Development and Validation Analytical RP-HPLC Method for the Simultaneous Estimation of Nirmatrelvir and Ritonavir in API Form and Pharmaceutical Dosage Form

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***Abstract*—A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Nirmatrelvir and Ritonavir in bulk and marketed pharmaceutical formulations. Separation of Nirmatrelvir and Ritonavir was successfully achieved on an X-Terra (4.6 ×150mm, 5µm particle size) in an isocratic mode utilizing Methanol: Acetonitrile: Water (50:35:15% v/v/v) at a flow rate of 1.0mL/min and eluates was monitored at 280nm, with a retention time of 1.689 and 3.238 minutes for Nirmatrelvir and Ritonavir respectively. The method was validated and the response was found to be linear in the drug concentration range of 20µg/mL to 100µg/mL for Nirmatrelvir and 5µg/mL to 25µg/mL for Ritonavir respectively. The values of the slope and the correlation coefficient were found to be 22649 and 0.999 for Nirmatrelvir and 19445 and 0.9991 for Ritonavir respectively. The LOD and LOQ for Nirmatrelvir were found to be 2.58µg/mL and 7.84µg/mL respectively. The LOD and LOQ for Ritonavir were found to be 1.08 µg/mL and 3.27µg/mL respectively. This method was found to be good %recovery for Nirmatrelvir and Ritonavir were found to be 99.3 and 100.7 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.**

***Index Terms*—Nirmatrelvir and Ritonavir, HPLC, Method Development, Accuracy, Validation.**

I. INTRODUCTION

Nirmatrelvir (PF-07321332) is an orally bioavailable 3C-like protease (3CLPRO) inhibitor that is the subject of clinical trial NCT04756531. 3CLPRO is responsible for cleaving polyproteins 1a and 1ab of SARS-CoV-2. Without the activity of the SARS-CoV-2 3CLPRO, nonstructural proteins (including proteases) cannot be released to perform their functions, inhibiting viral replication[1]. In the US, Europe, and Canada, Nirmatrelvir, in combination with ritonavir, is indicated for the treatment of mild-to-moderate coronavirus disease 2019 (COVID-19) in adults who are at high risk for progression to severe COVID-19, including hospitalization or death. In Europe, this therapeutic indication is approved under conditional marketing authorization[2]. Nirmatrelvir is a SARS-CoV-2 main protease inhibitor. It works by preventing the growth of the virus that causes COVID-19. Ritonavir increases ("boosts") the levels of Nirmatrelvir. This helps Nirmatrelvir work better[3]. The IUPAC name of Nirmatrelvir is 5 (1R, 2S, 5S)-N-[(1S)-1-cyano-2-[(3S)-2-oxo pyrrolidin-3-yl] ethyl]-3-[(2S)-3, 3-dimethyl-2-[(2, 2, 2-trifluoro acetyl) amino] butanoyl]-6, 6-dimethyl-3-azabi cyclo [3.1.0] hexane-

2-carboxamide. The Chemical Structure of Nirmatrelvir is shown in following figure-1.

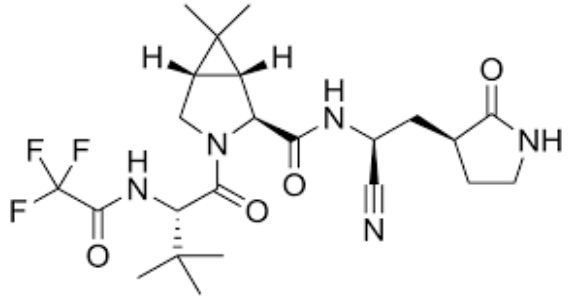

Fig-1: Chemical Structure of Nirmatrelvir

Ritonavir is an antiretroviral protease inhibitor that is widely used in combination with other protease inhibitors in the therapy and prevention of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS)[4]. Ritonavir can cause transient and usually asymptomatic elevations in serum aminotransferase levels and, rarely, can lead to clinically apparent acute liver injury. In HBV or HCV coinfected patients, highly active antiretroviral therapy with ritonavir may result of an exacerbation of the underlying chronic hepatitis B or C[5]. Ritonavir is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection. Ritonavir is used along with other medications to treat human immunodeficiency virus (HIV) infection. Ritonavir is in a class of medications called protease inhibitors. It works by decreasing the amount of HIV in the blood[6]. The IUPAC name of Ritonavir is 1, 3-thiazol-5-ylmethyl N-[(2S, 3S, 5S)-3-hydroxy-5-[[(2S)-3-methyl-2-[[methyl-[(2-propan-2-yl-1, 3-thiazol-4-yl) methyl] carbamoyl] amino] butanoyl] amino]-1, 6-diphenyl hexan-2-yl] carbamate. The Chemical Structure of Ritonavir is shown in follows

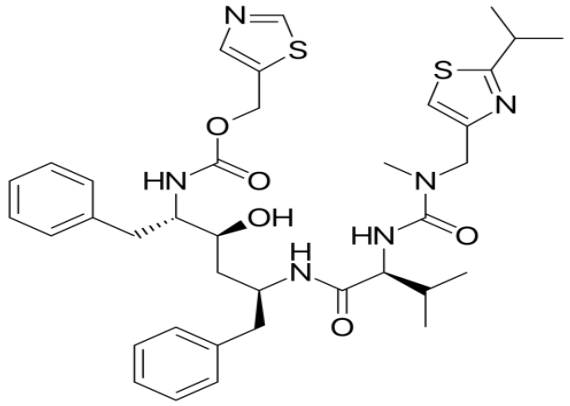

Fig-2: Chemical Structure of Ritonavir

The literature survey revealed that there are few stability indicating RP-HPLC and UV spectrophotometric methods are available for the simultaneous estimation of Nirmatrelvir and Ritonavir in bulk and pharmaceutical dosage forms[28-31]. Few analytical methods for Nirmatrelvir and Ritonavir drugs in biological fluids by LC-MS/MS have been reported. Hence, present work focused on the development and validation of a simple, rapid, robust and economical stability indicating RP-HPLC method.

II. EXPERIMENTAL

Table-1: Instruments Used

S.No.	Instruments and Glasswares	Model
1	HPLC	WATERS Alliance 2695 separation module, software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital Ultra Sonicator	Labman

Table-2: Chemicals Used

S.No.	Chemical	Brand Names
1	Nirmatrelvir	Synpharma Research Lab, Hyderabad
2	Ritonavir	Synpharma Research Lab, Hyderabad
3	Water and Methanol for HPLC	LICHROSOLV (MERCK)
4	Acetonitrile for HPLC	Merck

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Nirmatrelvir and Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines[27].

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Acetonitrile: Water in proportion 50:35:15 v/v respectively[7].

Optimization of Column:

The method was performed with various columns like C18 column, Symmetry and Zodiac column. X-Terra (4.6 ×150mm, 5µm particle size) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Preparation of Mobile Phase:

Accurately measured 500 ml (50%) of Methanol, 350 ml of Acetonitrile (35%) and 150ml of HPLC Grade water were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration[8].

Diluent Preparation:

The Mobile phase was used as the diluent.

Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[9].

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:

Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Nirmatrelvir and Ritonavir sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent[10].

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (20 ppm of Nirmatrelvir & 18ppm of Ritonavir):

Pipette out 0.2ml of Nirmatrelvir and 0.18ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (40 ppm of Nirmatrelvir& 24ppm of Ritonavir):

Pipette out 0.4ml of Nirmatrelvir and 0.24ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (60 ppm of Nirmatrelvir& 30ppm of Ritonavir):

Pipette out 0.6 ml of Nirmatrelvir and 0.3ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent[11].

Preparation of Level – IV (80 ppm of Nirmatrelvir& 36ppm of Ritonavir):

Pipette out 0.8 ml of Nirmatrelvir and 0.36ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (100 ppm of Nirmatrelvir& 42ppm of Ritonavir):

Pipette out 1.0ml of Nirmatrelvir and 0.42ml of Ritonavir stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient[12].

Precision

Repeatability

Preparation of Nirmatrelvir and Ritonavir Product Solution for Precision:

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits[13].

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Day 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Day 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

Accuracy:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.9375ml of the above Nirmatrelvir and 0.75ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml

of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of Nirmatrelvir and 1.5ml of Ritonavir from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Nirmatrelvir and Ritonavir and calculate the individual recovery and mean recovery values[14-16].

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.187ml of the above Nirmatrelvir and 1.5ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of Flow Conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded[17].

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Acetonitrile: HPLC Grade water was taken in the ratio and 55:30:15 and 45:40:15 instead (50:35:15), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

III. RESULTS AND DISCUSSION

Development of an Analytical Method:
Method Optimization: Optimization of the HPLC method was carried out in experiments investigating mobile phase, flow rate, column, pH of the mobile phase, ratio of mobile phase, injection volume, and temperature of the column and solvents. The final isocratic chromatographic conditions and Optimized Chromatogram shown in Table 3 and the figure-3 are optimized for simultaneous analysis of Nirmatrelvir and Ritonavir. Test and standard samples were prepared in mobile phase as diluent which consist of a Methanol: Acetonitrile: Water in ratio 50:35:15%v/v. The flow rate of 1.0 ml/min, injection volume of 10 µl, wavelength of 280 nm for Nirmatrelvir and Ritonavir is used at 42ºC temperature conditions[18]. Using this method, retention times of Nirmatrelvir and Ritonavir were 1.689 min and 3.238 min, respectively.

Table-3: Shows Optimized Chromatographic Conditions

PARAMETER	OPTIMIZED CHROMATOGRAPHIC CONDITIONS
Mobile phase :	Methanol: Acetonitrile: Water (50:35:15%v/v)
Column :	X-Terra (4.6mm ×150mm, 5µm particle size)
Flow rate :	1.0ml/min
Diluent	Methanol: Acetonitrile: Water (50:35:15%v/v)
Injection Volume	10 µl
Wavelength:	280 nm
Column temp:	42ºC
Run mode	Isocratic
Runtime	7minutes

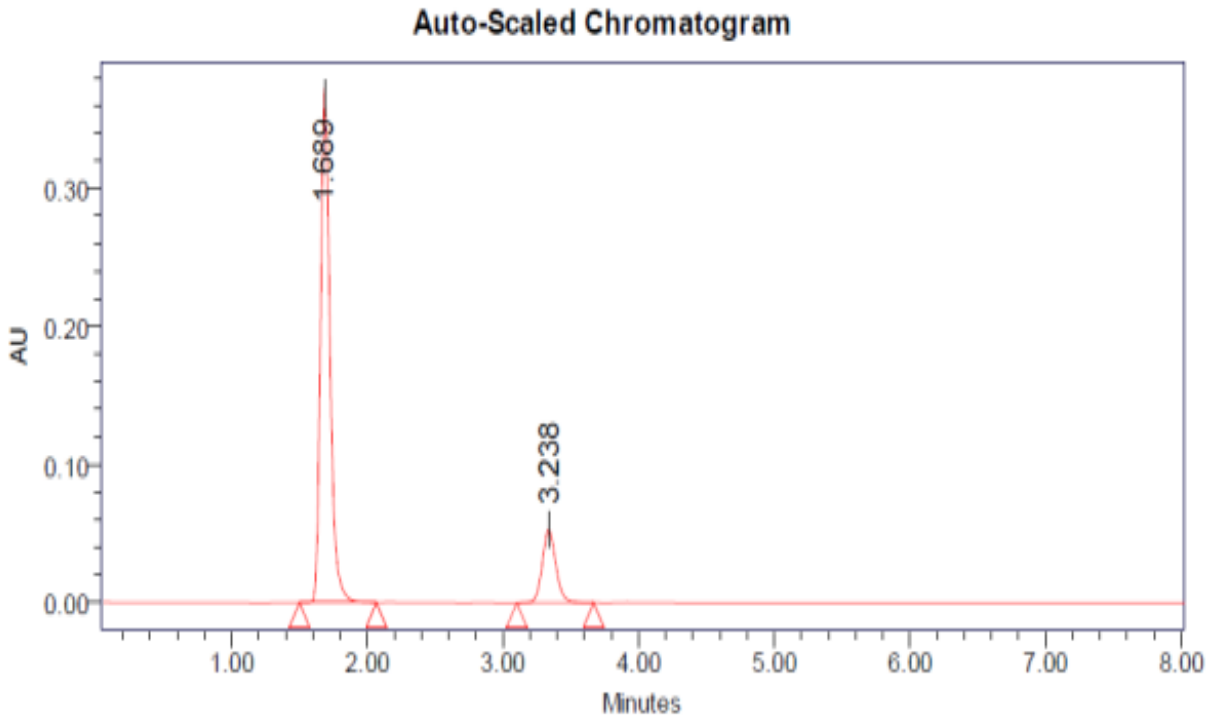

Fig-3: Optimized Chromatographic Condition

Analytical Method Validation:

The method was validated for linearity, accuracy, precision, specificity, LOD, and LOQ in accordance with ICH guidelines[27].

System Suitability Parameters:

Table-4: Observation of System Suitability Parameters

S. No.	Parameter	Nirmatrelvir	Ritonavir
1.	Retention Time (min)	1.694	3.234
2.	Theoretical Plates	6993	5735
3.	Tailing factor	1.23	1.12
4.	Area	1429524	300414
5.	Resolution	10.69	

The system suitability parameters were found to be within the specified limits for the proposed method[19].

Accuracy:

The degree of closeness between a test value and the realistic value is called accuracy. By collecting at least nine measurements across three concentration levels, accuracy was assessed. The acceptance criterion for accuracy is the RSD for all recovery values should not be more than 2%[20].

Table-5: Accuracy Observation of Nirmatrelvir

%Concentration (at Specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	713835.3	9.375	9.3	100	99.3%
100%	1406995	18.75	18.92	99.1	
150%	205468	28.12	27.86	99.05	

Table-6: Accuracy Observation of Ritonavir

%Concentration (at Specification Level)	Average Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	150840	7.5	7.59	100	100.7%
100%	300215	15	15.27	101.82	
150%	443109	22.5	22.62	100.5	

Precision:

Method Precision / Repeatability**:** The % RSD value for six replicate injection of an concentrations of 18.75 µg/mL of Nirmatrelvir and 15 µg/mL of Ritonavir carried out on the same day was found to be < 2% which indicate that the method repeatable[21]. The results for method precision are given in Table 7.
System Precision / Intermediate Precision: Intermediate precision was determined by measuring the peak area of six replicate was inject into the HPLC system and was analyzed and they were found within the acceptable limit (% RSD) intermediate precision given in Table 8 & 9.

Table-7: Observation of System Precision

S. No.	Sample Area 1	Sample Area 2
1	1429456	300557
2	1422446	300364
3	1424679	300377
4	1425211	300817
5	1426102	300227
Mean	1425578.8	300468.4
Std. Dev	2552.73	227.4
%RSD	0.1	0.07

Ruggedness:
Day 1:

Table-8: Observation of Robustness Day1

S. No.	Sample Area 1	Sample Area 2
1	1428595	300757
2	1426785	300826
3	1426465	300668
4	1426588	300119
5	1427757	300599
6	1426622	300181
Mean	1427135	300525
Std. Dev.	854.665	301.1717
% RSD	0.059887	0.100215

Day 2:

Table-9: Observation of Robustness Day2

S. No.	Sample Area 1	Sample Area 2
1	1426363	300918
2	1420494	300848
3	1428474	300199
4	1428574	300188
5	1426563	300198
6	1426568	300194
Mean	1426173	300424.2
Std. Dev.	2954.815	356.1204
% RSD	0.207185	0.118539

Linearity:
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a

suitable level of precision, accuracy and linearity. The linearity response was determined by analyzing independent levels of concentrations in the range of 20-100µg/ml and 5-25µg/ml for Nirmatrelvir and Ritonavir respectively[22]. Peak area of each solution was measured.

Table-10: Linearity Observation of Nirmatrelvir

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1.	I	20	504954
2.	II	40	958753
3.	III	60	1426583
4.	IV	80	1845498
5.	V	100	2272948
Correlation coefficient			0.999

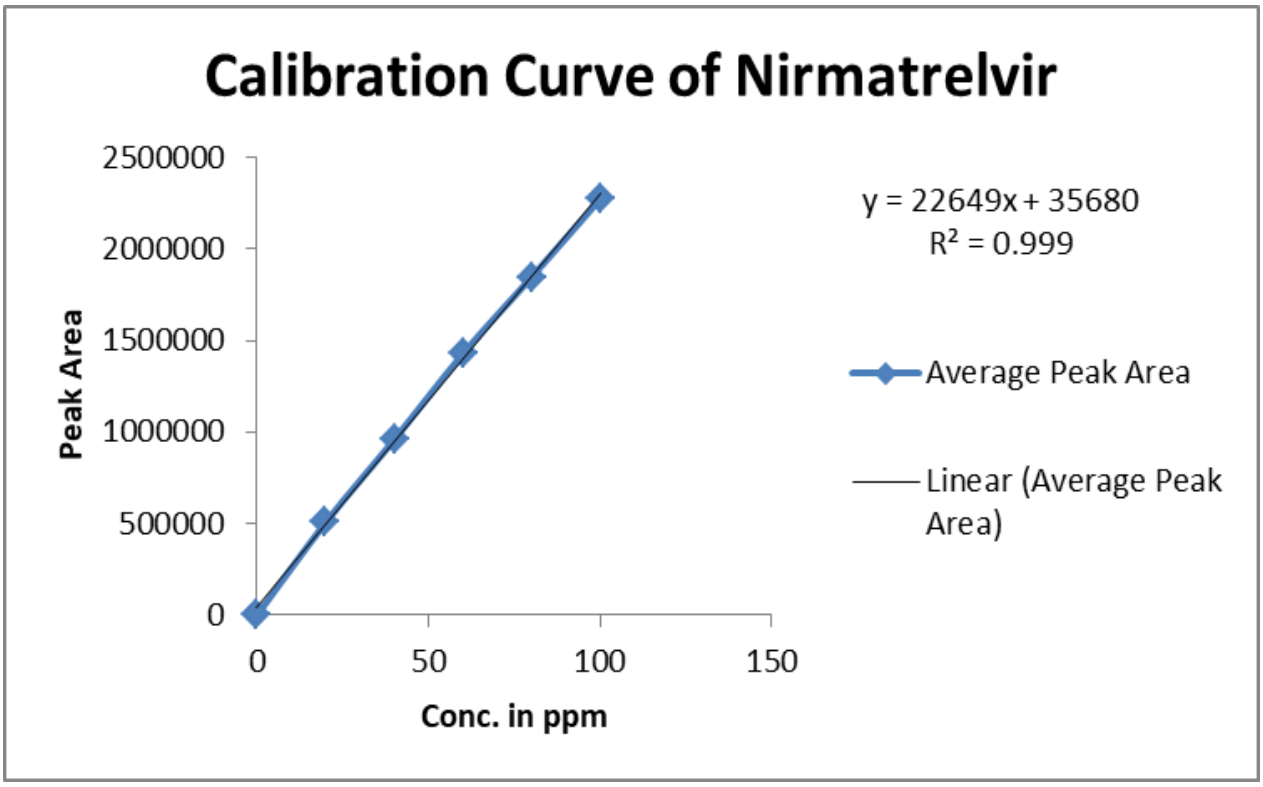

Fig-4: Calibration Curve for Nirmatrelvir

Table-11: Linearity Observation of Ritonavir

S. No	Concentration Level (%)	Concentration µg/ml	Average Peak Area
1	I	5	107359
2	II	10	191497
3	III	15	300389
4	IV	20	388105
5	V	25	490352
Correlation coefficient			0.999

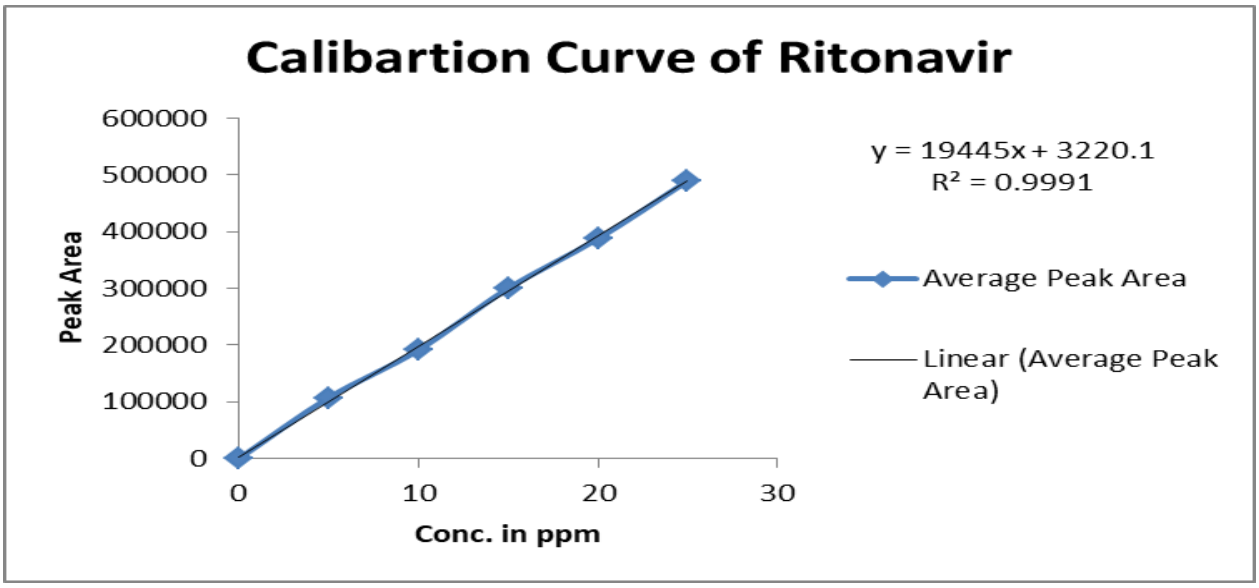

Fig-5: Calibration Curve for Ritonavir

Limit of Detection (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value[23].

LOD= 3.3 × S.D / Slope

Table-12: LOD Results of the Method

Drug	Amount (µg/ml)
Nirmatrelvir	2.58
Ritonavir	1.08

From the above, the LOD values of Nirmatrelvir and Ritonavir were found to be 2.58 and 1.081 µg/ml respectively.

Limit of Quantitation (LOQ):

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined[24].

LOQ = 10× S.D / Slope

Table-13: LOQ Results of the Method

Drug	Amount(µg/ml)
Nirmatrelvir	7.84
Ritonavir	3.27

From the above, the LOQ values of Nirmatrelvir and Ritonavir were found to be 7.84 and 3.27 µg/ml respectively.

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Ritonavir, Nirmatrelvir[25]. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Ritonavir, Nirmatrelvir were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count[26].

Table-14: System Suitability for Nirmatrelvir (Flow rate)

Flow Rate (mL/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	6728	1.23	1.868
Actual Flow rate	1.0	6993	1.23	1.694
More Flow rate	1.2	5285	1.21	1.544

Table-15: System Suitability for Ritonavir (Flow rate)

Flow Rate (mL/min)		System Suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less Flow rate	0.8	4402	1.11	3.621
Actual Flow rate	1.0	5735	1.12	3.234
More Flow rate	1.2	6509	1.12	2.998

Table-16: System Suitability Outcomes Nirmatrelvir (Organic Mobile Phase ratio)

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	45:35:20	6728	1.23	1.868
Actual organic phase	50:35:15	6993	1.23	1.694
More organic phase	55:40:5	6996	1.24	1.675

Table-17: System Suitability Interpretations Ritonavir (Organic Mobile Phase ratio)

Organic phase		System suitability Results		
		USP Plate Count	USP Tailing	Retention Time (min)
Less organic phase	45:35:20	4402	1.11	3.621
Actual organic phase	50:35:15	5735	1.12	3.234
More organic phase	55:40:5	4831	1.15	2.302

IV. SUMMARY AND CONCLUSION

A new method was established for simultaneous estimation of Nirmatrelvir and Ritonavir by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Nirmatrelvir and Ritonavir by using Phenomenex Luna C18 (4.6mm × 250mm, 5µm) particle size, flow rate was 1.0ml/min, mobile phase ratio was (45:55% v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid), detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 1.689mins and 3.238mins. The % purity of Nirmatrelvir and Ritonavir was found to be 99.87%. The system suitability parameters for Nirmatrelvir and Ritonavir such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Nirmatrelvir and Ritonavir was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r^2) was found to be 0.999 and 0.999, % recovery was found to be 100.710% and 100.350%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Nirmatrelvir and Ritonavir in API and Pharmaceutical dosage form.

REFERENCES

[1] https://go.drugbank.com/drugs/DB16691
[2] https://pubchem.ncbi.nlm.nih.gov/compound/Nirmatrelvir
[3] https://en.wikipedia.org/wiki/Nirmatrelvir
[4] https://go.drugbank.com/drugs/DB00503
[5] https://pubchem.ncbi.nlm.nih.gov/compound/Ritonavir
[6] https://en.wikipedia.org/wiki/Ritonavir
[7] Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
[8] A. Braith Wait and F. J. Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
[9] Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.

[10] Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
[11] Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
[12] Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
[13] Sahajwalla CG a new drug development, Vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
[14] D. H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66, 2012, 11-23.
[15] M. D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31, 2009.
[16] FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), 2000.
[17] Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3, 2012, 53-63.
[18] Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, 75, 2002, 49-50.
[19] Synder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.
[20] Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.
[21] Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20, 2002, 430-436.
[22] Chan CC, Leo YC, Lam H. Analytical method validation and Instrument Performance Validation. Vol-I, Wiley Inter science, 2004.
[23] Shabir GA, Lough WJ., Arain SA, Bradshaw TK. Evaluation and application of best practice in analytical method validation. J Chromatogr RT, 30, 2007, 311-333.
[24] Miller JN, Miller JC, Statistics and chemometrics for analytical chemistry Harlow, Pearson Prentice Hall, 2005, 263.
[25] Huber L. Validation of HPLC methods. BioPharm. Int, 12, 1999, 64-66.
[26] European-commission Quality control procedures for pesticide residues analysis. Report number SANCO/10232/2006.
[27] ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonization, IFPMA, Geneva, 2005.
[28] A. Venkata Suresh Babu1*, H. K Sharma2, Method Development and Validation of Ritonavir and Nirmatrelvir in Tablet Dosage Form by Using Rp-High Performance Liquid Chromatography, Eur. Chem. Bull. 2023, 12(Special Issue 5), 815 – 825.
[29] S. Pallavi, G. Sowjanya, Development and validation of a new RP-UPLC method for the simultaneous estimation of Nirmatrelvir and ritonavir in bulk and copacked tablet dosage forms, Research Journal of Pharmacy and Technology 2023; 16(9):4370-6. Doi: 10.52711/0974-360X.2023.00715.
[30] David Blessing Rani J1,2*, Asha Deepti C1, Development of a Simple Accurate Method, Validation and It's Degradation Studies of Nirmatrelvir, Ritonavir in Bulk and Marketed Formulation by RP-HPLC, IJPQA, Volume 14 Issue 3, July - September 2023, pages: 740-744.
[31] Vadivelan Ramachandran, Bhargav Bhongiri*, Raju Bairi, Raman Suresh Kumar.Potential Health Benefit of Isoflavones. Vadivelan Ramachandran et al., Int. J. Res. Pharm. Sci., 2020, 11 (SPL4), 2463-2467. IF-0.604.