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Development and validation of a new stability indicating RP-UPLC method for the simultaneous estimation of Nirmatrelvir and Ritonavir in presence of internal standard (Velpatasvir)

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ABSTRACT

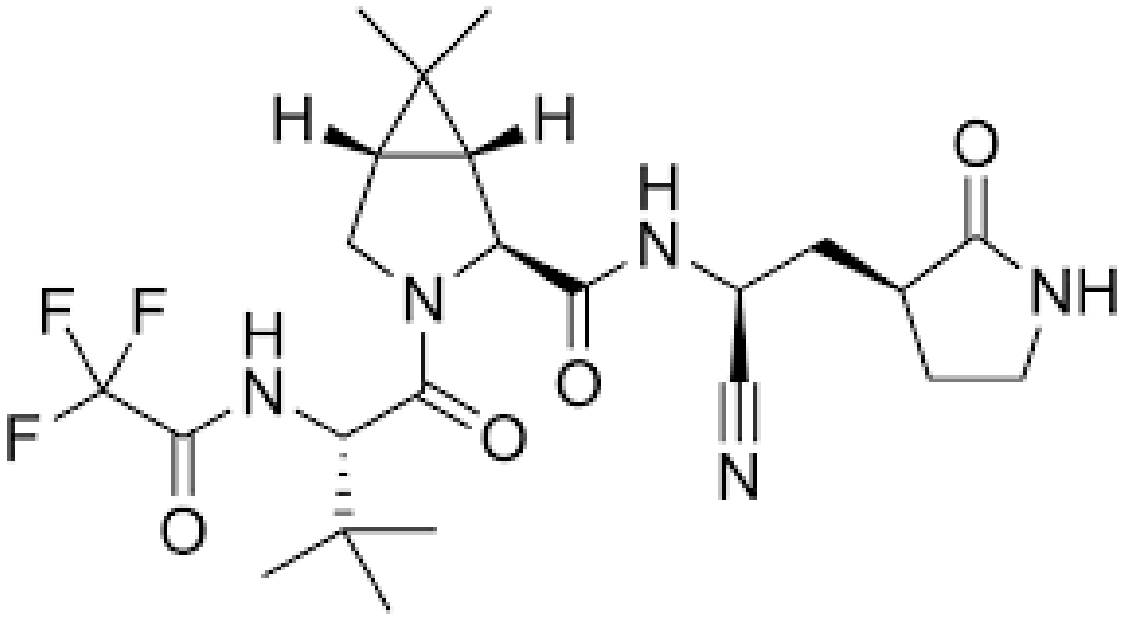
A new stability indicating RP-UPLC method has been proposed for the simultaneous estimation of Nirmatrelvir and Ritonavir in presence of an internal standard, Velpatasvir using Waters ACQUITY UPLC system with PDA detector and Hibar C18 (100 x 2.1 mm, 1.8µ) column. Mobile phase consisting of 0.01M Ammonium acetate and Acetonitrile (60:40, v/v) was used with flow rate 0.3 ml/min (Detection wavelength: 260 nm) (Injection volume: 1.0 µL) (Column temperature: 30°C) with run time 6 mins. The method was linear over the concentration range 30-180 µg/ml and 10-60 µg/ml for Nirmatrelvir and Ritonavir respectively. Forced degradation studies were performed and the method was validated as per ICH guidelines.

INTRODUCTION

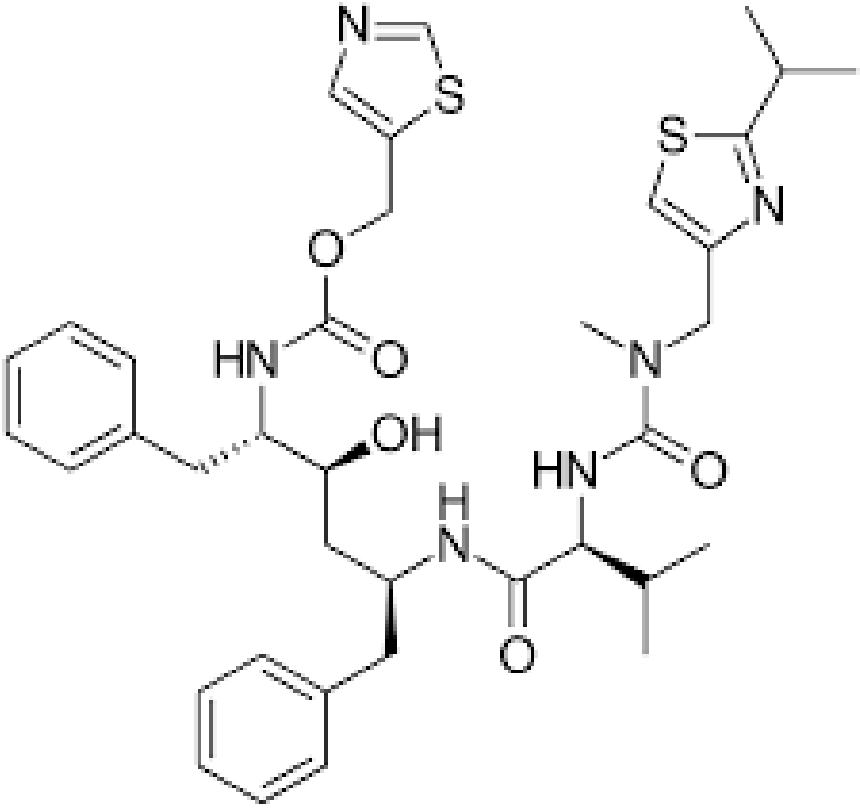
Nirmatrelvir (CAS: 2628280-40-8) is an antiviral prescription medication primarily used to treat chronic hepatitis B virus infection. It is chemically, (1R,2S,5S)-N-((1S)-1-Cyano-2-((3S)-2-oxopyrrolidin-3-yl) ethyl)-3-((2S)-3,3-dimethyl-2-(2,2,2-trifluoroacetamido) butanoyl)-6,6-

dimethyl-3- azabicyclo (3.1.0) hexane-2carnnboxamide.The molecular weight of Nirmatrelvir is 499.54 gm/mole with molecular formula $C_{23}H_{32}F_3N_5O_4$. It binds SARS-CoV-2 Mpro selectively and reversibly inhibits SARS-CoV-2 Mpro activity against human coronaviruses[1].

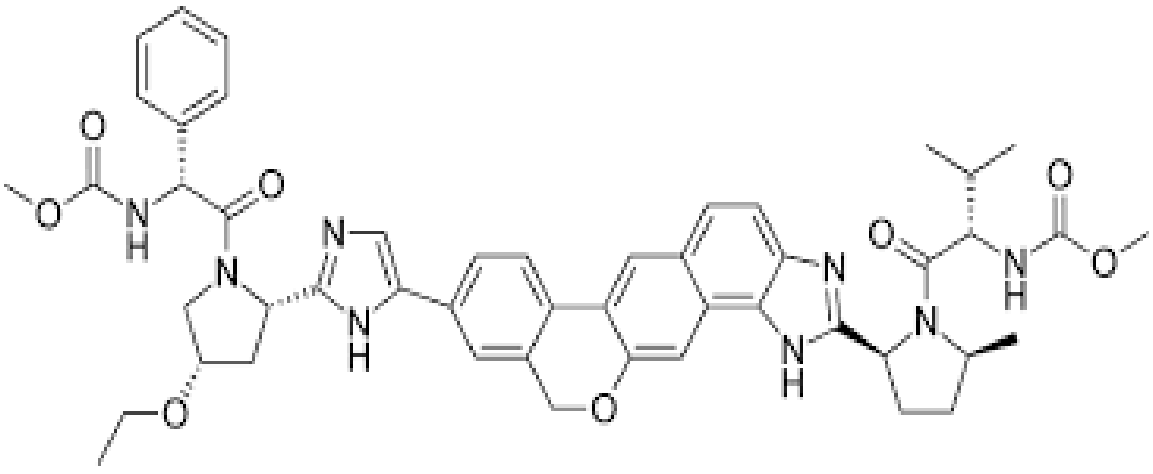
Ritonavir (CAS: 155213-67-5) is an anti-viral drug used in combination with other drugs in the treatment of highly active anti-retroviral therapy. It is chemically, 5-Thiazolylmethyl [(αS)-α-[(1S,3S)-1-hydroxy-3-[(2S)-2-[3-[(2-isopropyl-4-thiazolyl) methyl]-3-methyl ureido]-3-methylbutyramido]-4-phenylbutyl] phenethyl] carbamate. The molecular weight of Ritonavir 720.94 gm/mole with molecular formula $C_{37}H_{48}N_6O_5S_2$. It is a protease inhibitor[2] and inhibits the CYP3A-mediated metabolism of nirmatrelvir providing increased plasma concentrations of Nirmatrelvir. The combination[5] (Figure 1) of Nirmatrelvir and Ritonavir is an oral anti-viral medication used to treat mild to moderate COVID-19 patients.



Nirmatrelvir (NT)



Ritonavir (RV)



Velpatasvir (Internal standard) (IS)

Figure 1: Chemical structures of Nirmatrelvir, Ritonavir and Velpatasvir (Internal standard)

Sumalatha et al. have developed an RP-HPLC method[3] for the simultaneous estimation of Nirmatrelvir and Ritonavir using Zorbax Eclipse XDB C18 column and a mixture of 0.025 M KH_2PO_4 (pH 2.5) and Acetonitrile (Gradient mode) as mobile phase with 1.0 ml/min flow rate (UV detection 240 nm). The linearity was followed over the concentration range 5-15 µg/ml for Nirmatrelvir and 30-90 µg/ml for Ritonavir and the retention time for Nirmatrelvir was found to be 3.94 ± 0.08 min for Nirmatrelvir and 9.08 ± 0.1 min for Ritonavir.

Imam et al., have developed a RP-HPLC method[4] using green analytical procedure index and the AGREE evaluation method for the simultaneous determination of Nirmatrelvir and Ritonavir in pharmaceutical dosage form using BDS Hypersil C18 column (Isocratic mode) with mobile phase consisting of Ethanol: Water (80:20, v/v) with 1.0 ml/min flow rate (Detection wavelength 215 nm) and the linearity was followed over the concentration range 1.0-20 µg/ml for both Nirmatrelvir and Ritonavir and the retention time for Nirmatrelvir was found to be 4.9 min for Nirmatrelvir and 6.8 min for Ritonavir.

Nagajyothi and Sunitha have developed a stability indicating RP-HPLC method[5] for the simultaneous estimation of Nirmatrelvir and Ritonavir using Discovery C18 column and a mixture of KH_2PO_4 buffer and Acetonitrile (65:35, v/v) as mobile phase (Column temperature 30 °C) with flow rate 0.9 ml/min (UV detection 242 nm). The linearity was followed over the concentration range 1.5-105 µg/ml for Nirmatrelvir and 1-70 µg/ml for Ritonavir and the retention time for Nirmatrelvir was observed at 2.251 min and that of Ritonavir at 2.820 min.

Shaikh Zaheer et al, have developed a RP-HPLC method[6] for the simultaneous estimation of Nirmatrelvir and Ritonavir using C18 symmetric column and a mixture of 0.01M dibasic phosphate buffer (pH 3.0) and Methanol (60:40, v/v) as mobile phase (Column temperature 40 °C) with 1.0 ml/min flow rate (UV detection 215 nm). The linearity was followed over the concentration range 37.50-225 µg/ml for Nirmatrelvir and 25-150 µg/ml for Ritonavir and the retention time for Nirmatrelvir was found to be 2.612 min for Nirmatrelvir and 4.438 min for Ritonavir.

Yassin et al. have developed a stability indicating RP-HPLC method[7] for the simultaneous estimation of Nirmatrelvir and Ritonavir using VDSpher PUR 100 ODS column and a mixture of 0.03 M potassium di-hydrogen phosphate buffer (pH 4.0) and Acetonitrile (45:55, v/v) as mobile phase (Column temperature 40 °C) with 1.0 ml/min flow rate (UV detection 215 nm). The linearity was followed over the concentration range 1.5-105 µg/ml for Nirmatrelvir and 1-70 µg/ml for Ritonavir and the retention time for Nirmatrelvir was found to be 3.94 ± 0.08 min for Nirmatrelvir and 9.08 ± 0.1 min for Ritonavir.

In the present study the authors have proposed a new stability indicating RP-UPLC method for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablets in presence of an internal standard, Valacyclovir, an antiviral drug and the method was validated as per ICH guidelines.

MATERIALS AND METHODS

Nirmatrelvir and Ritonavir in presence of an internal standard, Velpatasvir API were procured from Pfizer as gift samples. The combination of Nirmatrelvir and Ritonavir is available as tablets with different brand names Paxista, Paxlovid (Pfizer), Paxzen, Paxbrook, Zencovid etc with label claim of 150 gm Nirmatrelvir and 100 mg Ritonavir. HPLC grade Acetonitrile was procured from Merck (India) and all other chemicals Ammonium acetate, Sodium hydroxide, Hydrochloric acid and Hydrogen peroxide (30% w/v) were purchased from Merck (India) and Milli Q water was used from Millipore system.

Preparation of stock and standard solutions

60 mg of Nirmatrelvir and 20 mg Ritonavir were accurately weighed and transferred in to two different 50 ml volumetric flasks and diluted with 10 ml diluent (Water: Acetonitrile) (50:50, v/v), sonicated for 10 min and then make up to the final volume with the diluent (1200 µg/ml of Nirmatrelvir, 400 µg/ml of Ritonavir).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with Diluent (120µg/ml of Nirmatrelvir and 40µg/ml of Ritonavir)

Internal standard preparation: Accurately 50 mg of Velpatasvir was transferred in to a 50 ml clean dry volumetric flask and 3/4 th volume of diluent was added, sonicated for 5 minutes and made up to the final volume with diluents and filter the solution with nylon 0.5 µm size filters (1000 µg/ml of Velpatasvir).

Final concentration: 1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with Diluent (100µg/ml of Velpatasvir)

Preparation of 0.01M Ammonium acetate buffer solution

0.77 gm of Ammonium acetate was accurately weighed and transferred into a 1000 ml volumetric flask and about 900 ml of Milli-Q water was added and sonicated to degas and finally the volume was made up to volume with Milli-Q water by adjusting the pH to 3.0 with dilute acetic acid.

Instrumentation and Chromatographic conditions

Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system with PDA detector and Hibar C18 (100 x 2.1 mm, 1.8µ) column were used for the chromatographic study. Mobile phase mixture consisting of 0.01M Ammonium acetate and Acetonitrile 60:40, v/v) was used with flow rate 0.3 ml/min (Detection wavelength: 260 nm) (Injection volume: 1.0 µL) (Column temperature: 30°C) with run time 3 mins for the chromatographic study. A mixture of water and

Acetonitrile (50:50, v/v) was used as diluent. 100 µg/ml of Velpatasvir solution was used as the internal standard.

Method validation[10]

Linearity study

A series of solutions containing a mixture of Nirmatrelvir (5-30 µg/ml) and Ritonavir (20-120 µg/ml) were prepared from the stock and working standard solutions were prepared along with the internal standard, Velpatasvir using the diluent (Water: Acetonitrile) (50:50, v/v) and each of these solutions were injected (n=3) into the UPLC system and the chromatograms were recorded. The peak area of each of the solutions injected was noted at its retention time and the mean peak area ratio (Analyte/IS) was calculated. A calibration curve was drawn by plotting the concentration of drug solution on the x-axis and the corresponding mean peak area ratio values on the y-axis. The LOD and LOQ were calculated from the signal to noise ratio (S/N). The LOD is 3.3 times the signal to noise ratio and that of LOQ is 10 times the signal to noise ratio.

Precision study

Precision of the method was evaluated intra-day and inter-day precision studies. A mixture of Nirmatrelvir (10 µg/ml) and Ritonavir (20 µg/ml) solutions were prepared (n=6) along with the internal standard (Velpatasvir) within the linearity range on the same day (intra-day precision) and on different consecutive days (inter-day precision) and the chromatographic study was performed. The mean peak area (n=3) and thereby the % RSD was calculated.

Accuracy study

Accuracy of the method was measured by spiking the formulation solution with a known concentration of standard drug (50, 100, 150%) containing Nirmatrelvir and Ritonavir and were injected thrice into the UPLC system after the addition of internal standard and the chromatograms were recorded. The mean peak area ratio (Analyte/IS) was calculated from the chromatograms obtained and finally the % RSD was calculated.

Assay of tablets

20 tablets of two different brands were weighed, and the average weight of each tablet was calculated and then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 50mL of diluent was added and then sonicated for 50 mins and further the volume made up with the diluent and filtered. The filtrate contains 250 µg/ml of Nirmatrelvir and 500 µg/ml of Ritonavir. 0.4 ml of the filtered solution was pipetted out into a 10 ml volumetric flask and the volume was made up to 10 ml with the diluent and the resultant solution contains 10 µg/ml of Nirmatrelvir and 20 µg/ml Ritonavir. 1µl of each of the marketed formulation solution along with the internal standard was injected into the UPLC system and the chromatogram was recorded and the amount of Nirmatrelvir and Ritonavir was calculated from the respective calibration curves.

Forced degradation studies[11]

The specificity of the method can be known from the stability studies. Forced degradation studies were performed to determine the stability of Nirmatrelvir and Ritonavir towards stress conditions such as acidic hydrolysis, alkaline hydrolysis, oxidation, neutral thermal and photolytic degradation.

Acid degradation

1 ml of stock solution of Nirmatrelvir and Ritonavir was taken and 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at at 60^0C. The resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir solution and 1µl of this solution was injected into the UPLC system and the chromatogram was recorded.

Alkaline degradation

1 ml of stock solution of Nirmatrelvir and Ritonavir was taken and 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at at 60^0C. The resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir solution and 1µl of this solution was injected into the UPLC system and the chromatogram was recorded.

Thermal (Dry heat) degradation

The standard drug solution was placed in oven at 105^0C for 6 Hrs to study dry heat degradation. The resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir solution and 1µl of this solution was injected into the UPLC system and the chromatogram was recorded.

Photolytic degradation

The photochemical stability was performed by exposing the solution (3000 µg/ml, 1000 µg/ml and 500 µg/ml) to UV light in in photo stability chamber (UV chamber) for 7 days or 200 Watt hours/m^2 in photo stability chamber.

The resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir solution and 1µl of this solution was injected into the UPLC system and the chromatogram was recorded.

Neutral degradation

Stress testing under neutral conditions was studied by refluxing the drug solution in water for 6 Hrs at a temperature of 60ºC and the resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir solution and 1µl of this solution was injected into the UPLC system and the chromatogram was recorded.

Oxidative degradation

1 ml of stock solution of Nirmatrelvir and Ritonavir was taken and 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately and the solutions were kept for 30 min at 60^0C. The resultant solution was diluted to obtain 120 µg/ml Nirmatrelvir and 40 µg/ml Ritonavir and 1µl of this solution was injected into the UPLC system and the chromatogram were recorded.

RESULTS AND DISCUSSION

The authors have proposed a new stability indicating RP-UPLC method for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablets and the method was validated as per ICH guidelines. Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC) system with PDA detector and Hibar C18 (100 x 2.1 mm, 1.8µ) column were used for chromatographic study. Mobile phase consisting of Ammonium acetate and Acetonitrile (60:40, v/v) was used with flow rate 0.3 ml/min (Detection wavelength: 260 nm) (Injection volume: 1.0 µL) (Column temperature: 30°C) with run time 3 mins. A mixture of water and Acetonitrile (50:50, v/v) was used as diluent. The present RP-UPLC method was compared with the previously published methods and some of the important observations were highlighted in Table 1.

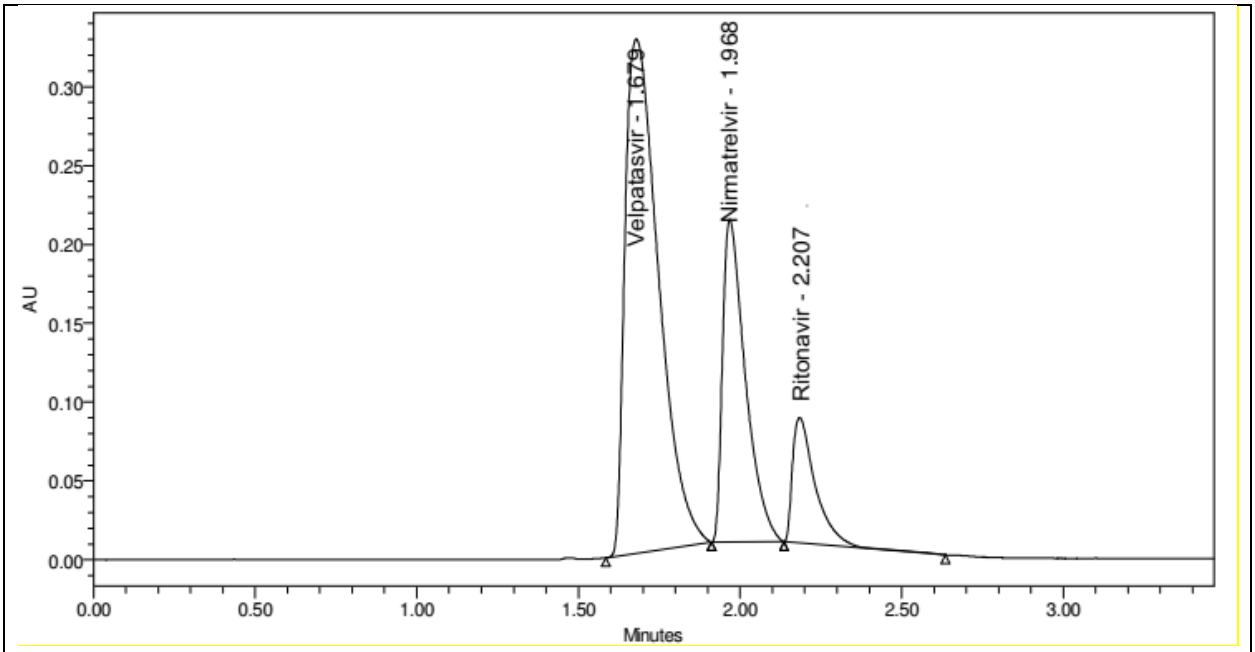
Table 1: Comparison of previously published methods with the present methods

Mobile phase(v/v)	Column	Rt (min)	Linearity (µg/ml)	Ref
RP-HPLC 0.025 M KH_2PO_4 (pH 2.5): Acetonitrile (Gradient mode)	Zorbax Eclipse XDB C18	3.94 ± 0.08 (NT) 9.08 ± 0.1 (RV)	5-15 (NT) 30-90 (RV)	3
RP-HPLC Ethanol: Water (80:20)	BDS Hypersil C18	4.9 (NT) 6.8 (RV)	1-20 (NT) 1-20 (RV)	4
KH_2PO_4 buffer: Acetonitrile (65:35)	Discovery C18	2.251 (NT) 2.820 (RV)	1.5-105 (NT) 1-70 (RV)	5
RP-HPLC 0.01M Dibasic phosphate buffer (pH 3.0): Methanol (60:40)	C18 symmetric	2.612 (NT) 4.438 (RV)	37.5-225 (NT) 25-150 (RV)	6

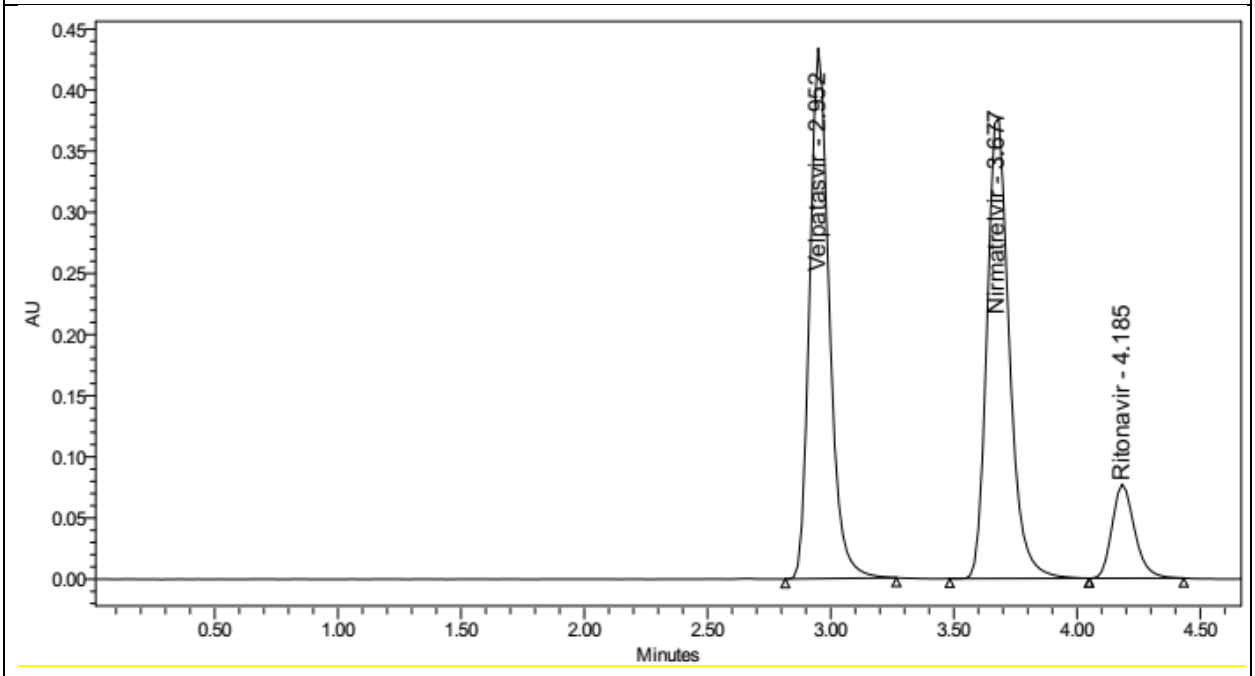
RP-HPLC 0.03 M Potassium di-hydrogen phosphate buffer (pH 4.0) and Acetonitrile (45:55)	VDSpher PUR 100 ODS	3.94 ± 0.08 (NT) 9.08 ± 0.1 (RV)	1.5-105 (NT) 1-70 (RV)	7
RP-HPLC Acetonitrile: Octane sulphonic acid Buffer pH 2.5(30:70)	Agilent Eclipse XDB	2.312 (NT) 4.238 (RV)	37-225 (NT) 25-150 (RV)	8
TLC Methanol: Water: 2% Urea solution of β-cyclodextrin (40:10:50) (Human plasma)	Aluminum silica gel plates	-	10-50 ng/band (NT & RV)	9
RP-UPLC 0.01M Ammonium acetate: Acetonitrile (60:40)	Hibar C18	2.312 (NT) 4.238 (RV)	30-180 (NT) 10-60 (RV)	Present method

Method optimization

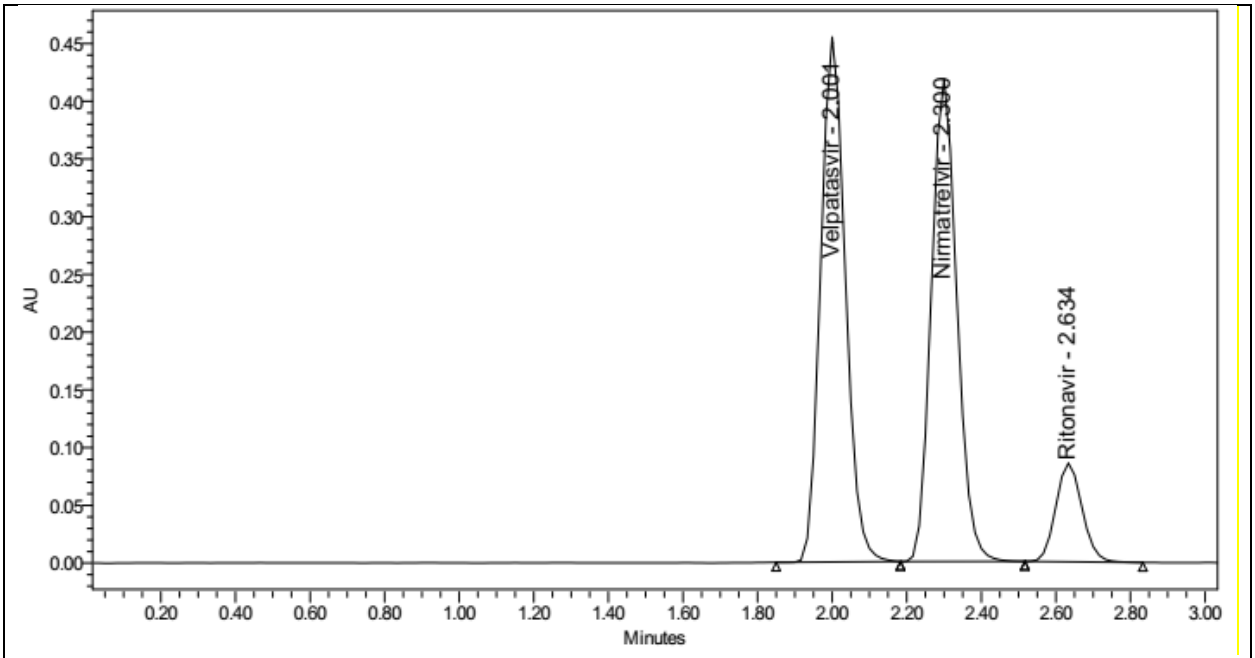
Initially, different columns and chromatographic conditions were applied to optimize the method and the trial runs (Figure 2) were shown in Table 2.



Trial 1



Trial 2



Trial 3

Figure 2: Typical chromatograms obtained during method optimization (Trial runs)

Table 2: Method optimization

Trial	Mobile phase (v/v)	Column	Result
1	Acetonitrile: KH_2PO_4 (60:40)	BEH C18	Peaks eluted but with less resolution and plate count.
2.	Acetonitrile: OPA (60:40)	BEH C18	Peaks eluted but the Obtained Rt was higher.
3	Acetonitrile: KH_2PO_4 (70:30)	Hibar	By changing column and buffer same as previous peaks eluted but the Obtained Rt was higher so further trials are carried out.

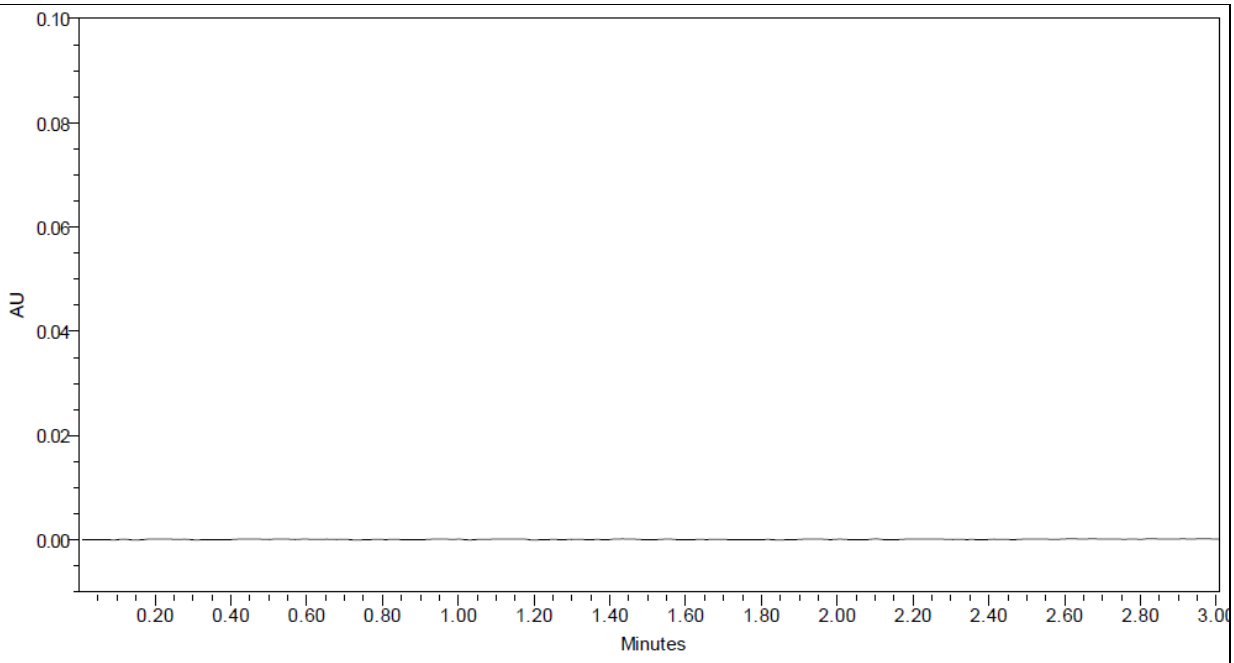
4	0.01M Ammonium acetate and Acetonitrile (60:40)	Hibar	Method optimized

Method validation

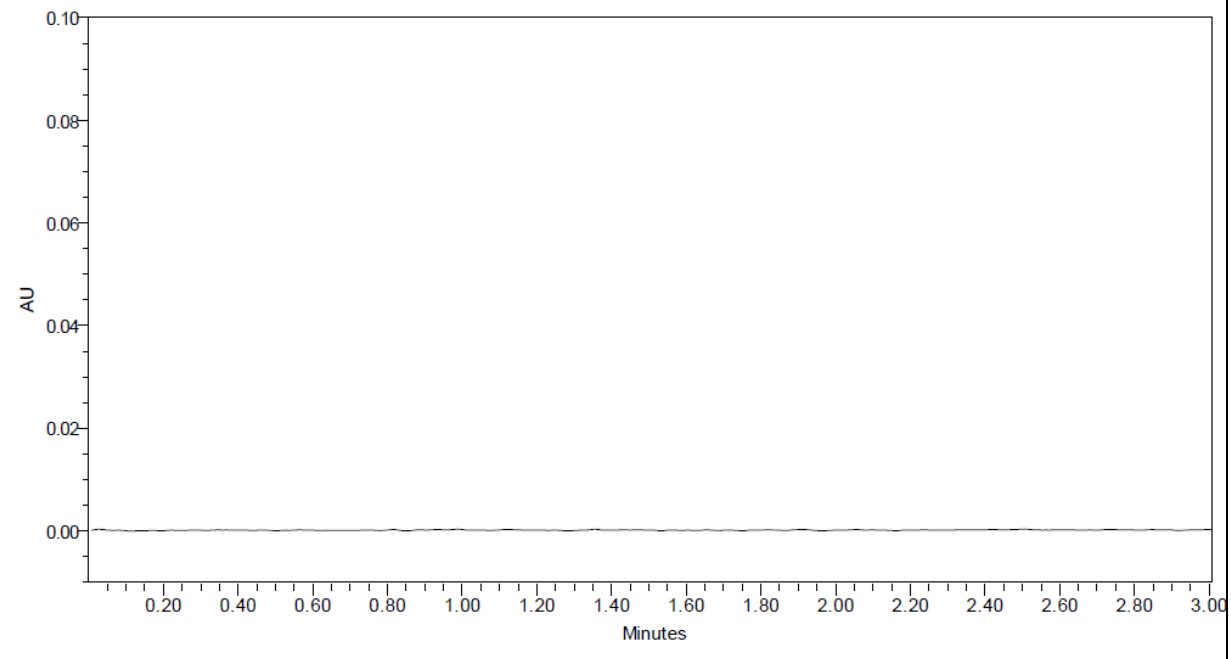
The representative chromatograms of blank, placebo and the combination of Nirmatrelvir and Ritonavir in presence of an internal standard, Velpatasvir were shown in Figure 3.

During the chromatographic study, it was found that the internal standard, Velpatasvir was eluted at 1.122 mins (Theoretical plates: 5101 and Tailing factor: 1.15), Nirmatrelvir at 1.311 mins (Theoretical plates: 5929; Tailing factor: 1.12 and Resolution: 3.2), Ritonavir at 1.528 mins (Theoretical plates: 7507; Tailing factor: 1.11 and Resolution: 3.3) and the system suitability parameters such as theoretical plates were greater than 2000, the tailing factor was less than1.5 and the resolution was greater than 2.0 which were all within the acceptable criteria.

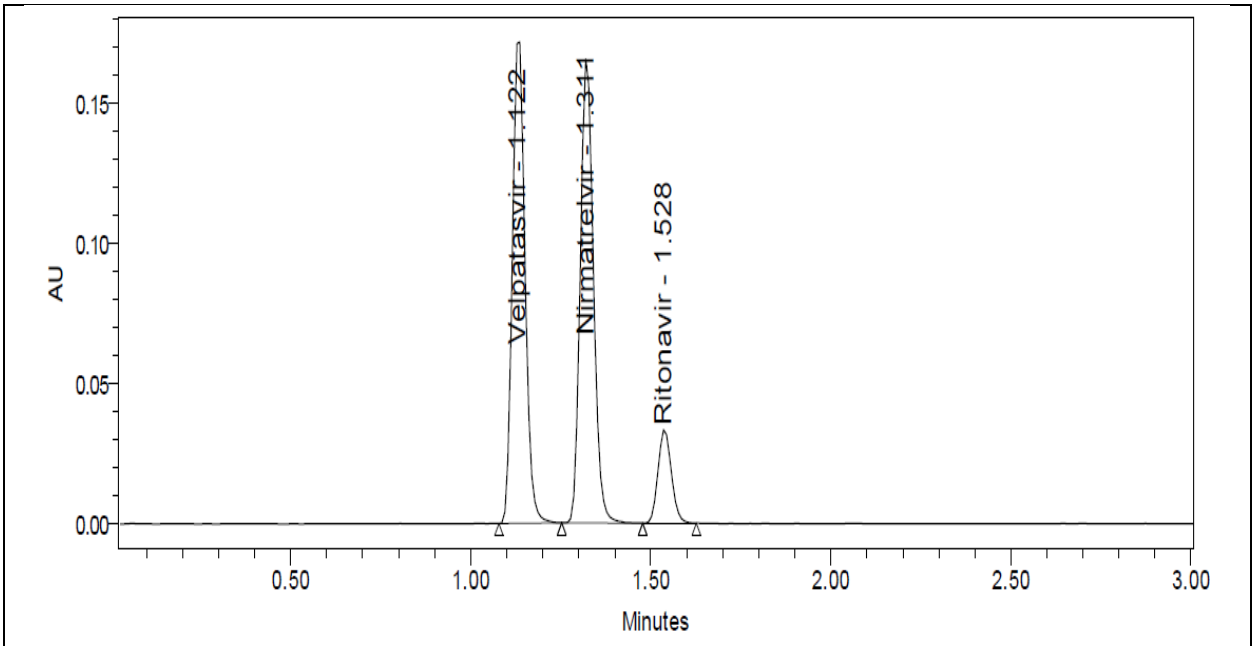
Nirmatrelvir has shown linearity over the concentration range 30-180 µg/ml with linear regression equation, $y = 0.0082x + 0.0018$ ($R^2 = 0.9998$). Ritonavir has shown linearity over the concentration range 10-60 µg/ml with linear regression equation, $y = 0.005x + 0.0014$ ($R^2 = 0.9995$) (Table 3). The LOD and LOQ were found to be 0.31 µg/ml and 0.94 µg/ml for Ritonavir respectively and the LOD and LOQ for Nirmatrelvir were found to be 0.45 µg/ml and 1.35 µg/ml respectively. The representative calibration curves were shown in Figure 4. The % RSD in intraday and inter day precision studies was found to be less than 2.0 (Table 4) indicating that the method is precise. The % RSD in accuracy studies was found to be less than 2 (Table 5) indicating that the method is accurate.



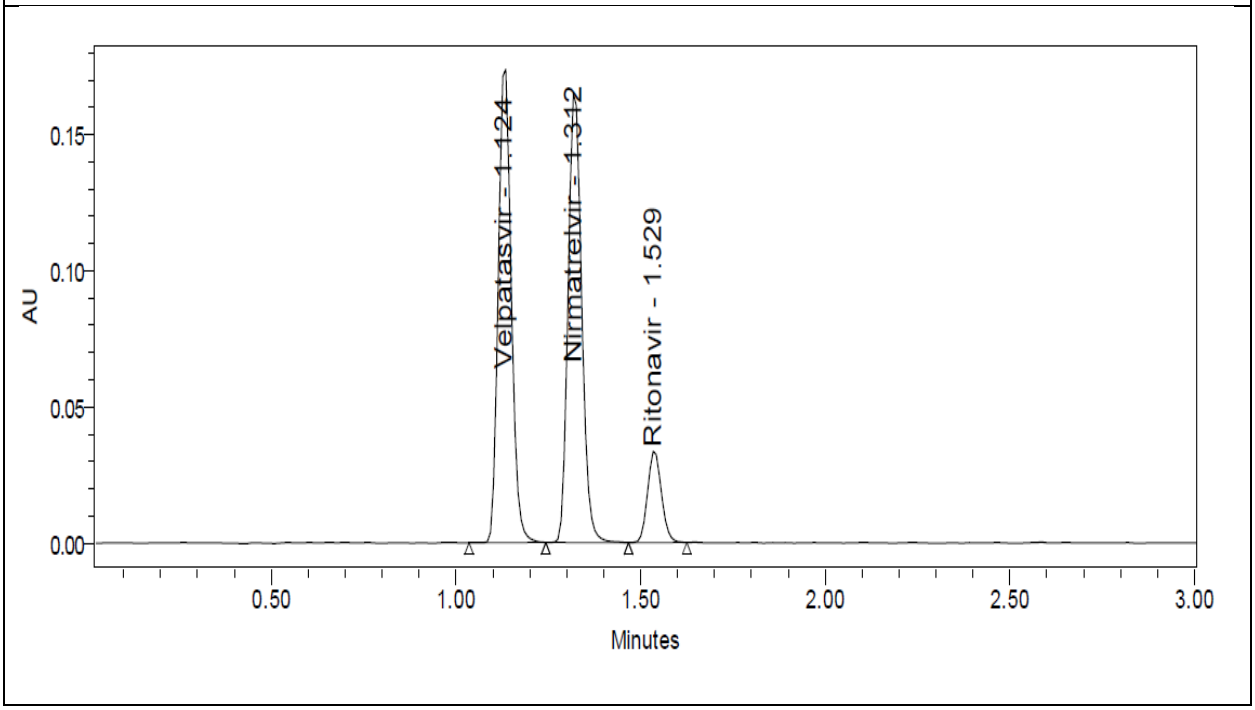
Blank



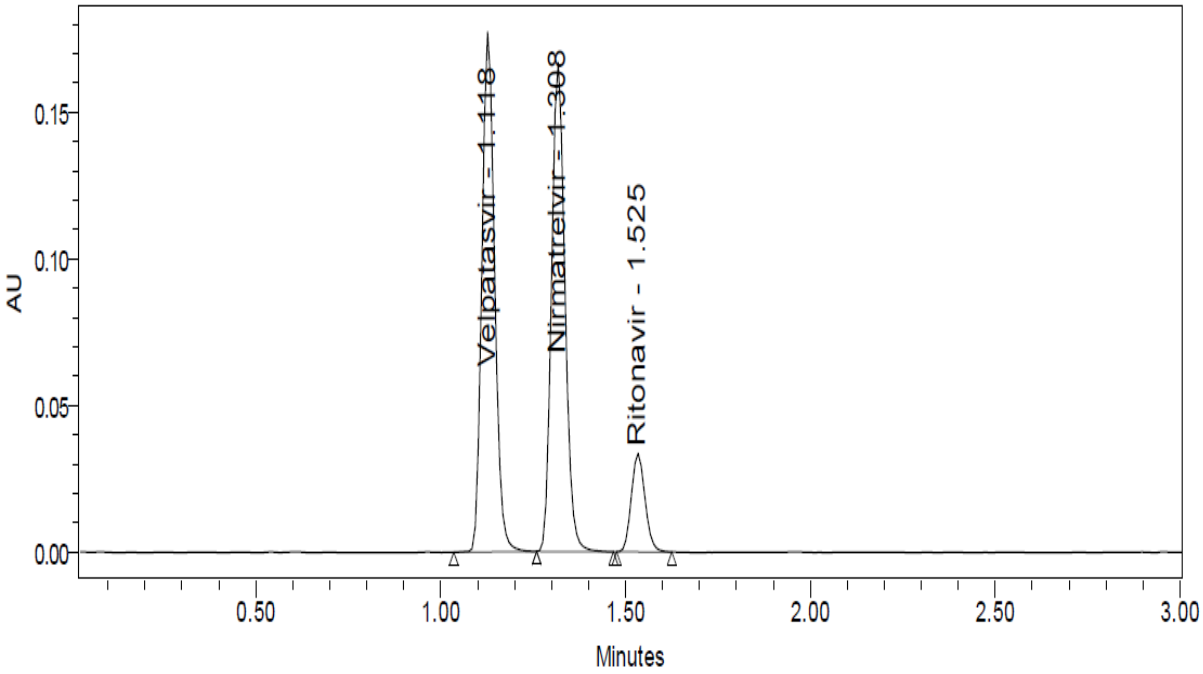
Placebo



Typical chromatogram of Nirmatrelvir and Ritonavir (API) in presence of internal standard (Velpatasvir) (Optimised method)



Typical chromatogram of Nirmatrelvir and Ritonavir tablet formulation in presence of internal standard (Velpatasvir) (Brand I)



Typical chromatogram of Nirmatrelvir and Ritonavir tablet formulation in presence of internal standard (Velpatasvir) (Brand II)

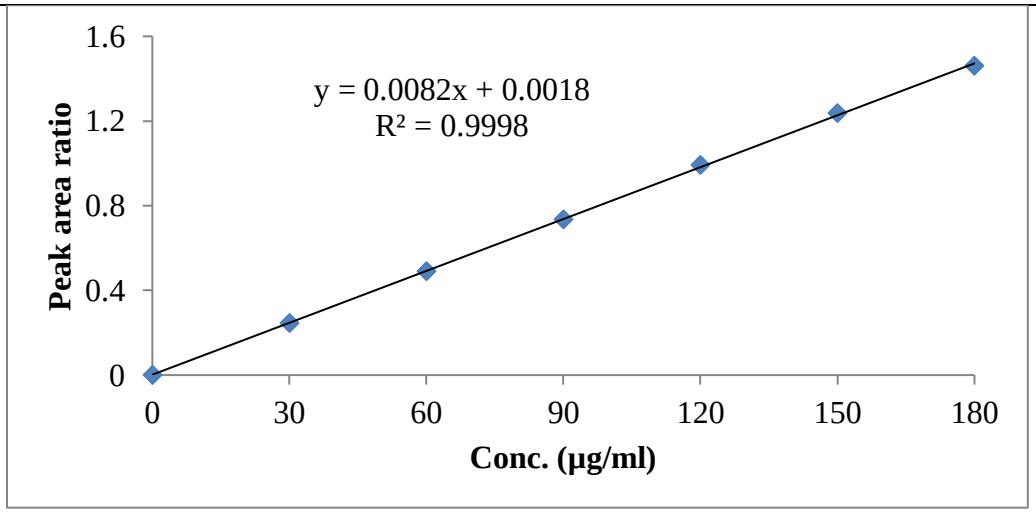
Figure 3: Typical chromatogram of Nirmatrelvir and Ritonavir in presence of internal standard (Velpatasvir)

Table 3: Linearity study

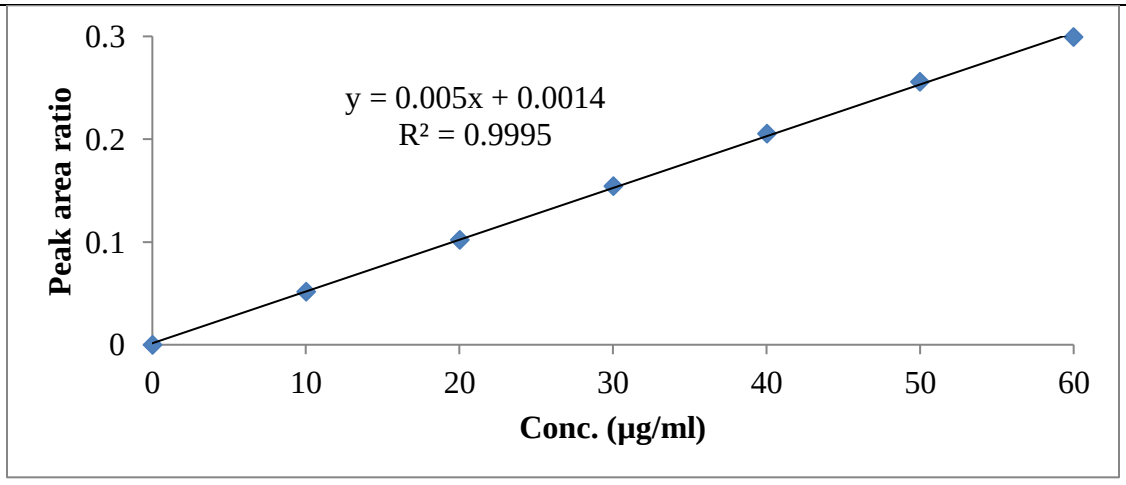
Conc. (µg/ml)			Peak area ratio (Analyte/IS)	
Nirmatrelvir	**Ritonavir**	**IS**	**Nirmatrelvir / IS**	**Ritonavir / IS**
30	10	100	0.25	0.05

60	20	100	0.49	0.10
90	30	100	0.74	0.15
120	40	100	0.99	0.21
150	50	100	1.24	0.26
180	60	100	1.46	0.30

*Mean of three replicates



Nirmatrelvir (NT)



Ritonavir (RV)

Figure 4: Calibration curves

Table 4: Precision study

Nirmatrelvir							
Intraday precision					**Interday precision**		
Conc. (µg/ml)		**Peak area**		**Peak area ratio**	**Peak area**		**Peak area ratio**
NT	IS	NT	IS	Analyte / IS	NT	IS	Analyte / IS
120	100	832785	842248	0.98876	830265	837790	0.99102
120	100	835936	845284	0.98894	828371	835485	0.99149
120	100	839055	843534	0.99469	831110	836473	0.99359
120	100	837157	842582	0.99356	835796	843775	0.99054
120	100	836605	844506	0.99064	835154	842716	0.99103
120	100	833309	849438	0.98101	829640	835819	0.99261
*Mean peak area ratio ± SD (% RSD) = 0.990 ± 0.005 (0.490)					*Mean peak area ratio ± SD (% RSD) 0.9917 ± 0.001 (0.1)		
Ritonavir							
Intraday precision					**Interday precision**		
Conc. (µg/ml)		**Peak area**		**Peak area ratio**	**Peak area**		**Peak area ratio**
RV	IS	RV	IS	Analyte / IS	RV	IS	Analyte / IS

40	100	172056	842248	0.20428	171364	837790	0.20454
40	100	172090	845284	0.20359	171852	835485	0.20569
40	100	172634	843534	0.20466	171711	836473	0.20528
40	100	172411	842582	0.20462	171080	843775	0.20276
40	100	172644	844506	0.20443	171352	842716	0.20333
40	100	172607	849438	0.20320	171492	835819	0.20518
*Mean peak area ratio ± SD (% RSD) = 0.20 ± 0.0 (0.3)					*Mean peak area ratio ± SD (% RSD) 0.20 ± 0.00 (0.6)		

*Mean of three replicates

Table 5: Accuracy study

Nirmatrelvir			
Spiked drug Conc. (µg/ml)	**Drug Formulation (µg/ml)**	***Drug recovered (µg/ml)**	**% Recovery**
60	120	58.91444	98.19
	120	58.94871	98.25
	120	59.68751	99.48
120	120	119.3966	99.50
	120	119.5802	99.65
	120	119.8959	99.91
180	120	178.9353	99.41

	120 120	177.8781 177.205	98.82 98.45
*Mean % Recovery ± SD (% RSD) = 99.07 ± 0.65 (0.7)			
Ritonavir			
Spiked drug Conc. (µg/ml)	**Drug Formulation (µg/ml)**	***Drug recovered (µg/ml)**	**% Recovery**
20 20 20	40 40 40	19.87318 19.80271 19.99642	99.37 99.01 99.98
40 40 40	40 40 40	40.14584 40.49711 40.3519	100.36 101.24 100.88
60 60 60	40 40 40	59.90708 59.86609 59.65466	99.85 99.78 99.42
*Mean % Recovery ± SD (% RSD) = 99.99 ± 0.73 (0.73)			

*Mean of three replicates

Assay of tablets

The tablet formulations contain 150 mg of Nirmatrelvir and 100 mg of Ritonavir. The assay for the estimation of Nirmatrelvir and Ritonavir was performed for two of the marketed brands with optimized chromatographic conditions and the percentage of purity was found to be 99.22-99.64 for Nirmatrelvir and 99.52-99.77 for Ritonavir (Table 6). The typical chromatograms obtained for

the two tablet formulations were shown in Figure 3 in presence of the internal standard, Valacyclovir.

Table 6: Assay of tablets

S. No.	Brand name	Label claim (mg)		*Observed amount (%w/w)		% Recovery*	
		NT	RV	NT	RV	NT	RV
1	Brand I	150	100	149.46	99.77	99.64	99.77
2	Brand II	150	100	148.83	99.52	99.22	99.52

*Mean of three replicates

Forced degradation studies

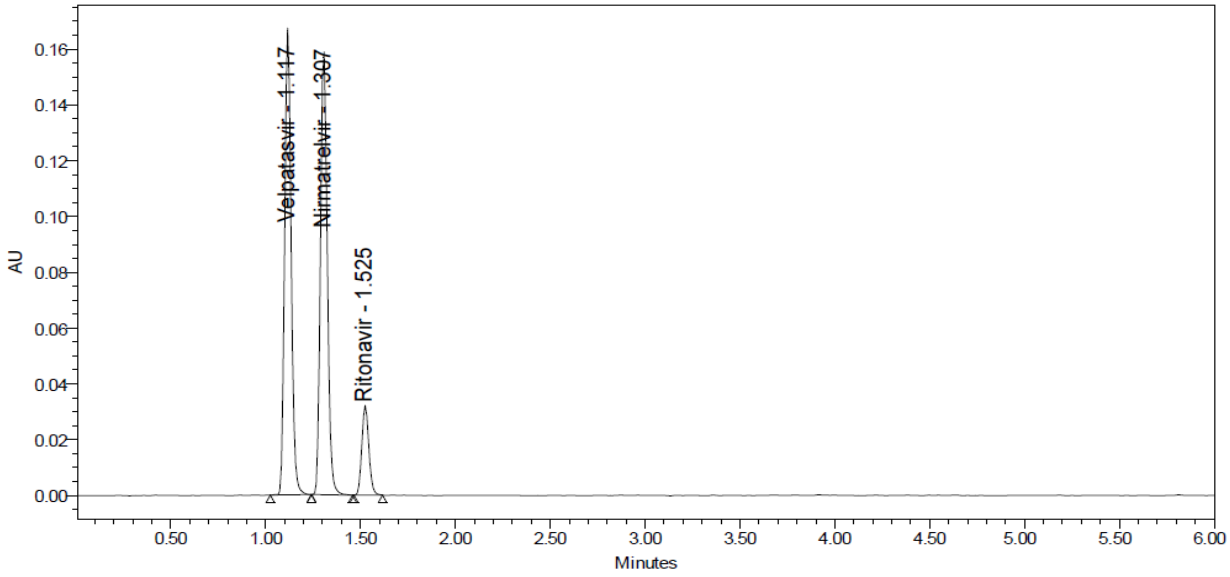
The respective chromatograms obtained during the forced degradation studies were shown in Figure 5 and the other details were shown in Table 7.

Table 7: Forced degradation studies

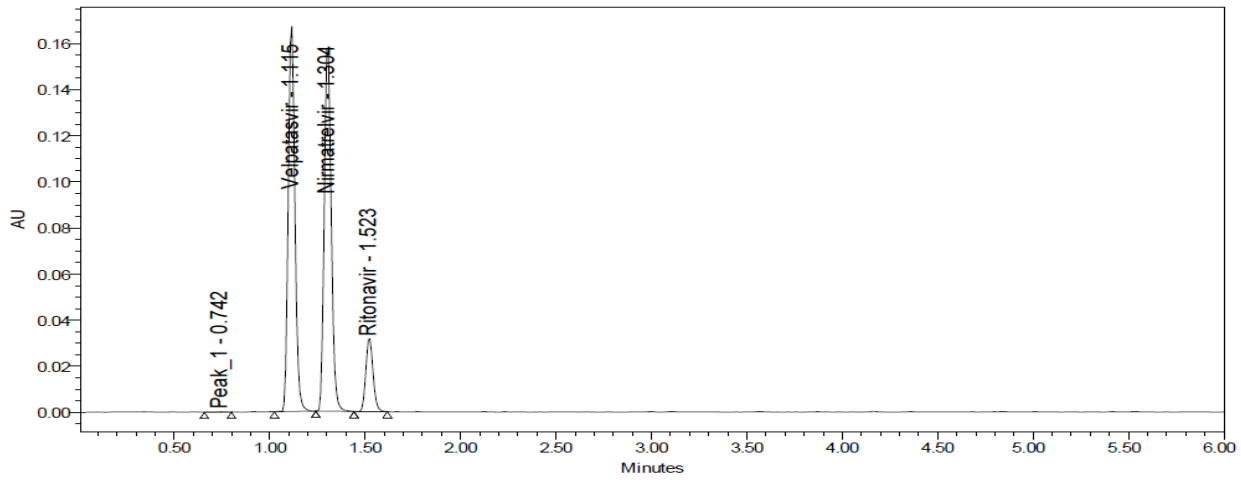
Stress Conditions	Rt (min)			Peak area ratio (Analyte/IS)		*Drug recovered (%)		*Drug decomposed (%)	
	IS	NT	RV	NT	RV	NT	RV	NT	RV
Standard	1.122	1.311	1.528	0.990	0.204	100	100	-	-
Acidic degradation	1.117	1.307	1.525	0.957	0.195	96.49	95.50	3.51	4.50
Alkaline degradation	1.117	1.308	1.524	0.941	0.192	94.91	94.02	5.09	5.98

Oxidative degradation	1.115	1.304	1.523	0.939	0.193	96.47	94.25	3.53	5.75
Thermal degradation	1.121	1.310	1.528	0.968	0.199	97.64	97.41	2.46	2.59
Photolytic degradation	1.116	1.306	1.524	0.976	0.200	98.41	97.88	1.59	2.22
Neutral degradation	1.112	1.302	1.520	0.991	0.203	99.90	99.13	0.10	0.87

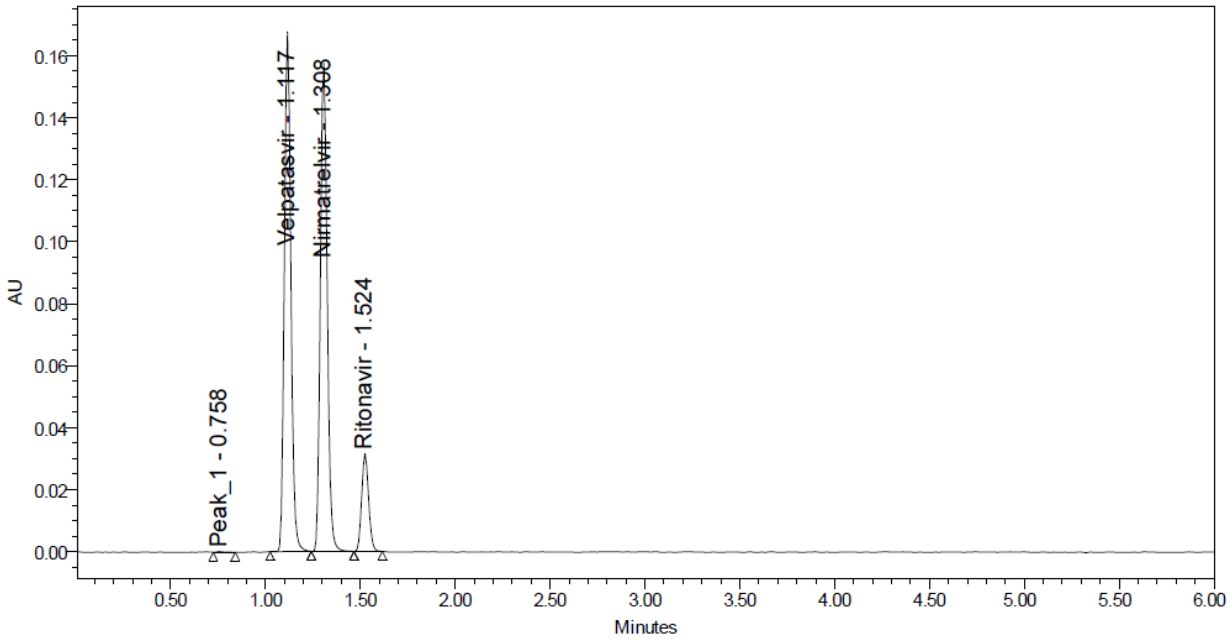
*Mean of three replicates



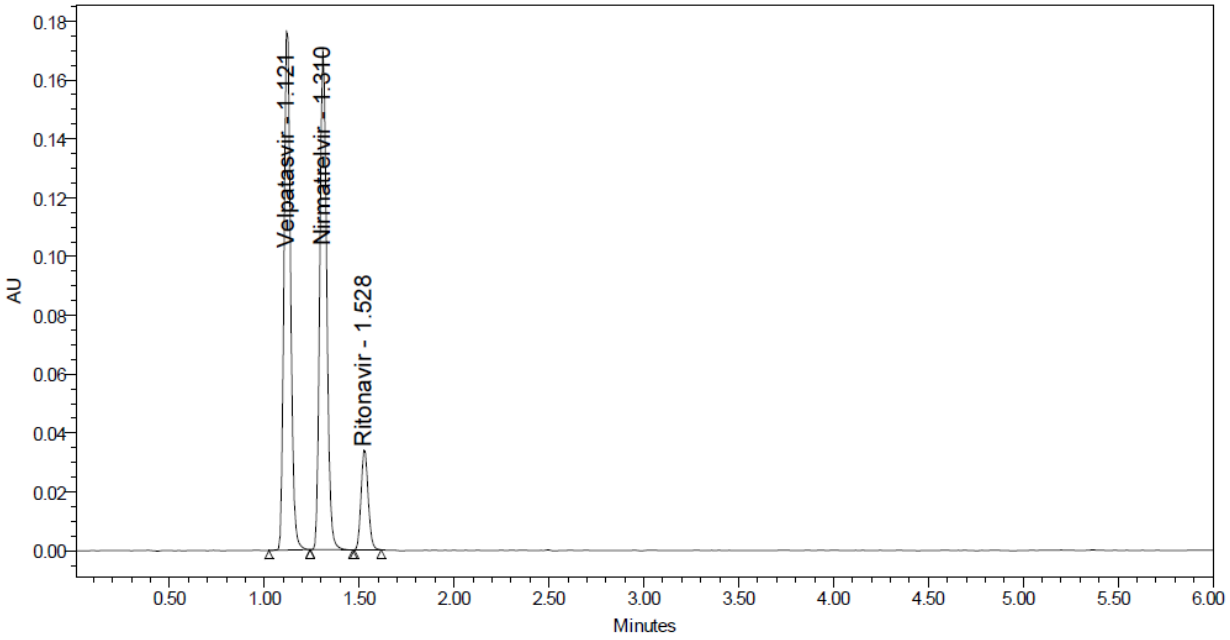
Acidic degradation



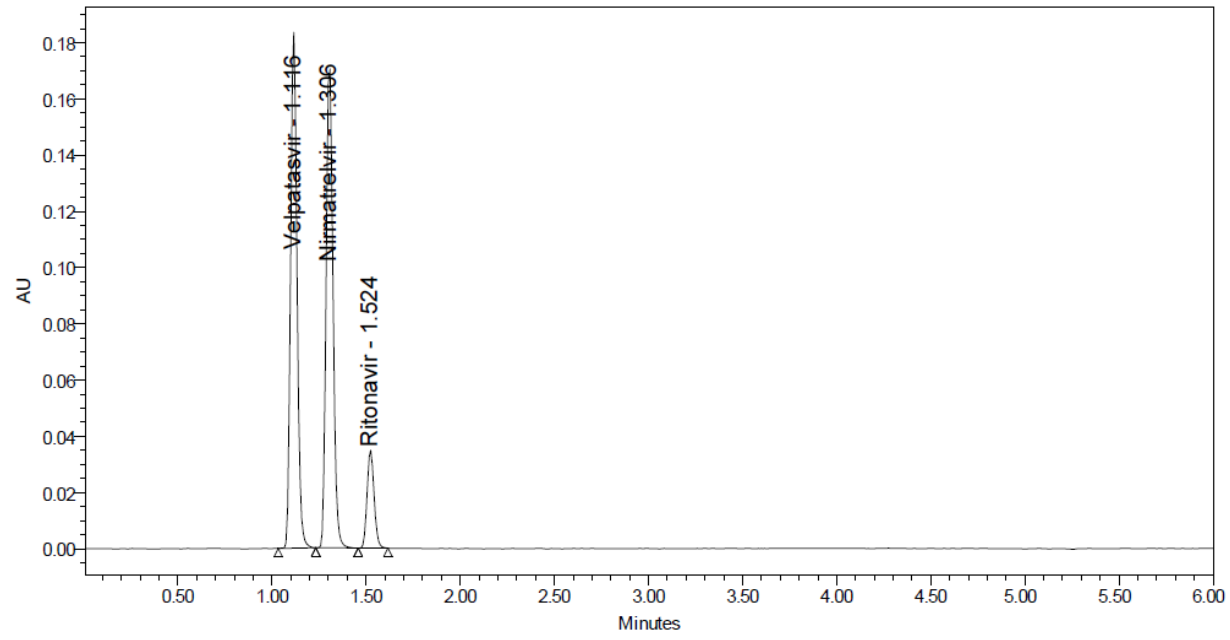
Oxidative degradation



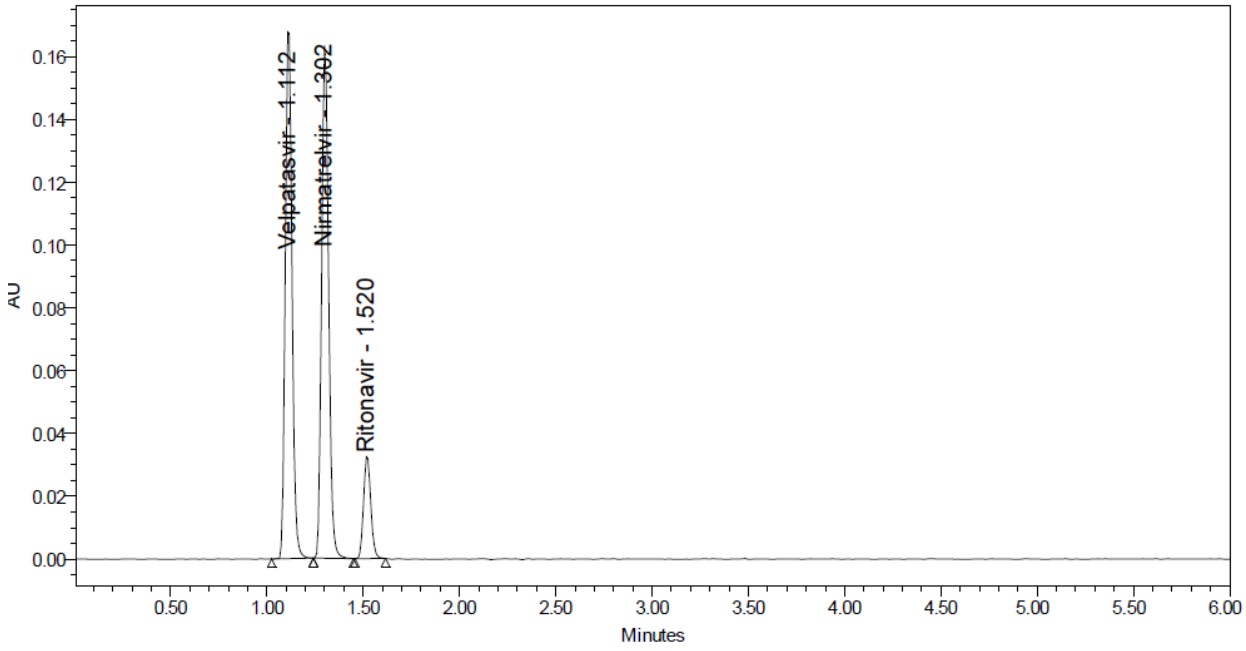
Alkaline degradation



Thermal degradation



Photolytic degradation



Neutral degradation

Figure 5: Chromatograms of Nirmatrelvir and Ritonavir in presence of the internal standard (Velpatasvir) during forced degradation studies

CONCLUSIONS

A new stability indicating RP-UPLC method has been developed for the estimation of the combination of Nirmatrelvir and Ritonavir and validated as per ICH guidelines. The method is specific and there is no interference of excipients used in the tablet formulation. The proposed method is simple precise, accurate and robust and can be applied for the pharmaceutical formulations successfully.

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REFERENCES

1) Anastassopoulou C, Hatziantoniou S, Boufidou F, Patrinos GP and Tsakris A. The role of oral antivirals for COVID-19 treatment in shaping the pandemic landscape. J Pers Med 2022; 12: 439.

2) Zeldin R and Petruschke RA. Pharmacological and therapeutic properties of ritonavir-boosted protease inhibitor therapy in HIV-infected patients. The Journal of Antimicrobial Chemotherapy 2004; 53(1): 4-9.
3) Sumalatha C, Srinivas M and Jitender Kumar M. Application of RP-HPLC method for the simultaneous determination of Nirmatrelvir and Ritonavir in tablets dosage form. International Journal of Zoological Investigations 2024; 10(2): 227-238.
4) Imam MS, Batubara AS, Gamal M. et al. Adjusted green HPLC determination of Nirmatrelvir and Ritonavir in the new FDA approved co-packaged pharmaceutical dosage using supported computational calculations. Sci Rep 2023; 13: 137.
5) Nagajyothi K and Sunitha B. Development and validation of an HPLC method for the simultaneous detection of Nirmatrelvir and Ritonavir in pharmaceutical formulations. World J Pharm Sci 2025; 13(4): 209-218.
6) Shaikh Zaheer, Abdul Ahad, M Zameer Ahmed and Maqdoom Farooqui. Simultaneous quantification of anti-viral drugs Nirmatrelvir and Ritonavir used for the treatment of Covid-19 by HPLC method in bulk and dosage forms. International Journal of Pharmaceutical Sciences and Drug Research. 2024; 16(5): 825-834.
7) Yassin MG, Roshdy A and Marie AA. Stability indicating RP-HPLC technique for simultaneous estimation of Nirmatrelvir and Ritonavir in their new co-packaged dosage form for COVID-19 treatment. Sci Rep 2025; 15: 2281.
8) Sindhu M, Mohammed Farana, Bhavani M, Santhoshi Priya D, Shanthi Priya DK and Vasudha Bakshi. Stability indicating RP-HPLC method for simultaneous estimation of Nirmatrelvir and Ritonavir in bulk and tablets. Research Journal of Pharmacy and Technology. 2025; 18(2): 594-598.
9) Imam MS, Abdelazim AH, Batubara AS. et al. Simultaneous green TLC determination of Nirmatrelvir and Ritonavir in the pharmaceutical dosage form and spiked human plasma. Sci Rep 2023; 13: 6165.
10) ICH, Validation of analytical procedures: Text and methodology Q2 (R1). International Conference on Harmonization. Geneva; 2005.
11) ICH, Stability testing of new drug substances and products Q1A (R2). International Conference on Harmonization. Geneva; 2003.