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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF NIRMATRELVIR AND RITONAVIR IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT:
A simple, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablet dosage forms. Chromatographic separation was achieved using an Inertsil ODS-4 column (150 mm × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% orthophosphoric acid buffer and acetonitrile in the ratio 30:70 (v/v), delivered at a flow rate of 1.0 mL/min. Detection was carried out at 254 nm, and the column temperature was maintained at 30°C. The retention times for Nirmatrelvir and Ritonavir were found to be 2.4 minutes and 3.8 minutes, respectively. The method was validated in accordance with ICH Q2(R1) guidelines for parameters such as system suitability, linearity, precision, accuracy, sensitivity, robustness, and assay. The %RSD for repeatability and intermediate precision was less than 2%, confirming the method's precision. The method showed excellent linearity with correlation coefficients (r^2) of 0.999 for both analytes. The recovery values ranged from 98.11% to 101.5%, confirming accuracy. LOD and LOQ were found to be 0.24 µg/mL and 0.72 µg/mL for Nirmatrelvir, and 0.14 µg/mL and 0.41 µg/mL for Ritonavir, respectively. The validated method was successfully applied to the assay of marketed tablet formulations, confirming its suitability for routine quality control analysis.

INTRODUCTION

Pharmaceutical analysis plays a vital role in the development, quality control, and regulatory approval of drug formulations. It ensures that pharmaceutical products meet the required standards of quality, safety, and efficacy throughout their lifecycle. Among the various analytical techniques available, high-performance liquid chromatography (HPLC) has emerged as a powerful and versatile tool in drug analysis due to its high resolution, sensitivity, and reproducibility.

Reverse-phase HPLC (RP-HPLC) is particularly preferred for the separation and quantification of polar and semi-polar compounds. Method development in RP-HPLC involves the careful selection of stationary and mobile phases, detection wavelength, flow rate, and temperature to achieve efficient separation of analytes. Moreover, method validation is essential to confirm that the developed method is suitable for its intended purpose.

Nirmatrelvir is a novel antiviral drug that acts as a selective inhibitor of the SARS-CoV-2 main protease (Mpro/3CLpro), which is essential for viral replication. Ritonavir, originally developed as an antiretroviral for HIV treatment, is co-administered with Nirmatrelvir to boost its plasma levels by inhibiting CYP3A-mediated metabolism. The combination, marketed as Paxlovid, has become a widely prescribed oral therapy for mild to moderate COVID-19 in high-risk patients.

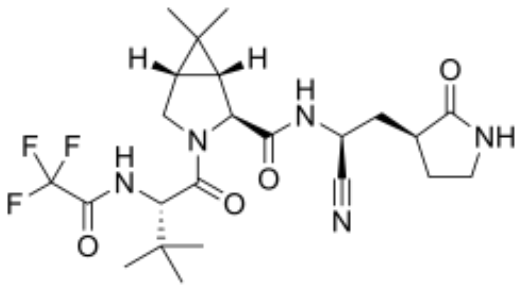

Fig 1. Chemical Structure of Nirmatrelvir

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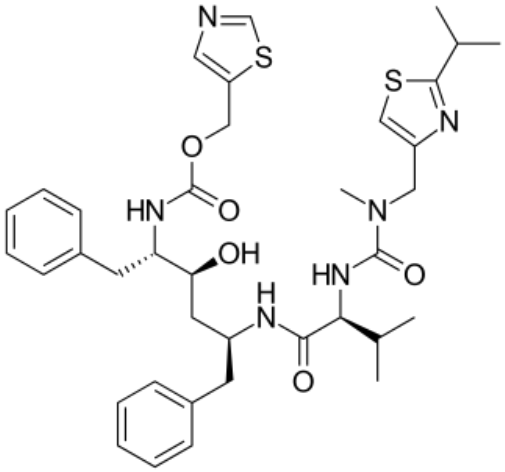

Fig 2. Chemical structure of Ritonavir

2. Materials and Methods

Chemicals and Reagents:

Nirmatrelvir and Ritonavir working standards, combination tablets (PAXZEN), HPLC-grade methanol, acetonitrile, and 0.1% orthophosphoric acid buffer were used.

Instrumentation:

A Waters HPLC 2965 system equipped with a photodiode array (PDA) detector and Empower 2 software was used for chromatographic analysis.

Standard Preparation:

Accurately Weighed and transferred 15mg of Nirmatrelvir and 10mg of Ritonavir working Standards into 100ml clean dry volumetric flasks, add 3/4 ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (Final concentrations 15 µg/ml of Nirmatrelvir and 10 µg/ml of Ritonavir).

Sample Preparation:

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 500 mL volumetric flask, diluent added up to 500ml and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.1 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

3. Results and Discussion

Optimized Chromatographic Conditions:

For the HPLC method development, an Inertsil ODS-4 column (150 mm × 4.6 mm, 5 µm) was employed to achieve efficient separation. The mobile phase consisted of a mixture of 0.1% orthophosphoric acid (OPA) buffer and acetonitrile in a 30:70 (v/v) ratio, providing optimal resolution and peak shape. The flow rate was maintained at 1.0 mL/min to ensure consistent elution. Detection was carried out at a wavelength of 254 nm using a UV detector, allowing for precise quantification of the analyte. A 10 µL sample volume was injected for each run, and the column temperature was set at 30°C to enhance reproducibility and chromatographic performance.

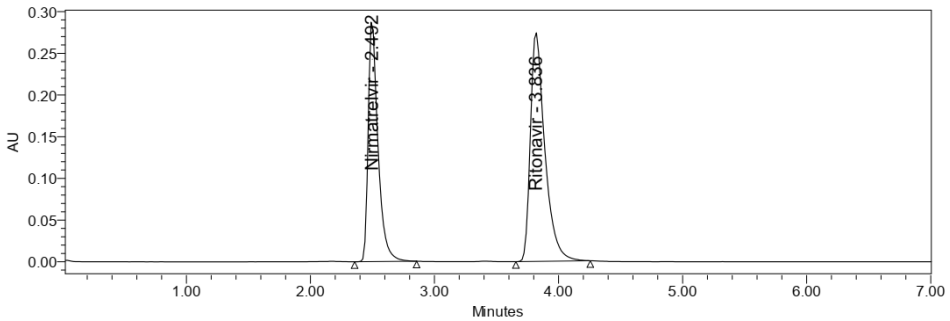

Fig 3 Optimized chromatogram of Nirmatrelvir and Ritonavir

System Suitability

The system suitability parameters complied with ICH criteria. Nirmatrelvir and Ritonavir were well resolved with retention times of 2.4 min and 3.8 min, respectively. Theoretical plate counts were 7548 and 6652, and the tailing factors were 1.2 and 1.4, indicating good column efficiency and peak symmetry.

Linearity

Linearity was evaluated in the range of 3.75–22.5 µg/mL for Nirmatrelvir and 2.5–15 µg/mL for Ritonavir. The regression equations were:

- Nirmatrelvir: y = 73370x + 6411
- Ritonavir: y = 97714x - 12296

Correlation coefficients for both drugs were 0.999, confirming excellent linearity.

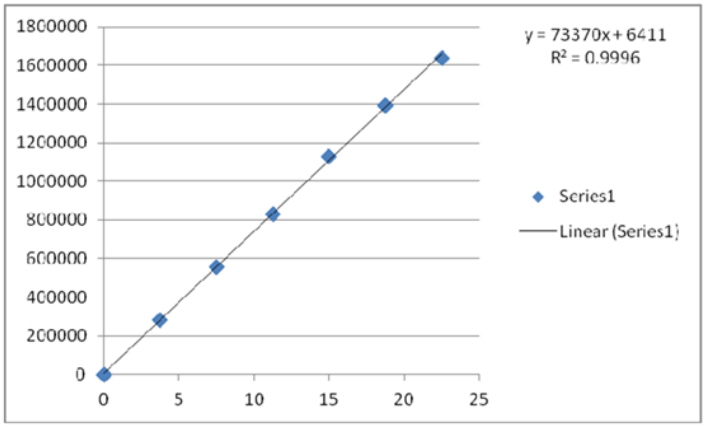

Fig: 4 Calibration curve of Nirmatrelvir

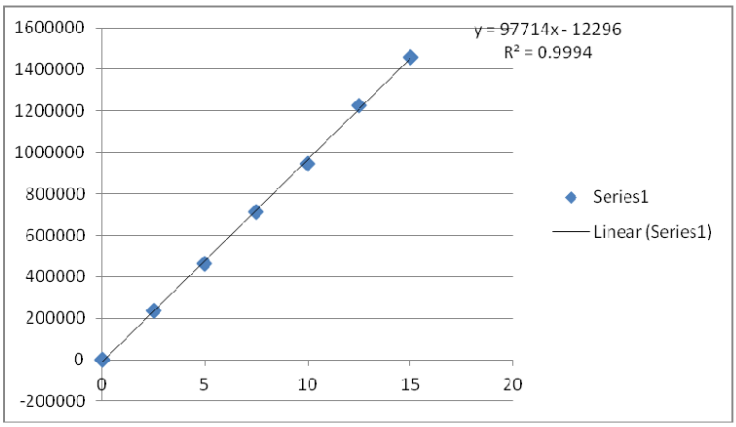

Fig: 5 Calibration curve of Ritonavir

Precision

Precision was assessed through intra-day and inter-day studies. The %RSD for Nirmatrelvir and Ritonavir were 0.84% and 0.88% (intra-day), and 0.82% and 0.99% (inter-day), respectively. These low RSD values indicate good repeatability and reproducibility.

Accuracy

Accuracy was determined using recovery studies at 50%, 100%, and 150% of target concentrations. Recoveries ranged from:

- Nirmatrelvir: 99.07% to 101.07% (average 101.5%)
- **Ritonavir: 97.33% to 98.60% (average 98.11%)**

This demonstrates the method's accuracy and minimal interference from excipients.

Table: 1. Accuracy results of Nirmatrelvir and Ritonavir

Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	Average
Nirmatrelvir	7.5	7.58	101.07	101.5
	15	14.86	99.07	
	22.5	22.57	100.31	
Ritonavir	5	4.92	98.40	98.11
	10	9.86	98.60	
	15	14.6	97.33	

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined based on the standard deviation of the response and slope:

- Nirmatrelvir: LOD = 0.24 µg/mL, LOQ = 0.72 µg/mL

- Ritonavir: LOD = 0.14 µg/mL, LOQ = 0.41 µg/mL

These values reflect the method's high sensitivity.

Robustness

Robustness was tested by introducing minor deliberate changes to the flow rate, mobile phase composition, and temperature. The %RSD values remained below 2% for both analytes, indicating that the method is robust and unaffected by small variations in method parameters.

Assay of Formulation

The validated method was applied to the assay of Nirmatrelvir and Ritonavir tablets. The average assay results were found to be 99.41% for Nirmatrelvir and 100.04% for Ritonavir, which falls within the acceptable limits, confirming the method's applicability for routine analysis.

4. Conclusion

A robust, simple, and reliable RP-HPLC method has been successfully developed and validated for the simultaneous estimation of Nirmatrelvir and Ritonavir in pharmaceutical dosage forms. The method demonstrated excellent accuracy, precision, linearity, and sensitivity. It meets all ICH Q2(R1) guidelines and is suitable for use in routine quality control of fixed-dose combination tablets of Nirmatrelvir and Ritonavir.

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