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Original Article

# QUANTITATIVE ESTIMATION OF NIRMATRELVIR AND RITONAVIR MALEATE IN TABLET DOSAGE FORMS BY RP-HPLC METHOD

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***ABSTRACT***

*A Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and optimized for the simultaneous estimation of Nirmatrelvir and Ritonavir in tablet dosage forms. The method was carefully developed by studying various chromatographic parameters to ensure optimal separation and accurate quantification of both drugs. The maximum absorbance for both drugs was observed at 220 nm, with excellent peak purity. A Sunfire C18 column (4.6×250 mm, 5 µm) was used to achieve good peak resolution. The flow rate was fixed at 0.9 mL/min, and the mobile phase was optimized as a mixture of Water and Acetonitrile (60:40% v/v), providing symmetrical peaks and satisfactory separation. The injection volume was set to 10 µL, which resulted in good peak area and resolution, while the temperature was maintained at 35 ºC for optimal drug stability. The method showed excellent linearity in the concentration range of 5-25 µg/mL for Nirmatrelvir and 10-50 µg/mL for Ritonavir. The method demonstrated high precision, with recovery values ranging from 98.0-102% and both system and method precision found to be accurate within acceptable limits. The total run time for the analysis was 6 minutes, with Nirmatrelvir and Ritonavir eluting 3.0 and 3.8 ± 0.02 minutes, respectively. The method passed robustness and ruggedness tests, with relative standard deviations remaining well within satisfactory limits. This RP-HPLC method provides a reliable, accurate, and efficient approach for the quantitative analysis of Nirmatrelvir and Ritonavir in tablet dosage forms, suitable for routine quality control and regulatory compliance.*

***Keywords:*** *Nirmatrelvir and Ritonavir, RP-HPLC, Validation, Accuracy.*

## INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them[1-7]. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure [8-14].

Nirmatrelvir is administered alongside ritonavir, a potent inhibitor of CYP3A enzymes, to inhibit its metabolism and increase plasma nirmatrelvir concentrations. While therapeutically beneficial, the use of ritonavir poses a significant risk of drug interaction due to its potent inhibition profile - patients and clinicians should consult the prescribing information for Paxlovid (nirmatrelvir and ritonavir) to evaluate any potential for drug interaction with existing medications prior to the initiation of Paxlovid [15-21].

Ritonavir belongs to the class of organic compounds known as n-carbamoyl-alpha amino acids and derivatives. These are compounds containing an alpha amino acid (or a derivative thereof) which bears a carbamoyl group at its terminal nitrogen atom. It is used alone or in combination with other medicines for the treatment of the infection caused by the human immunodeficiency virus (HIV). HIV is the virus that causes acquired immune deficiency syndrome (AIDS). The aim of this study is to develop and validate a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Nirmatrelvir and Ritonavir Maleate in tablet dosage forms [21-26].

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Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life[27]. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body[28-32]. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy[33-38]. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. The method aims to provide a reliable, accurate, and efficient approach for routine analysis and quality control of these drugs in pharmaceutical formulations.

**Materials**

Nirmatrevir and Ritonavir received as Kind Gift sample from Hetero Drugs company, Hyderabad. Triethanolamine purchased from Merck Company.All the Organic solvents used are HPLC grade.

**Methodology**

**HPLC METHOD DEVELOPMENT:**

**OPTIMIZED CHROMATOGRAPHIC CONDITIONS:**

Instrument used: Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature: 35ºC

Column: Sunfire C18 (4.6×250mm) 5µ

Mobile phase: Acetonitrile: Water (40:60v/v)

Flow rate: 0.9 ml/min

Wavelength: 220 nm

Injection volume: 10 µl

Run time: 6min

**Analytical method development**

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) were mixed and degassed in digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:** The Mobile phase was used as the diluent.

**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 pipette0.15ml of the Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flaskand dilute up to the mark with Methanol.

**Optimization of Column:** The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**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 solvent39. Further pipette 0.15ml of the Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

**SPECIFICITY**

Preparation of Standard Solution:Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard intoa 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve itcompletely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Nirmatrelvir and 0.3ml of the Ritonavir stock solutions into a 10mlvolumetric flask and dilute up to the mark with Diluent[40].

**LINEARITY**

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard intoa 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve itcompletely and make volume up to the mark with the same solvent[41].

**PRECISION**

Accurately weigh and transfer 10 mg of Nirmatrelvir and 10mg of Ritonavir working standard intoa 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.

**ROBUSTNESS**

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

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mark with the same solvent. (Stock solution). Further pipette 0.15ml of the Nirmatrelvir and 0.45ml of the Ritonavir stock solutions into a 10mlvolumetric flask and dilute up to the mark with Diluent[42].

## Results & Discussion

**Table 1: Chromatographic Data for Linearity Study for Nirmatrelvir:**

| % concentration level | Concentration (ug/ml) | Peak area |
|---|---|---|
| 33.3 | 5 | 230247 |
| 66.6 | 10 | 462332 |
| 100 | 15 | 659905 |
| 133.3 | 20 | 892989 |
| 166.6 | 25 | 1101075 |

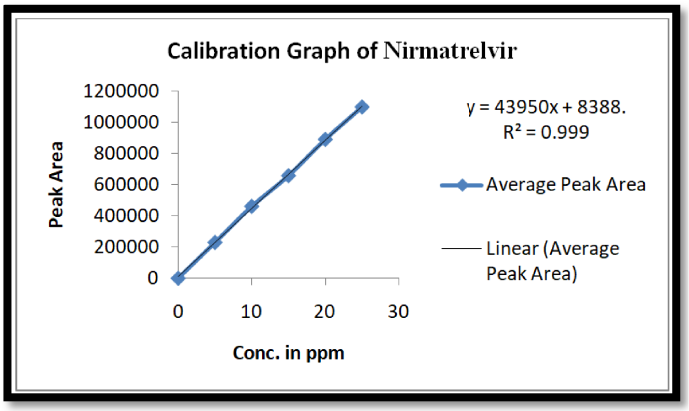



**Figure 1: Nirmatrelvir calibration graph**

**Table 2: Chromatographic Data for Linearity Study for Ritonavir**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 33.3 | 10 | 1215225 |
| 66.6 | 20 | 2135937 |
| 100 | 30 | 3020839 |
| 133.3 | 40 | 4078841 |
| 166.6 | 50 | 5058145 |

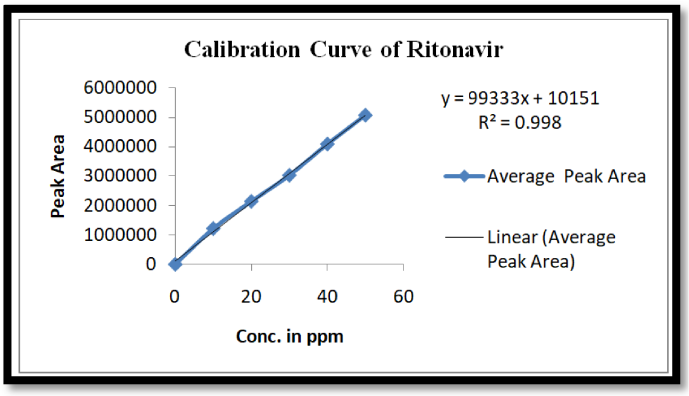



**Figure 2: Ritonavir calibration curve**

**Table 3: The accuracy results for Nirmatrelvir**

| % Conc. (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | % Mean Recovery |
|---|---|---|---|---|---|
| 50% | 331938 | 7.5 | 7.3 | 99.88 | 100.166 |
| 100% | 658274 | 15 | 14.7 | 98.89 | |
| 150% | 970963 | 22.5 | 22.2 | 101 | |

**Table 4: The accuracy results for Ritonavir**

| % Conc. (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | % Mean Recovery |
|---|---|---|---|---|---|
| 50% | 209357 | 7.5 | 7.3 | 99.7 | 99 |
| 100% | 420697.7 | 15 | 14.9 | 99 | |
| 150% | 420697.7 | 22.5 | 22.2 | 99 | |

**REPEATABILITY**

**Table 5: Results of repeatability of Nirmatrelvir**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Nirmatrelvir | 3.003 | 654426 | 61521 | 8474 | 1.1 |
| 2 | Nirmatrelvir | 3.005 | 659862 | 61937 | 8262 | 1.2 |
| 3 | Nirmatrelvir | 3.007 | 650837 | 62018 | 8117 | 1.1 |
| 4 | Nirmatrelvir | 3.008 | 651433 | 61893 | 7917 | 1.2 |
| 5 | Nirmatrelvir | 3.005 | 652752 | 61867 | 8011 | 1.1 |
| Mean | | | 653862 | | | |
| Std.dev | | | 3626.323 | | | |
| %RSD | | | 0.554601 | | | |

**Table 6: Results of repeatability of Ritonavir**

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Ritonavir | 3.857 | 3028176 | 381011 | 9583 | 1.1 |
| 2 | Ritonavir | 3.859 | 3018373 | 381645 | 8927 | 1.2 |
| 3 | Ritonavir | 3.857 | 3018462 | 381663 | 8465 | 1.1 |
| 4 | Ritonavir | 3.861 | 3081711 | 381746 | 9222 | 1.2 |
| 5 | Ritonavir | 3.853 | 3075143 | 381193 | 8462 | 1.1 |
| Mean | | | 3044373 | | | |
| Std.dev | | | 31427.07 | | | |
| %RSD | | | 1.0323 | | | |

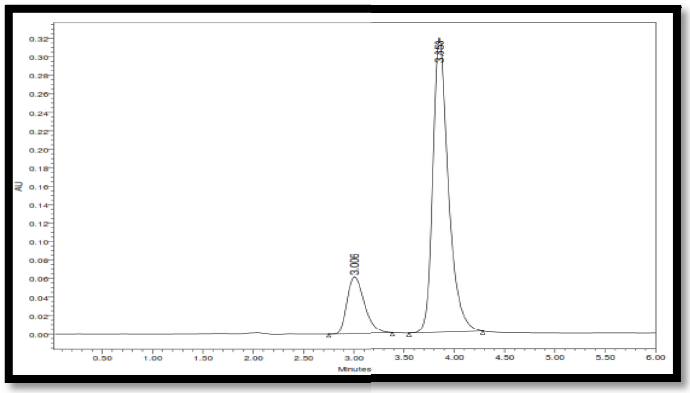



**Figure 3: Optimized Chromatogram (Standard)**

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**Table7: Optimized Chromatogram (Standard)**

| S.no | Name | RT | Area | height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1. | Nirmatrelir | 3.006 | 731322 | 61677 | 1.2 | 8574 |
| 2. | Ritonavir | 3.853 | 3421257 | 319786 | 1.1 | 9664 |

**Table 8: Results for Robustness -Nirmatrelvir**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 0.9mL/min | 658211 | 3.006 | 8793 | 1.2 |
| Less Flow rate of 0.8mL/min | 621077 | 3.441 | 7269 | 1.3 |
| More Flow rate of 1.0mL/min | 642190 | 2.663 | 9446 | 1.2 |
| Less organic phase | 542402 | 3.185 | 8126 | 1.1 |
| More organic phase | 642112 | 2.867 | 5854 | 1.3 |

**Table 9: Results for Robustness -Ritonavir**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 0.9mL/min | 429069 | 3.853 | 5224 | 1.59 |
| Less Flow rate of 0.8mL/min | 472673 | 4.426 | 6328 | 1.58 |
| More Flow rate of 1.0mL/min | 392497 | 3.415 | 6217 | 1.54 |
| Less organic phase | 391379 | 4.291 | 6996 | 1.61 |
| More organic phase | 391703 | 3.583 | 6120 | 1.50 |

**Discussion:** A 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. The method was optimized by evaluating several chromatographic parameters to ensure optimal separation and accurate quantification. The maximum absorbance for both drugs was observed at 220 nm, and a Sunfire C18 column (4.6×250 mm, 5 µm) was used for efficient peak resolution. The mobile phase consisted of a mixture of Water and Acetonitrile (60:40% v/v), and the flow rate was fixed at 0.9 mL/min. The injection volume was 10 µL, and the temperature was maintained at 35 ºC for improved drug stability. The analysis was completed in 6 minutes, with Nirmatrelvir and Ritonavir eluting at 3.0 and 3.8 ± 0.02 minutes, respectively. The method demonstrated excellent linearity in the concentration range of 5-25 µg/mL for Nirmatrelvir and 10-50 µg/mL for Ritonavir, with recovery values between 98.0-102%, indicating high accuracy and precision. Additionally, the method passed robustness and ruggedness tests, ensuring its reliability for routine quality control applications.

**Conclusion:**

The developed RP-HPLC method for the simultaneous estimation of Nirmatrelvir and Ritonavir is reliable, accurate, and efficient. The method is characterized by excellent linearity, precision, and recovery, making it suitable for routine quantitative analysis of these drugs in tablet dosage forms. The optimized chromatographic conditions, including the choice of mobile phase, flow rate,and column, resulted in good separation and peak symmetry. With a short analysis time of 6min, this method is not only time-efficient but also meets the stringent requirements for regulatory compliance and quality control in pharmaceutical laboratories. The method's robustnessand ruggedness further confirm its applicability for consistent and reproducible analysis in routine quality assessments.

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