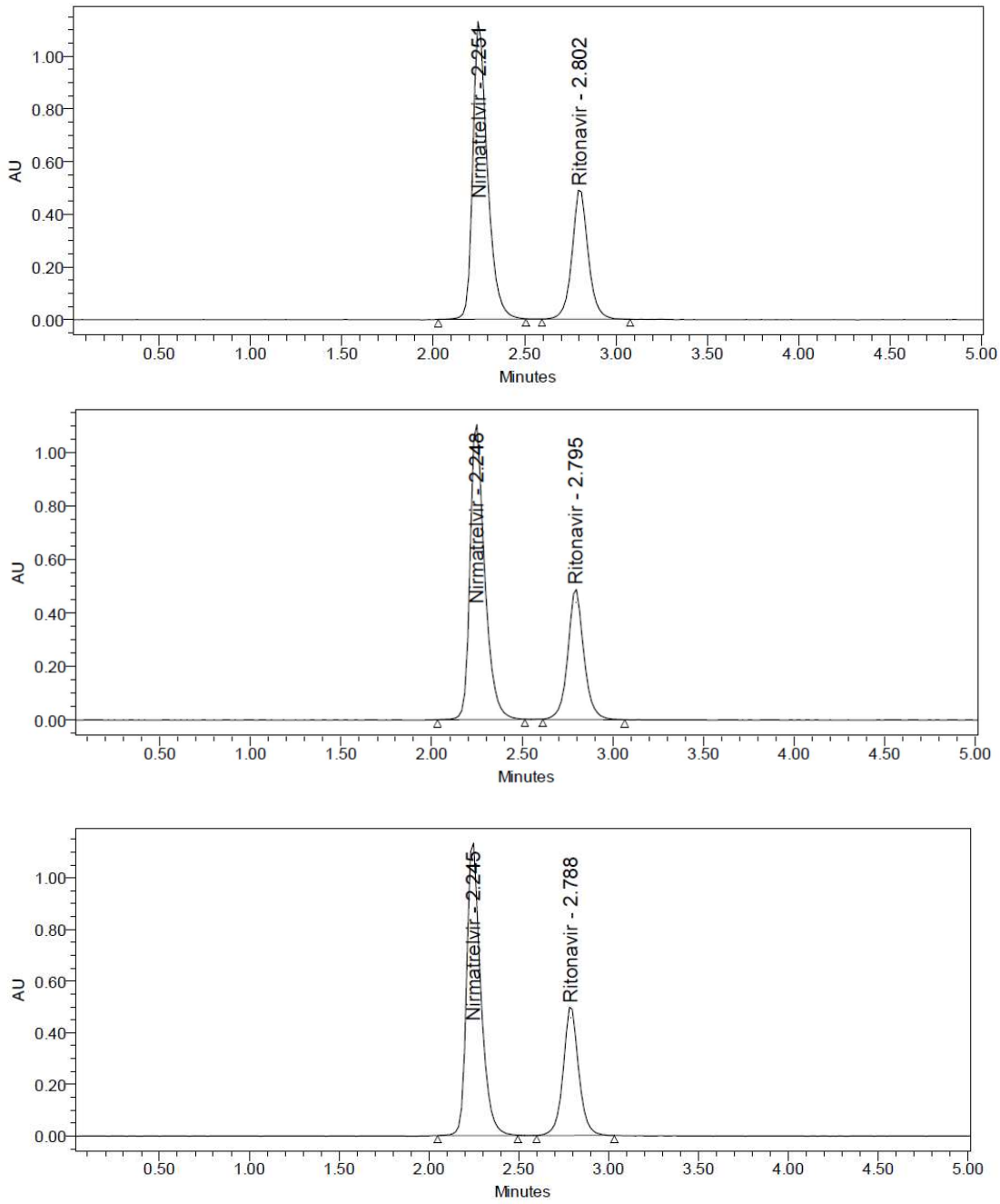


Fig 23: Accuracy 150% Chromatogram of Nirmatrelvir and Ritonavir

Sensitivity

Table 8: Sensitivity table of Nirmatrelvir and Ritonavir

Molecule	LOD	LOQ
Nirmatrelvir	0.21	0.65
Ritonavir	0.16	0.48

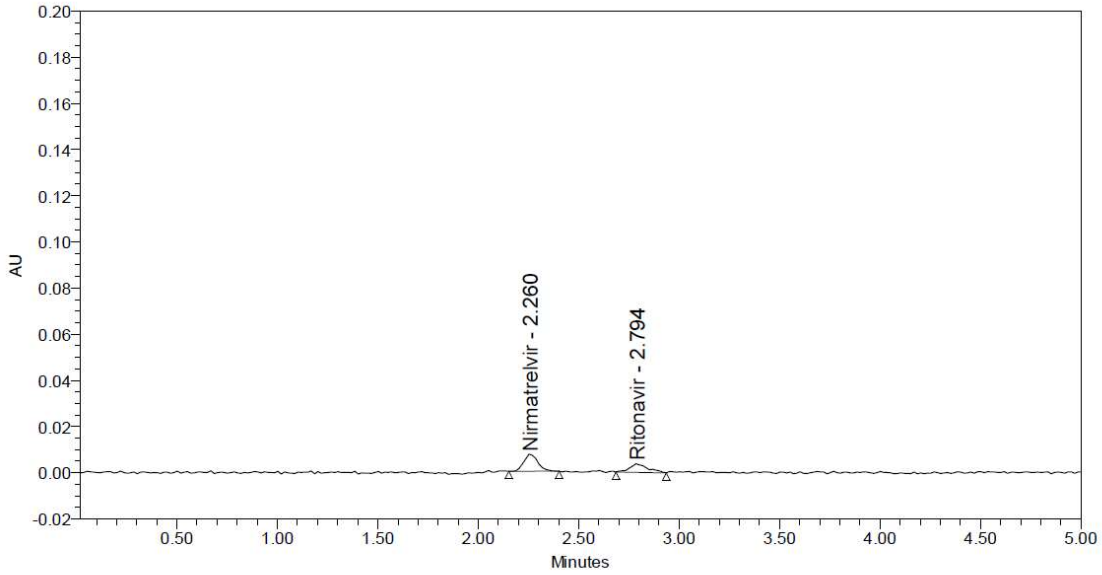


Fig 24: LOD Chromatogram of Standard

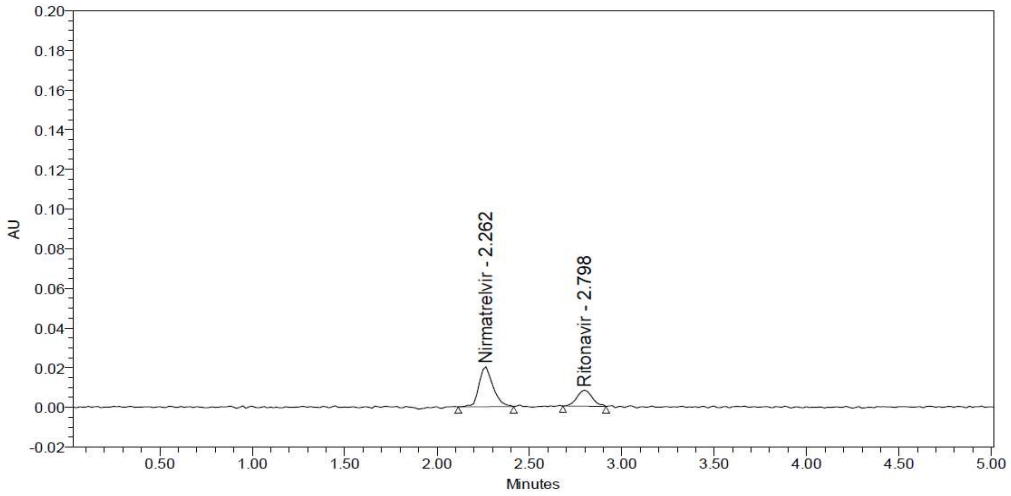


Fig 25: LOQ Chromatogram of of Standard

Robustness

Table 9: Robustness data forNirmatrelvir and Ritonavir

S.no	Condition	%RSD of Nirmatrelvir	%RSD of Ritonavir
1	Flow rate (-) 0.7ml/min	0.2	0.2
2	Flow rate (+) 0.9ml/min	0.4	0.5
3	Mobile phase (-) 55B:45A	0.4	0.5
4	Mobile phase (+) 65B:35A	0.2	0.1
5	Temperature (-) 25°C	0.1	0.3
6	Temperature (+) 35°C	0.3	0.7

Samples were injected in duplicate under robustness settings such as flow minus (0.9 ml/min), flow plus (1.1 ml/min), mobile phase minus (45B:55A), mobile phase plus (55B:45A), temperature minus (25°C), and temperature plus (35°C). All of the system suitability parameters passed with little to no impact. %RSD was not over the upper bound.

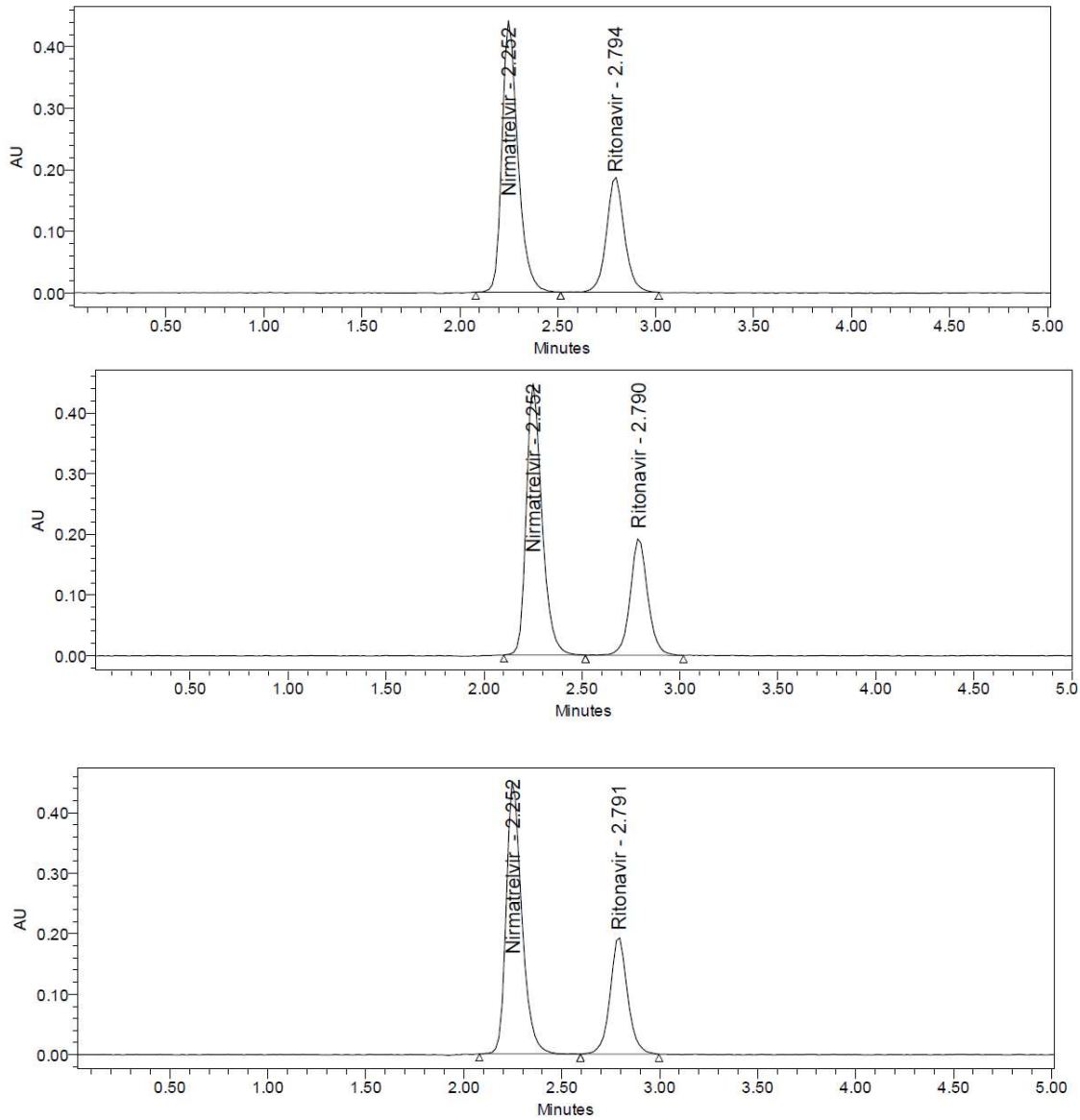
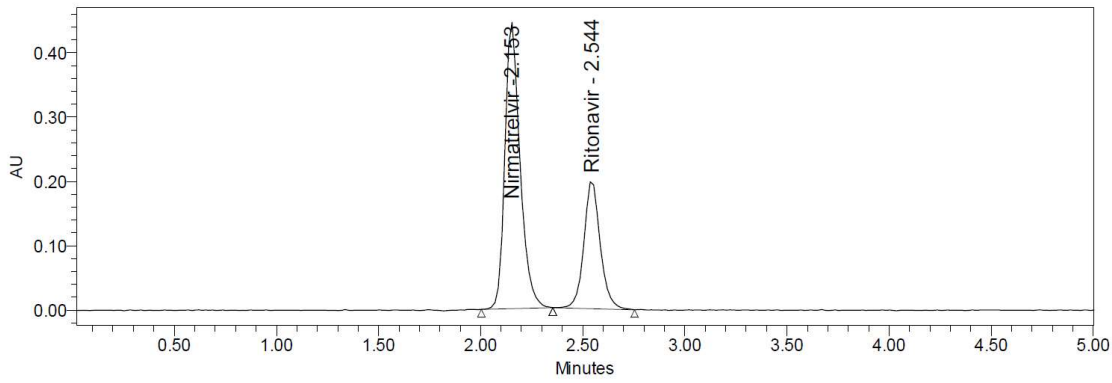


Fig 26: Flow minus Chromatogram of Nirmatrelvir and Ritonavir



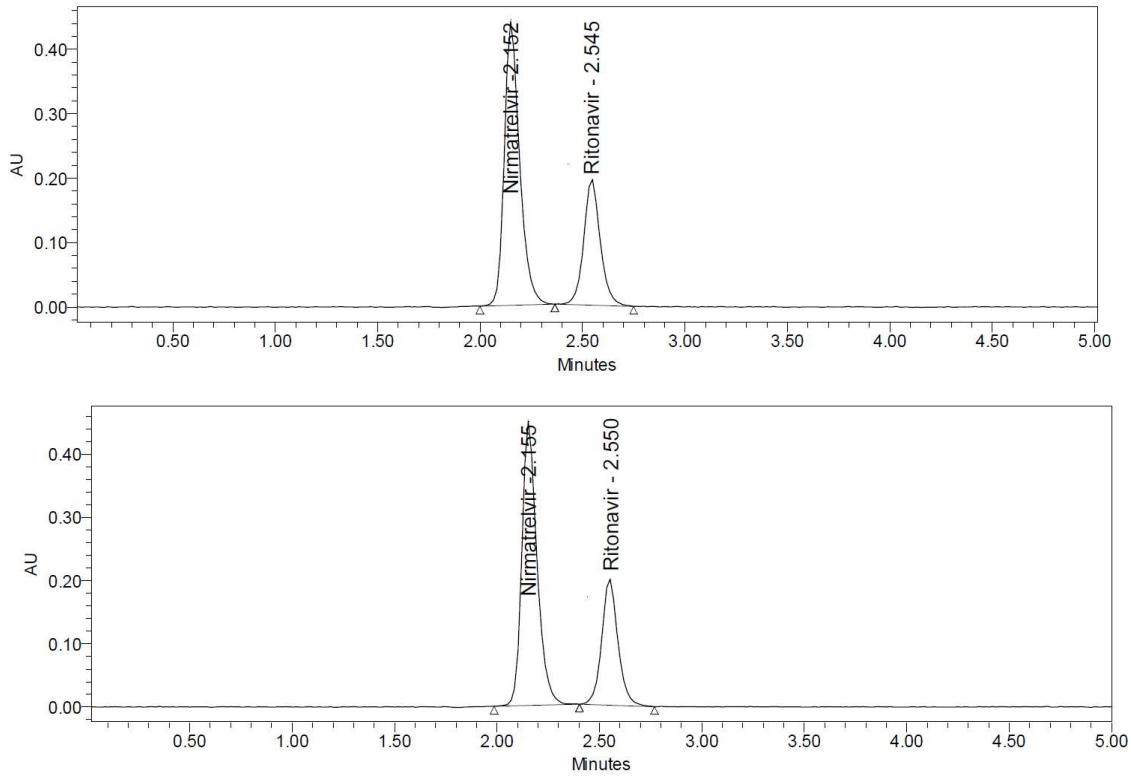
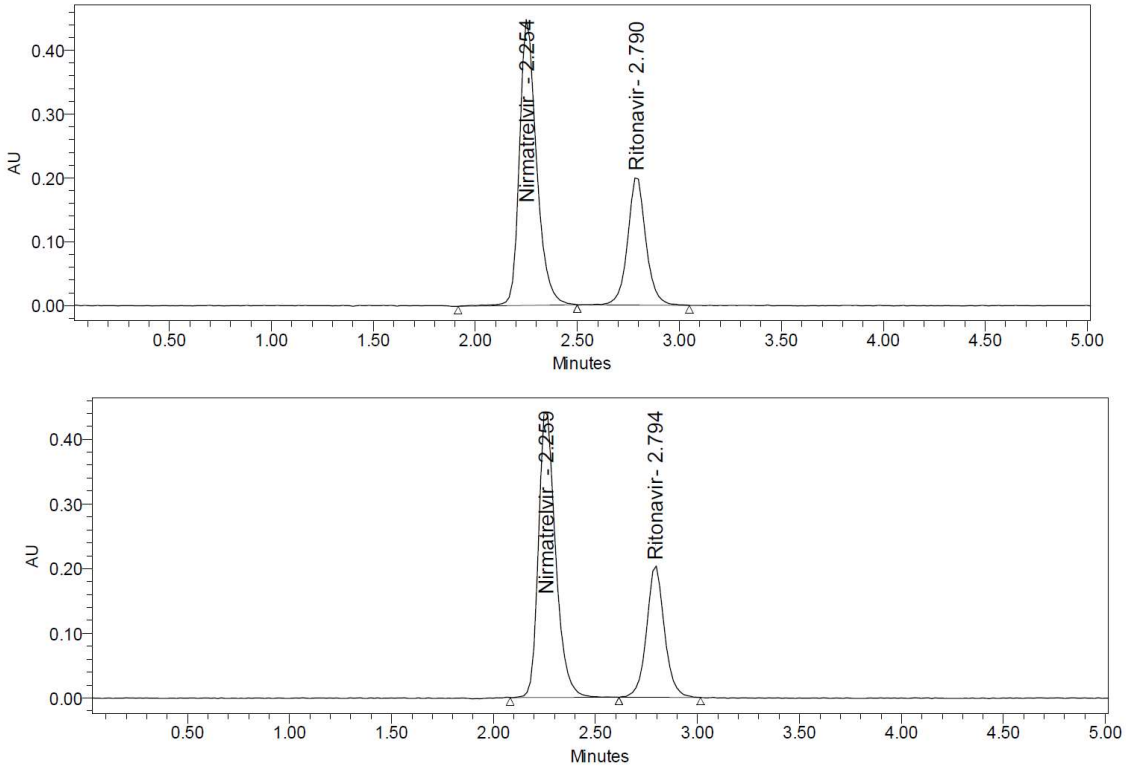


Fig 27: Flow plus Chromatogram of Nirmatrelvir and Ritonavir.



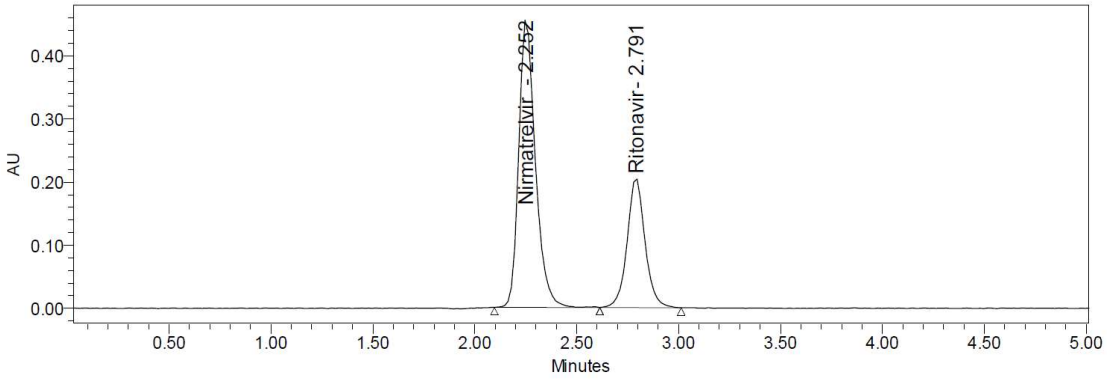


Fig 28: Mobile phase minus Chromatogram of Nirmatrelvir and Ritonavir

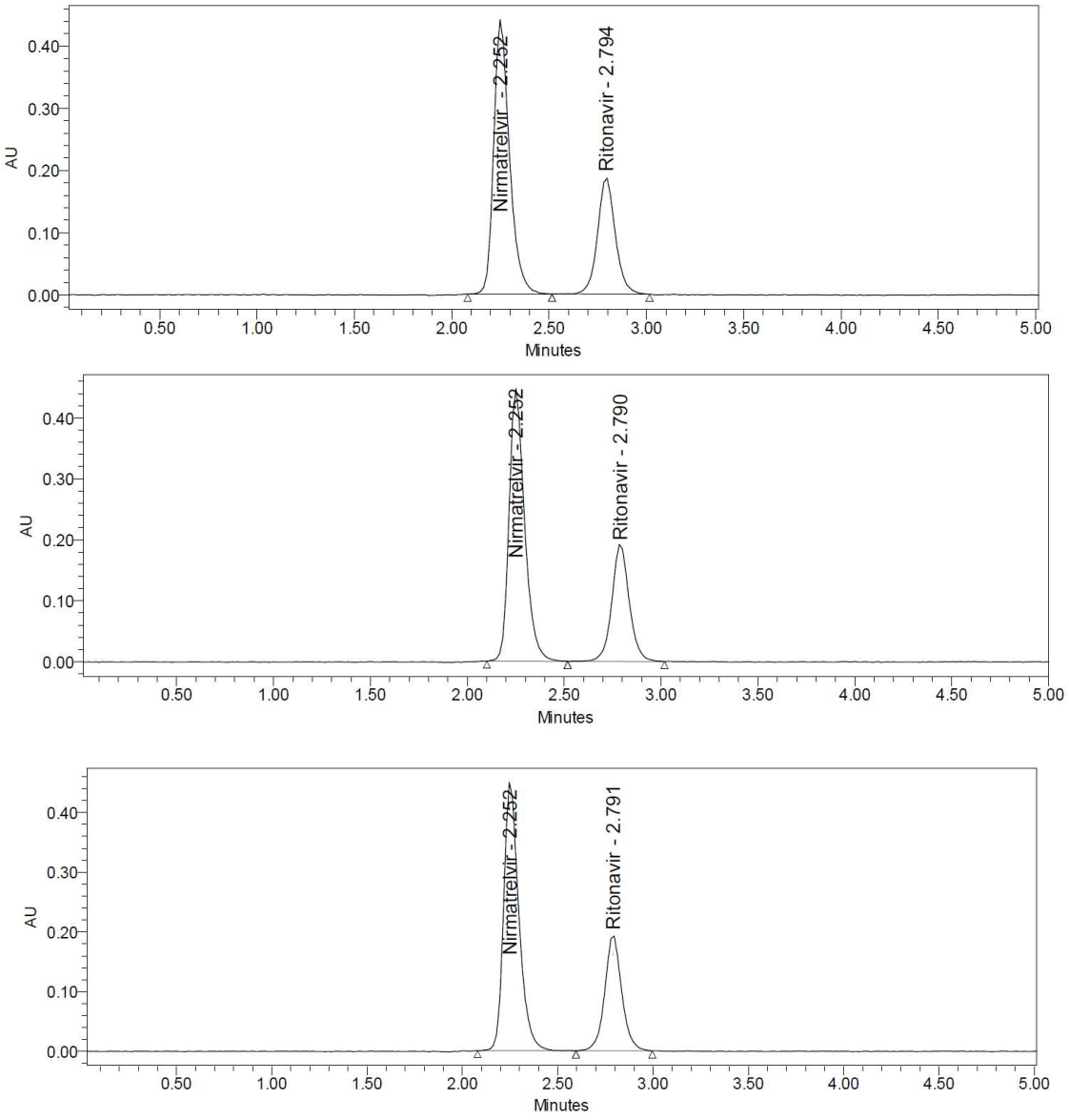


Fig 29: Mobile phase Plus Chromatogramof Nirmatrelvir and Ritonavir.

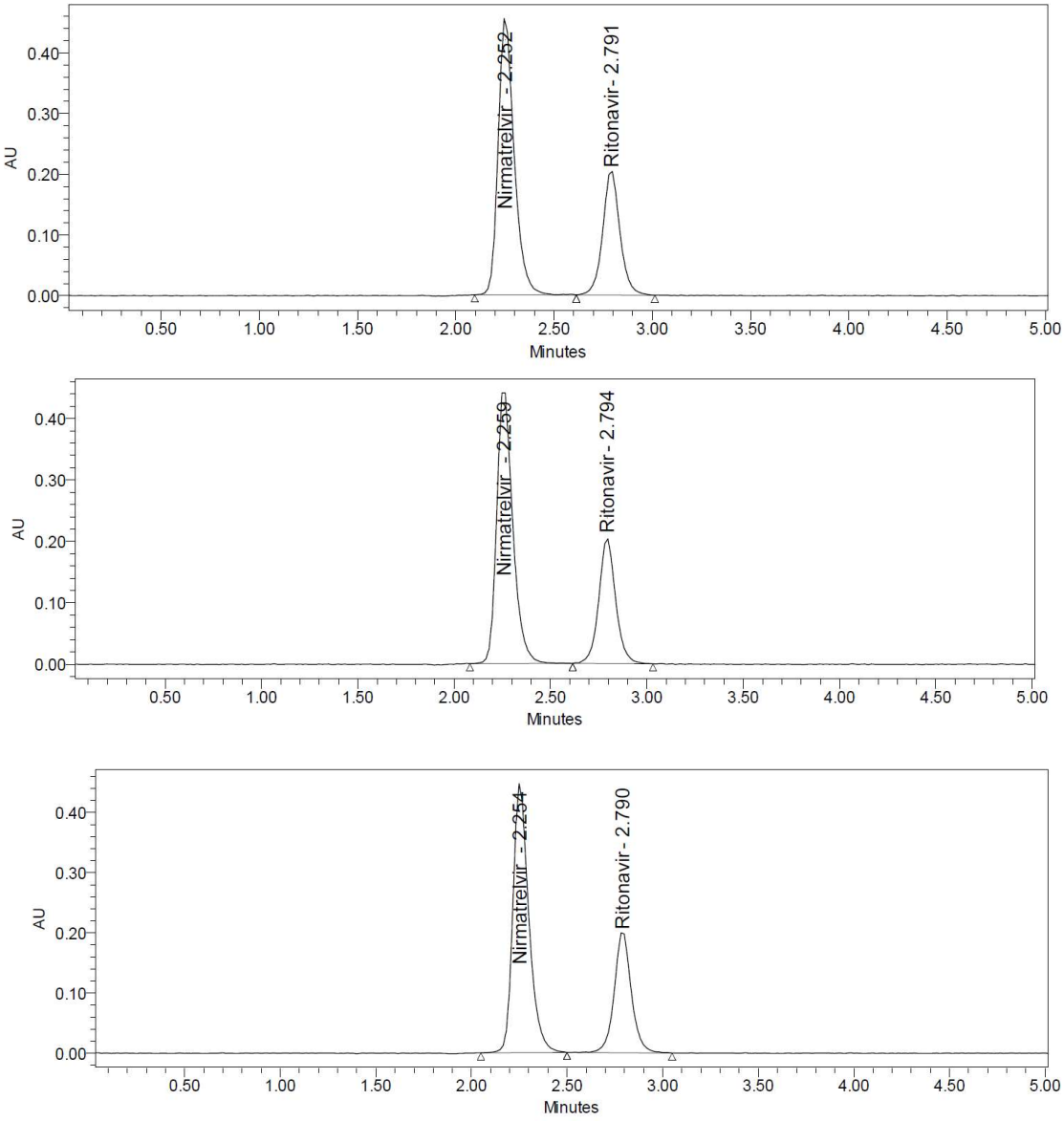
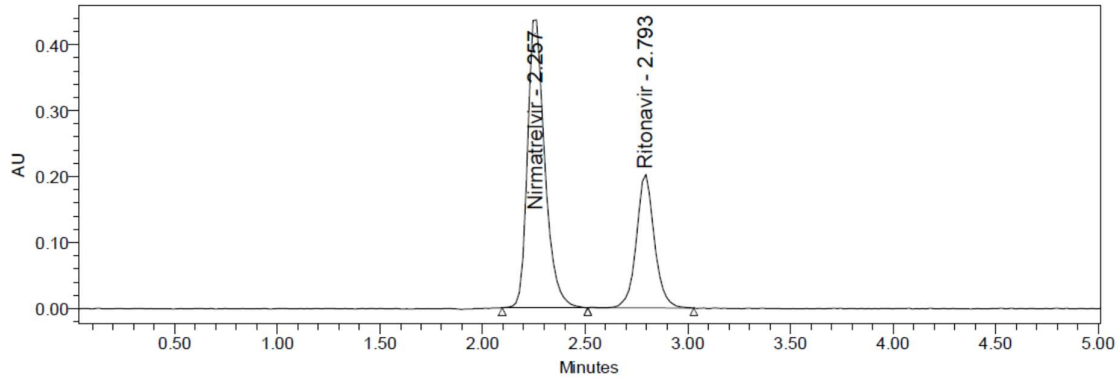


Fig 30: Temperature minus Chromatogram of Nirmatrelvir and Ritonavir



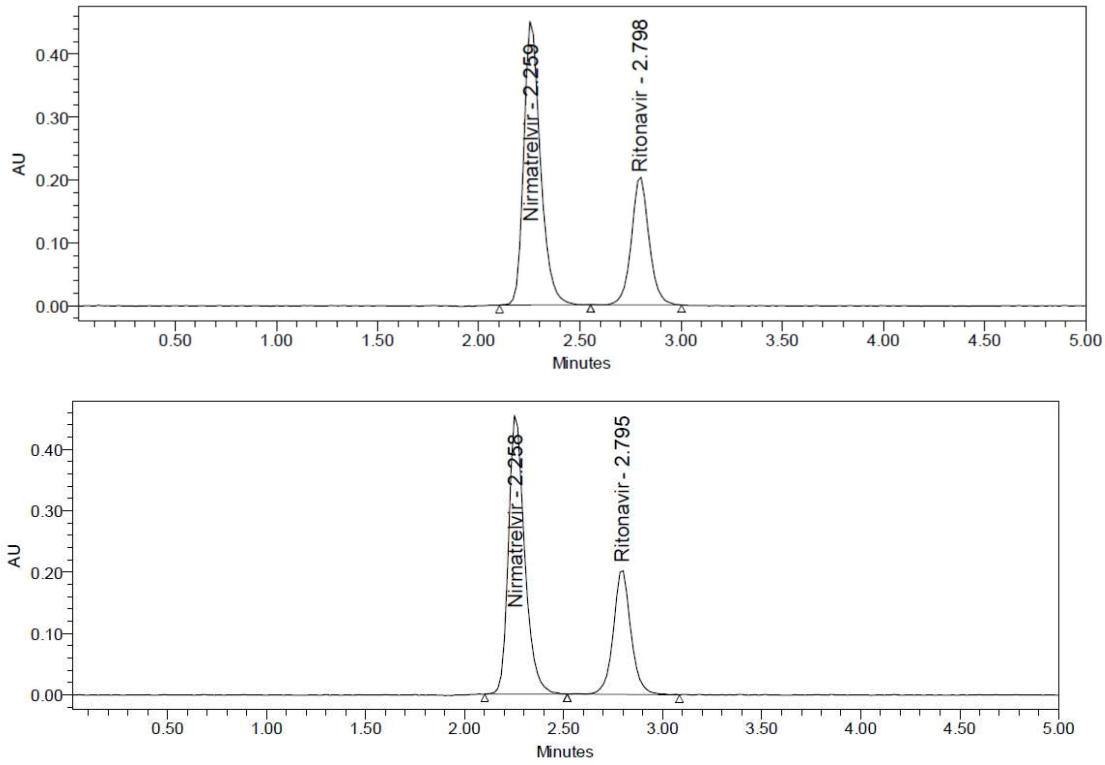


Fig 31: Temperature plus Chromatogram of Nirmatrelvir and Ritonavir

Assay

with the claim Nirmatrelvir 150 mg, Ritonavir 100 mg on the label. The aforementioned formulation was used for the assay. The average percentage of assay results for nimatrelvir and ritanavir were 99.84% and 99.74%, respectively.

Table 10: Assay Data of Nirmatrelvir

S.no	Standard Area	Sample area	% Assay
1	252676	251414	99.17
2	252661	252509	99.60
3	252638	252274	99.51
4	252495	256473	101.16
5	254357	253323	99.92
6	254772	252751	99.70
Avg	253267	253124	99.84
Stdev	1016.0	1756.1	0.69
%RSD	0.4	0.7	0.7

.

Table 11: Assay Data of Ritonavir

S.no	Standard Area	Sample area	% Assay
1	119169	118516	99.62
2	119165	118393	99.51
3	118338	118673	99.75
4	119901	118782	99.84
5	118480	118796	99.85
6	118065	118832	99.88
Avg	118853	118665	99.74
Stdev	681.3	176.1	0.15

%RSD	0.6	0.1	0.1

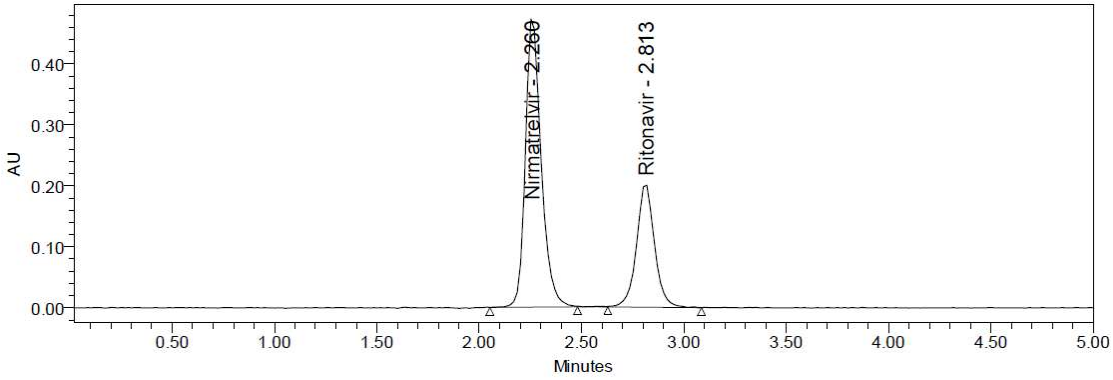


Fig 32: Chromatogram of working standard solution

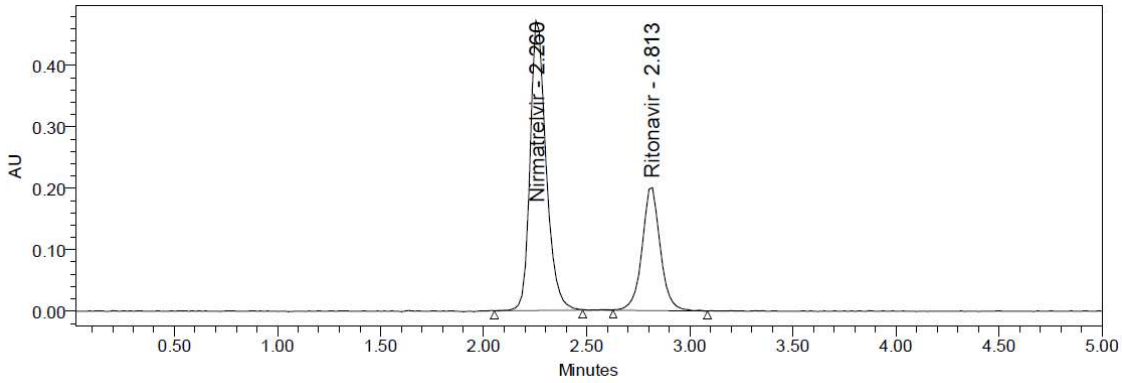


Fig 33: Chromatogram of working sample solution

Degradation studies

The proportion of drug degraded in solution is determined by injecting standard sand-degraded samples and applying various conditions such as acidic, alkaline, oxidative, photolytic, thermal, and neutral analysis.

Table 12: degradation data

Type of degradation	Nirmatrelvir			Ritonavir		
	AREA	% RECOVERED	% DEGRADED	AREA	% RECOVERED	% DEGRADED
Acid	240493	94.86	5.14	112076	94.20	5.80
Base	242104	95.50	4.50	113067	95.04	4.96
Peroxide	243912	96.21	3.79	115019	96.68	3.32
Thermal	246873	97.38	2.62	116020	97.52	2.48
Uv	249523	98.42	1.58	117346	98.63	1.37
Water	251418	99.17	0.83	118126	99.29	0.71

SUMMARY AND CONCLUSION

Table 13: Summary Table

Parameters	Nirmatrelvir	Ritonavir	LIMIT
Linearity Range(µg/ml)	3.75-22.5µg/ml	2.5-15µg/ml	
Regression coefficient	0.999	0.999	
Slope(m)	16802	16802	R< 1

Intercept(c)		995.5	995.5	
Regression equation (Y=mx+c)		y = 16802x + 995.5	y = 16802x + 995.5	
Assay (% mean assay)		99.84%	99.74%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.4	0.6	NMT 2.0%
Method precision %RSD		0.7	0.1	NMT 2.0%
Accuracy% recovery		99.15%	99.77%	98-102%
LOD		0.21	0.16	NMT 3
LOQ		0.65	0.48	NMT 10
Robustness	**FM**	0.2	0.2	%RSD NMT 2.0
	FP	0.4	0.5	
	MM	0.4	0.5	
	MP	0.2	0.1	
	TM	0.1	0.3	
	TP	0.3	0.7	

CONCLUSION

Nirmatrelvir and Ritonavir in pharmaceutical dosage form were simultaneously estimated using a straightforward, accurate, and precise procedure. Ritonavir and Nirmatrelvir were shown to have retention times of 2.816 and 2.241 minutes, respectively. The percentage RSD of Ritonavir and Nirmatrelvir was discovered to be 0.6 and 0.4, respectively. %Ritonavir and Nirmatrelvir showed recovery rates of 99.77% and 99.15%, respectively. The regression equations for Ritonavir and Nirmatrelvir yielded LOD and LOQ values of 0.21, 0.16, and 0.65, 0.48, respectively. y = 16802x + 995.5 for Nirmatrelvir and y = 16802x + 995.5 for Ritonavir are the regression equations. The method that was created was easy to use and cost-effective, making it suitable for routine quality control testing in industries. Both the retention times and the run time were reduced.

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