Int. J. Pharm. Investigation, 2025; 15(3):1039-1044.
https://www.jpionline.org

Original Article

Simultaneous Determination of Nirmatrelvir and Ritonavir in Bulk and Tablet Dosage Form by RP-HPLC Method

Rohit Gawali[1], Rahul Keshav Godge[1,*], Kavita Dhamak[1] , Arshu Patel[2]

[1]Department of Pharmaceutical Quality Assurance Technique, Pravara Rural College of Pharmacy, Pravaranagar, Rahta, Ahmednagar, Maharashtra, INDIA.
[2]Department of Pharmacognosy, Pravara Rural College of Pharmacy, Pravaranagar, Rahta, Ahmednagar, Maharashtra, INDIA.

ABSTRACT

Aim: A Rational and time-efficient, as well as, selective and gentle approach based on simultaneous estimation to the RP-HPLC verification in compliance with the most recent requirements for nirmatrelvir and ritonavir. **Materials and Methods:** In the process of developing and validating a easy and efficient RP-HPLC method that adheres to the Q2R1 guideline of the International Conference for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, several factors such as linearity, precision, limit of detection, limit of quantification, and accuracy have been considered. For data collected from different aliquot runs for Agilent Tech, statistical data analysis was done. Gradient System: Auto sampler, UV (DAD) detector installed. **Results:** The analysis of medications and degradants was done with 20 µL injection loop at the wavelength of 234 nm, using a reverse phase C18 Agilent column with dimensions 4.6×250 mm, 5 µm particle size, and software Chemstation 10.1. Acetonitrile and 0.1% OPA buffer in the ratio of 41:59 volume per volume at room temperature and the flow According to the outcomes of the study, the LOD and the LOQ were displayed within limit as described by ICH. **Conclusion:** The devised approach is quick, simple, accurate, precise, economical, and selective for the confirmation test, according to the validation experiments. Therefore, the aforementioned approach is straightforward and frequently used to determine the levels of ritonavir and nirmatrelvir in tablet dosage forms.

Keywords: Nirmatrelvir, Ritonavir, RP-HPLC.

Correspondence:
Dr. Rahul Keshav Godge
Associate Professor, Department of Pharmaceutical Quality Assurance Technique, Pravara Rural College of Pharmacy Pravaranagar, Rahta, Ahmednagar-413736, Maharashtra, INDIA.
Email: rahulgodge@gmail.com

Received: 14-10-2024;
Revised: 02-12-2024;
Accepted: 22-03-2025.

INTRODUCTION

Ritonavir is a protease inhibitor designed to treat HIV/AIDS. It works by inhibiting the HIV 1 protease, which is important for the growth of the virus. By blocking this enzyme, ritonavir prevents the virus from multiplying in the host. Although ritonavir was first used to treat HIV, it has been discovered that it can be used in combination with other protease inhibitors based on pharmacokinetics because it can inhibit the cytochrome P450 enzyme (CYP3A4), which metabolizes many drugs. In the treatment of COVID-19, ritonavir is often used as a booster for nimatrelvir, helping to break down nirmatrelvir and maintain it at higher levels in the body. 2. This virus is responsible for SARS-CoV-2. It targets the primary protease (Mpro) of SARS-CoV-2, an enzyme needed by the virus to convert its polyprotein into a functional virus. By inhibiting this protease, nivolumab can prevent the virus from growing and multiplying, thereby reducing a decreased rate of infection and contribute in decreasing the flow of the infections. Nimaprevir acts directly on the virus by inhibiting the activity of the viral protease, while ritonavir slows down the metabolism of nimaprevir, increasing its effectiveness and thus maintaining healthy nimaprevir levels. Clinical studies have shown that this combination can reduce the risk of hospitalization and death in patients at high risk of COVID-19. Structure of ritonavir and nirmatrelvir shown in Figure 1. Studies have shown that the RP HPLC method is better for ritonavir (Gandla *et al.*, 2024; Gandhi *et al.*, 2016; Badar *et al.*, 2019; Bhirud *et al.*, 2015) and the combination of ritonavir and nimaprevir (Kale *et al.*, 2024) and the Rp HPLC method is better for the other drugs (Godge *et al.*, 2020).

DOI: 10.5530/ijpi.20250328

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

MATERIALS AND METHODS

Materials

Chemicals and Reagents

The supplier of Ritonavir and Nirmatrelvir (API) were to be Swapnaroop Drugs and Pharmaceuticals, Chhatrapati Sambhajinagar, Among solvents used were HPLC quality. The chemicals and reagents employed were of analytical grade.

Instruments

The HPLC system applied was purchased from Analytical Technologies Ltd., HPLC Workstation Software and UV-DAD detector.

Chromatographic conditions

Column used was C18 (250×4.6 mm id, particle size: Sample temperature: The sample temperature is 22°C Concentration: 10 μM) absorptivity (A): 0.368 (±0.005) Wavelength: 5 μ, 234 nm Acetonitrile: 0.1% OPA (49:51) in water/acetonitrile was used as a mobile phase, with a flow rate of 0.7 mL/min. The Injection volume is 20 μL.

Methods

Preparation of Buffer

0.1% OPA Buffer-Take 1 mL orthophosporic acid in 1000ml volumetric flask and add 1000 mL of HPLC grade water to give 0.1% OPA.

Standard Stock Preparation

Preparation of Nimatravir

Assay: 15 mg nimatravir added in Methanol was added to a 100 mL volumetric flask until the final content was 100 mL. gives a concentration of 1500 μg/mL. Dilute the drug in serial manner to achieve nimaprevir concentration of 15-75 μg/mL (Madhuri *et al.*, 2024).

Preparation of Ritonavir

Add 10 mg of ritonavir into 100 mL volumetric flask, dissolve the content with methanol to 100 mL to give a stock solution containing 1000 μg/mL of the substance. Dilute the drug in serial manner to achieve ritonavir concentration of 10-50 μg/mL (Bhaskaran *et al.*, 2022; Jyothi *et al.*, 2019).

Preparation of mobile phase

The mobile phase was made by combining 410 volumes of acetonitrile with 590 L of 0.1% OPA (41:59), which was then diluted in distilled water to create a 1000 mL volumetric solution in a 1000 mL volumetric flask. The mobile phase was subjected to ultrasonication for 10 min to release dissolved gases and later filtered through 0.45 μ membrane filter using vacuum filtration.

Selection of Analytical Wavelength

To determine the appropriate analytical wavelength for this complex matrix, it is crucial to consider the presence of metal ions. Preparing the suitable diluted standard stock solution of drug with its UV spectrum of 10 μg/mL of ritonavir and nirmatrelvir. For RP-analysis, these diluted solutions were scanned in the range from 200 to 400 nm. Both drugs had significant absorbance at 234 nm. It was opted for as determination wavelength.

Method Development

The development method for ritonavir and nirmatrelvir were tested at various mobile phases including different concentrations of acetonitrile, methanol and buffers; the final ratio was 49:51% v/v acetonitrile and 0.1% OPA, preferring to deliver ritonavir and Nirmatrelvir. Appropriate studies were performed to determine the peak asymmetry, theoretical plates and resolution of the sample as shown in Table 1. Chromatographic separation was performed using the AGILENT (1100) equipped with a 20 μL sample injection loop and UV-visible detector. A mixture of acetonitrile and 0.1% orthophosphoric acid was used. 234 nm

Table 1: System suitability test.

Parameter	Method
Column	id 4.6 x250 mm length
Particle size	5.0 m
Stationary-Phase	C_{18}(AGILENT)
Mobile-Phase	Acetonitrile: 0.1 % OPA 49: 51
Detection of wavelength	234 nm
Flow rate	0.7 mL/min
Room Temperature	33
Size of sample	20 l

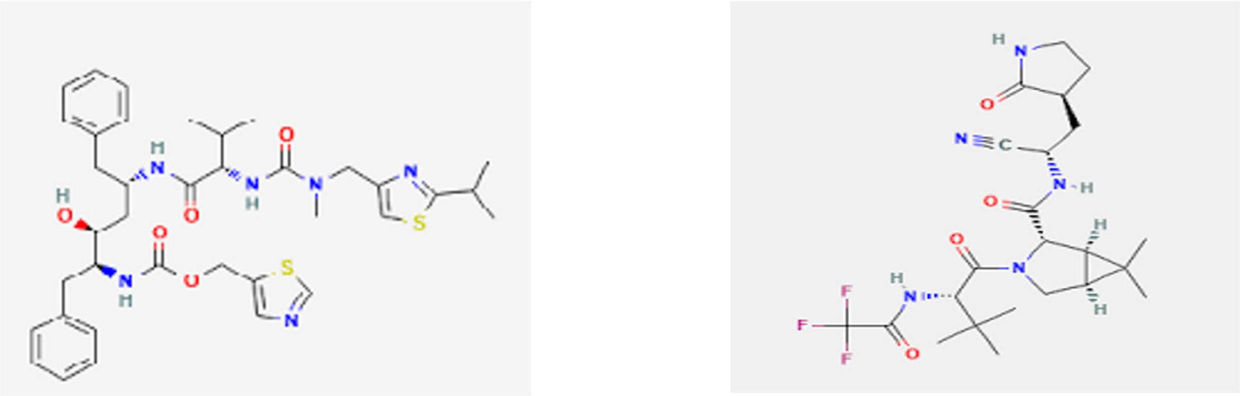

Figure 1: Structure of Ritonavir and Nirmatrelvir.

was the detecting wavelength, and 0.7 mL/min was the flow rate. Pass the cells through a 0.2 μM filter and degas before use. Analysis was conducted at room temperature in a 20 μL volume.

Calibration Curve

Linearity studies of the ritonavir and nirmatrelvir were done by dilution of the stock solution to get the nimaprevir concentration of 15-75 μg/mL and 10-50 μg/mL for ritonavir. The calibration curves are constructed to illustrate the variation of the peak area against the concentration of ritonavir and nimaprevir to demonstrate in Figures 2 and 3, respectively. The regression equations were y=34.858x 59.097 (R^2=0.9992) for ritonavir and y=42.271x 33.657 (R^2=0.9986) for nimaprevir. Diluting the stock solution to obtain a nimaprevir concentration of 15 75 μg/mL and 10 50 μg/mL for ritonavir.

Preparation of sample solution

Weigh 20 tablets, then powder the tablets, accurately transfer 599.99 mg of the powder into a 100 mL volumetric flask, treat the flask with methanol and ultrasonically shake for 15 min. The solution were finally filtered through Millipore syringe filter (0.42 μ). Duplicated portion of the pure solution was then run into the HPLC. It was demonstrated that the preparation is selective and its preparation is relatively unaffected by capsules such as lactose. A linear regression equation was used to quantify the assay. The result analysis revealed that there is a correlation between the labelled claim concentration and the drug content quantified in the study. This relationship confirms that the content of the formulations being studied must be analyzed using the analytical method to show that it is accurate and reproducible. Like quantity, detection, linearity, accuracy, precision and robustness as per the guideline from the ICH. Altogether, these tests proved that the model is appropriate for use and can provide good, accurate, and reliable outcomes across multiple test facilities. Chromatogram of ritonavir and Nirmatrelvir shown in Figure 4.

Method development

The validation of this method includes the evaluation of parameters such as quantity, detection, linearity, accuracy, precision and flexibility as recommended by ICH. Together, these tests confirm that the model is suitable for use and can produce good, accurate and reliable results at multiple test sites.

RESULTS

Linearity and Range

The linearity of the ritonavir was establish at concentration ranges of 10-50 μg/mL and calibration curve is y=34.858x-59.097) with (R^2=0.9992) and for nirmatrelvir establish at concentration ranges of 15-75 μg/mL and calibration curve is y=42.271x-33.657) with (R^2=0.9986).

Precision and Accuracy

Studies on the difference between day and midday were accurate. In a study involving three consecutive injections of a sample drug on the same day. The %RSD value was obtained by testing the difference between days when the sample and the sample solution

Table 2: Intraday and Interday Precision study (Method precision).

Concentration (μg/mL)	Ritonavir		Concentration (μg/mL)	Nirmatrelvir	
	% RSD			% RSD	
	Intraday	Interday		Intraday	Interday
20	0.20	0.11	30	0.103	0.26
30	0.11	0.35	45	0.23	0.09
40	0.33	1.11	60	0.103	1.41

Table 3: Accuracy data for Ritonavir.

Conc (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered (ppm)	% Recovery	% Mean recovery
80%	10	8	18.03	100.1667	100.037
	10	8	17.98	99.88889	
	10	8	18.01	100.0556	
100%	10	10	20.09221	100.4611	100.3124
	10	10	20.06524	100.3262	
	10	10	20.03	100.15	
120%	10	12	22.04621	100.21	100.2873
	10	12	22.08341	100.3791	
	10	12	22.06	100.1667	

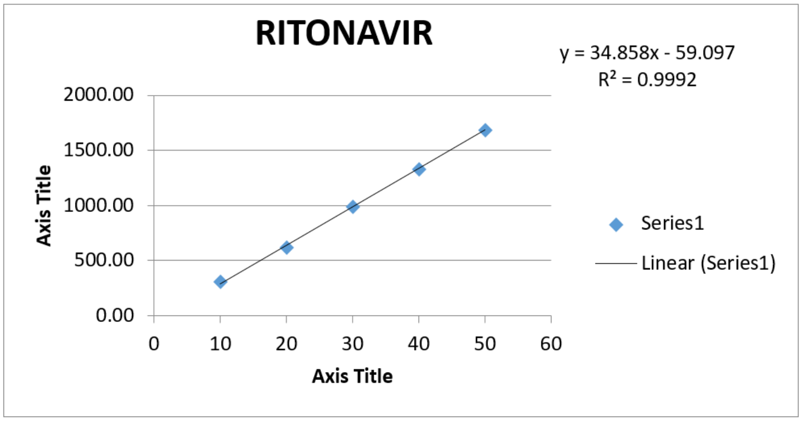

Figure 2: Calibration Curve of Ritonavir.

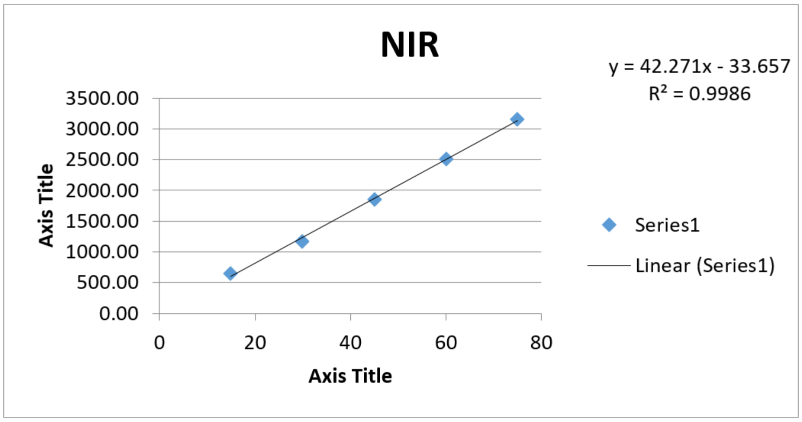

Figure 3: Calibration Curve of Nirmatrelvir.

Table 4: Accuracy data for Nirmatrelvir.

Conc (%)	Sample amount (ppm)	Amount added (ppm)	Amount recovered (ppm)	% Recovery	% Mean recovery
80%	15	12	26.97	99.76	99.49
	15	12	26.93	99.46	
	15	12	26.8	99.25	
100%	15	15	30.0896	100.60	100.51
	15	15	30.13499	100.90	
	15	15	30.01	100.03	
120%	15	18	32.9557	99.75	100.36
	15	18	33.02771	100.15	
	15	18	33.07	100.21	

Table 5: Robustness Study for Ritonavir and Nirmatrelvir.

Sl. No.	Parameter		Ritonavir			Nirmatrelvir		
			Conc. (PPM)	SD	%RSD	Conc. (PPM)	SD	%RSD
1	Flow Rate (ml)	0.6	30	2.81	0.24	45	0.74	0.03
		0.8	30	0.84	0.10	45	072	0.05
2	Wavelength (nm)	233	30	1.70	0.17	45	15.73	0.88
		235	30	2.86	0.29	45	12.84	0.72
3	Mobile Phase Composition (v/v)	40:60	30	3.34	0.34	45	2.64	0.15
		42:58	30	6.75	0.85	45	3.27	0.21

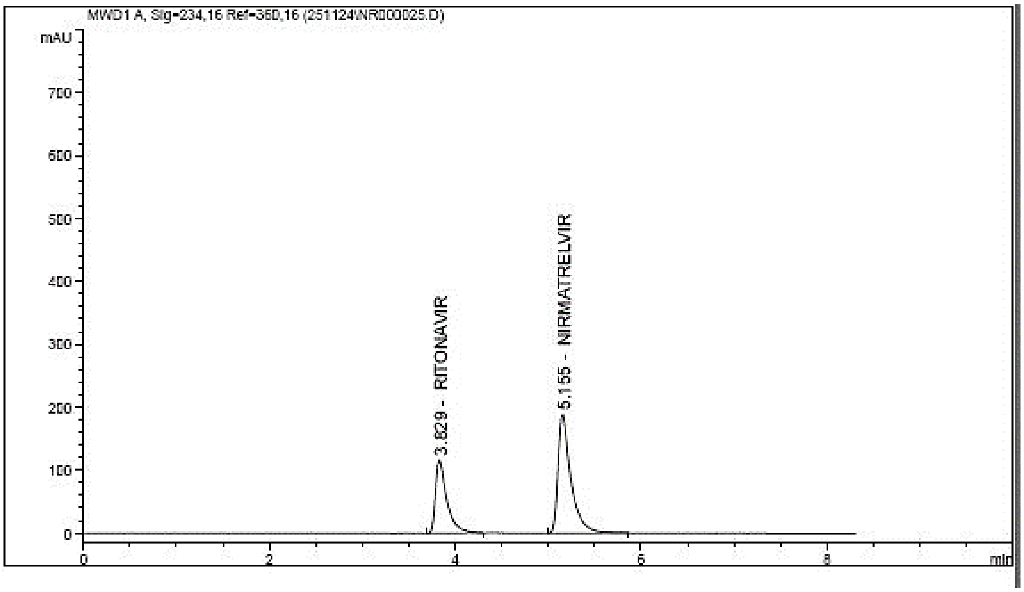

Figure 4: Chromatogram of Ritonavir and Nirmatrelvir.

were injected three times in succession over several days shown in Table 2. The data collected showed that the RPPHLC method produced high accuracy with an RSD percentage of less than 1.5%. The recovery results are within 100±2%, supporting the precision of this approach. Accuracy of the method is evaluated by testing two drugs at three different recovery levels at 80%, 100% and 120% of the recovery percentage are given in Tables 3 and 4 for ritonavir and nirmatrelvir respectively.

Robustness

Robustness assesses the reliability of an analytical method in response to minor alterations in method parameters. It constitutes a component of the method validation procedure. Strength of drug evaluated by making small targeted changes to the test protocol like pH, wavelength, and flow rate are deliberately varied and results are recorded. The results are presented in Table 5.

Limit of Detection and Quantification

In addition, the Limit of Quantification (LOQ) and the Limit of Detection (LOD) were determined. LOD=(3.3×standard deviation/slope of the calibration curve) is the formula used to calculate the LOD. This is the lowest test level that produces visible results. The LOD values for ritonavir and nirmavir were found to be 0.4382 μg/mL and 0.5618 μg/mL, respectively. The lower limit of analytical precision (LOQ) is expressed as: LOQ=(10×standard deviation/slope of the calibration curve). The LOQ values for ritonavir and nirmavir were 1.3278 μg/mL and 1.7025 μg/mL, respectively.

CONCLUSION

The proposed approach was determined to be simple, exact, accurate, and rapid for the analysis of Ritonavir and Nirmatrelvir, individually from pure pharmaceutical formulations. The mobile phase was simpler to prepare, and the run time was under 6 min, and the consumption of less than 6 mL of the mobile phase only proved that the method was inexpensive. The observed sample recoveries in all formulations enhanced well in label claims; this implied non-interference in the estimation. Consequently, the approach can be consistently employed for analysis of Ritonavir and Nirmatrelvir in their combined dosage forms. This degree of simplicity renders the RP-HPLC approach accessible for the quantification of Ritonavir and Nirmatrelvir in tablet formulations and pure pharmaceutical preparations. The mobile phase is easy to produce, and the runtime was under 6 min, utilizing less than 6 mL of mobile phase. The phase indicates that the strategy was cost-effective. The sample recoveries in all formulations were consistent with their individual label claims, indicating no interference in the estimation. Consequently, the method can be readily and conveniently employed for the regular analysis of Ritonavir and Nirmatrelvir in mixed dosage forms. The method's simplicity guarantees its applicability for the measurement of Ritonavir and Nirmatrelvir in tablet dosage forms using RP-HPLC. The aforementioned chromatographic peak resolutions indicate that the procedure was accurate, precise, linear, robust, and rugged.

ACKNOWLEDGEMENT

The authors would also like to thank the Principal and Management for supporting our efforts in developing analytical methods.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RP-HPLC: Reverse Phase High Performance Liquid Chromatography; **UV:** Ultra Visible Spectroscopy; **DAD:** Diode Array Detector; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **HCl:** Hydrochloride; **NaOH:** Sodium Hydroxide; **ICH:** International Council for Harmonization; **OPA:** Orthophosphoric Acid; **Amt:** Amount; **SD:** Standard Deviations; **RSD:** Relative Standard Deviation; **HT:** Hydroxyltryptamine; **PPM:** Parts per million.

SUMMARY

The proposed method there fore passed the test and its linear regression correlation between 10 to 50 μg/mL and 15 to 75 μg/mL of ritonavir and nirmavir respectively. This was done for accurate and precise tests and the percent of RSD value obtained was less than 1.5%. Additional flow rate test was also conducted, where by the system was found to be stable even when the flow rate was changed and wavelength was also varied, also finding of varying the mobile phase composition was also done and system has also been found to be strong at this area and it has been also shown that the system is sensitive only to temperature, pH and flow rate where it can slightly be affected. The two drugs achieved satisfactory linear relationship ($r \geq 0.9996$) with low values of LOD and LOQ. Comparison of developed method with other reported methods for determination of Co-administered drug The created technique were effectively utilized and proven effective in the determination of Co-administered drug. Therefore, the method demonstrates sensitivity. Altogether, these data show that this technique is sensitive and specific.

REFERENCES

Bhaskaran, N., & Shirodkar, R. K. (2022). Development and validation of Rp-HPLC method for quantification of total, free and entrapped ritonavir in lipid nanocarriers and drug content of film coated fixed dose formulation. Indian Journal of Pharmaceutical Education and Research, 56(3S), S547–S558.doi: 10.5530/ijper.56.3s.164.

Ayeen, F. Q., Yasmeen, R., & Badar, H. (2019). Development and validation of Rp-HPLC method for determination of ritonavir and lopinavir. Research Journal of Pharmacy and Technology, 12(7), 3413–3417. https://doi.org/10.5958/0974-360X.2019.00577.8

Gandhi, S. V., & Korhale, R. (2016). A Rp-HPLC method development and validation for the estimation of ritonavir in bulk and pharmaceutical dosage form. Journal of Chemical and Pharmaceutical Research, 8(7), 901–904.

Godge, R. K., Shinde, G. S., & Bhosale, M. S. (2020). RP-HPLC method for estimation of alogliptin and glibenclamide in synthetic mixture. Research Journal of Pharmacy and Technology, 13(2), 555–559. https://doi.org/10.5958/0974-360X.2020.00104.3

Hiremath, S. N., & Bhirud, C. H. (2015). Development and validation of a stability indicating HPLC method for the simultaneous analysis of lopinavir and ritonavir in fixed-dose combination tablets. Journal of Taibah University Medical Sciences, 10(3), 271–277. https://doi.org/10.1016/j.jtumed.2014.11.006

Jyothi, B., & Madhavi, N. (2019). Rp-HPLC method for simultaneous estimation of ritonavir, ombitasvir and paritaprevir in tablet dosage forms and their stress degradation studies. International Journal of Applied Pharmaceutics, 11(2), 193–210.

Kale, S. S., & Satpute, B. S. (2024). Analytical method development and validation of Rp-HPLC for nirmatrelvir and ritonavir in combined tablet dosage form by using simultaneous estimation method. International Journal of Novel Research and Development, 9(6), C753–C774.

Madhuri, S., & Sudha, N. (2024). Statistical optimization of Rp-HPLC for sensitive estimation of nirmatrelvir by doe approach. Afr. J. Biogr. Sci., 6(10), 6760–6770.

Veerareddy, V., & Gandla, K. (2024). Development and validation of a new Rp-HPLC method for the simultaneous estimation of nirmatrelvir, ritonavir and molnupiravir in formulated nanosponges, plasma samples and its pharmacokinetic study. Indian Journal of Pharmaceutical Education and Research, 58(4), 1299–1310. https://doi.org/10.5530/ijper.58.4.142.

Cite this article: Gawali R, Godge RK, Dhamak K, Patel A. Simultaneous Determination of Nirmatrelvir and Ritonavir in Bulk and Tablet Dosage Form by RP-HPLC Method. Int. J. Pharm. Investigation. 2025;15(3):1039-44.