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DEVELOPMENT AND VALIDATION OF A NOVEL RP-HPLC METHOD FOR THE QUANTITATIVE ANALYSIS OF MOLNUPIRAVIR IN BULK AND TABLET DOSAGE FORMS

[1]K. Harika,[2] B. Priyanka, [3]S. Gayathri,[4]P. Manisha, [5]K. Harshini and Srinivas Chinta*

[1,2,3,4,] *Department of Pharmaceutical Analysis, Vaagdevi pharmacy college, Bollikunta, Warangal, TG-506005

*Corresponding Author Email: cchinthasrinivas@gmail.com

ABSTRACT

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the rapid assay of Molnupiravir in tablet dosage forms. The method employed isocratic elution at a flow rate of 1.0mL/min using a Symmetry C18 column (250 × 4.6 mm, 5 µm particle size) at ambient temperature. The mobile phase is composed of ethanol and Phosphate Buffer pH-4 adjusted with Orthophosphoric acid solution in the ratio of 25:75 (v/v), in an isocratic mode. Detection was carried out at a UV wavelength of 235 nm, with a sample injection volume of 20 µL. The retention time for Molnupiravir was recorded at 4.63 minutes. The precision and accuracy of the method were found to be robust, with a percentage relative standard deviation (RSD) of less than 2%. Validation was performed in accordance with ICH guidelines, confirming the method's reliability. This RP-HPLC method is suitable for routine analysis of Molnupiravir in tablet dosage forms, affirming its effectiveness in quality control applications.

Key words:
Molnupiravir, RP-HPLC, Isocratic elution, ICH guidelines, validated method.

INTRODUCTION

A prodrug of the synthetic nucleoside derivative N4-hydroxyl cytidine, molnupiravir [1, 2] works against many viruses by introducing copying during the production of their RNA. It is an antiviral medication that inhibits the Replication of certain RNA viruses. It is used to treat COVID-19 in those infected by SARS-CoV-2 [3, 4]. Molnupiravir is [(2R, 3S, 4R, 5R) chemically]3-(4Z)-4-(hydroxyimino)-3,4-dihydroxy-5-[2,3,4,6-tetrahydropyrimidin-1-yl-2-oxo-1oxolan-2-yl2-methylpropanoate methyl (Figure 1). Molnupiravir has a molecular weight of 329.309 gm/mole and the chemical formula $C_{13}H_{19}N_3O_7$. It is a white, crystalline solid that dissolves easily in methanol, ethanol, and DMSO as well as water [5-7]. Hetero is the manufacturer of MOVFOR 200mg, a brand name under which molnupiravir is sold. A review of the literature [8, 9] on Molnupiravir provides the details fits chemical and physical characteristics as well as a range of analytical techniques used both independently and in conjunction with other Molnupiravir. A literature review [9–11] reveals that specific chromatographic techniques are published for the determination of Molnupiravir, and that RP-HPLC provides a single method for such estimation [12, 13]. Efforts were made to create a straightforward, and accurate, analytical approach for the simultaneous estimate of Molnupiravir and to expand it for their determination in the formulation in light of the necessity for an appropriate RP-HPLC method for the routine analysis of Molnupiravir in formulations.

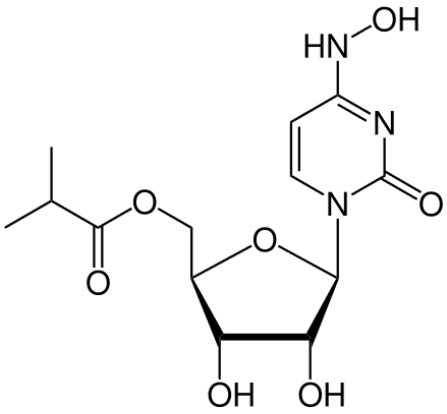

Fig.1. Chemical Structure of Molnupiravir

2. MATERIAL AND METHODS

2.1. Instrumentation and analytical condition

A single pump, PDA Detector, and Lab solution software are all included along with the HPLC. A 1cm quartz cell and a shimadzu series separation module are used in this twin beam UV-visible spectrophotometer. The Symmetry ODS C18 (250×4.6mm, 5µm) column is used to make the separation [13].

2.2. Chemicals and Reagents

In this method AR grade solvents are utilized.

2.3. Chromatographic conditions

2.3.1. The Mobile phase consists of Ethanol

Phosphate Buffer pH-4 was adjusted in an isocratic method using a 25:75% v/v solution of orthophosphoric acid. From the solvent reservoir to the column, the mobile phase was adjusted to flow at a rate of 1.0 ml/ min. And the injection volume was 20µl injection volume at 235 nm, the eluents were found [13]. Before usage, the mobile phase is freshly prepared and sonicated for about 5min, to remove any remaining gases.

2.4. Preparation of Potassium Dihydrogen Phosphate Buffer PH 4

Dissolve 6.8 grams of potassium dihydrogen phosphate in 1000ml of HPLC grade water and adjust the pH at 4 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultrasonication [13].

2.5. Preparation of Mobile Phase

A precise mixture of 350 ml of ethanol and 650 ml of phosphate buffer was prepared, and after 15 minutes of degassing in a digital ultra sonicator, the mixture was filtered through a 0.45 µ filter using vacuum filtration [12].

2.6. Preparation of standard solution

Weigh precisely, and then transfer 10 mg of the working standard of Molnupiravir into a 10 ml clean, and dry volumetric flask. Next, add around 7 ml of ethanol, sonicate to dissolve the air completely, and then add more amount of ethanol to get the volume up to the desired level. The abovementioned Molnupiravir stock solutions should be pipetted again, this time 0.6 ml, into a 10 ml volumetric flask and diluted with diluent to the appropriate level.

2.7. Preparation of sample solution

Weigh 10 tablets and powder them to determine the average weight of the tablets. Pipette 0.6 milliliters of Molnupiravir above stock solution into a 10-milliliter volumetric flask and dilute with diluent. Weigh 10 mg equivalent weight of Molnupiravir sample into a 10-milliliter clean dry volumetric flask. Add approximately 7 milliliters of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [6, 14].

3. RESULTS AND DISCUSSION

Molnupiravir was dissolved in diluent at a concentration of 10µg/ml. The drug shows maximum absorption at wavelength of 235nm, which led to the selection of this wavelength as the detection range. After considering all system suitability parameters, Methanol: Phosphate Buffer pH-4.2 adjusted with Orthophosphoric acid solution at a ratio of 25:75% v/v in an isocratic mode was used. The injection volume and mobile-phase flow rates were 10 µL and 1 ml/min, respectively. The retention period of Molnupiravir was determined to be 4.63 mins (Figure 2).

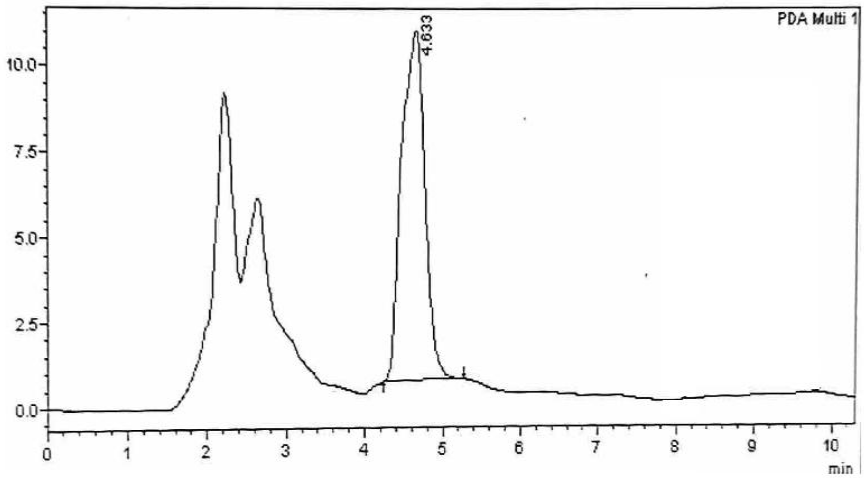

Fig.2. Chromatogram showing the retention time of Molnupiravir

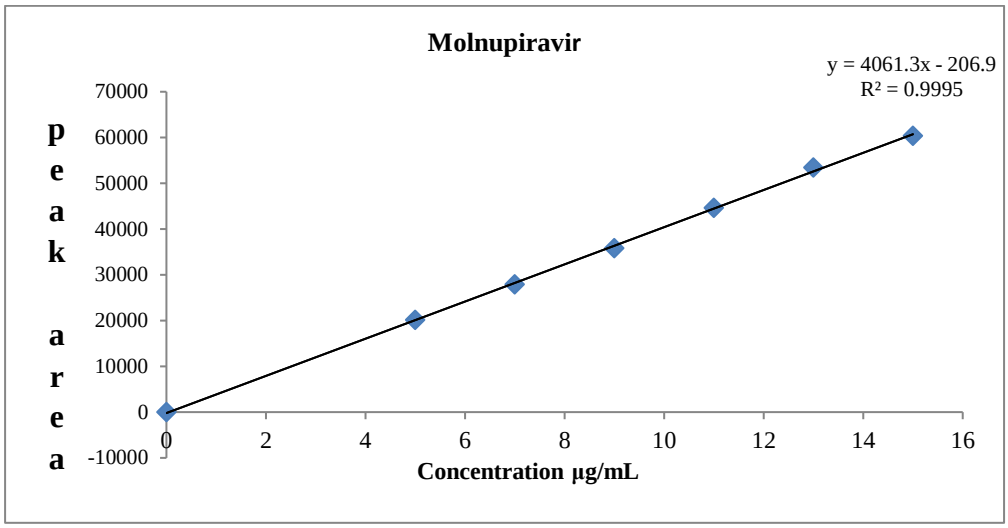

Fig. 3. Calibration curve of Molnupiravir

3.1. Method validation

3.1.1. Linearity

The calibration was done by using the external calibration method, with the optimum chromatographic conditions (Figure 3). Standard stock solutions of Molnupiravir were prepared by using ethanol and various concentrations have been prepared in the range of 5-15µg/ml of Molnupiravir in the diluent. 10µl of each solution was injected individually and the corresponding chromatogram was recorded at 235nm. The calibration curve has been plotted using concentration against peak area. This procedure has been repeated three times. The value was found to be 0.999 indicating that the concentration of Molnupiravir has good linearity. The calibration graph was shown in Table 1

Table1. Linearity of Molnupiravir.

S. No	Concentration, µg/mL	Peak area
1	5	20156
2	7	27887
3	9	35792
4	11	44639
5	13	53409
6	15	60345

Six-point graph was constructed covering a concentration range 5.0–15.0 µg/mL (three independent determinations were performed at each concentration.) Linear relationships between the peak area signal of Molnupiravir and the corresponding drug concentration were observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 2.

Table 2: Regression analysis of the calibration curve.

Parameters	Values
Calibration range (µg/mL)	5-15
Slope	4061
Intercept	206.9
Correlation coefficient (r^2)	0.999

Table 3: System suitability and validation parameters

Parameters	Result
Theoretical plates (N)	5578.5
Retention time (min)	4.633
Asymmetric factor	1.25
LOD (ng/mL)	20
LOQ (ng/mL)	39
Accuracy (%)	99.57
R.S.D. (%)	1.5

3.1.2. Precision

This validated method was used for the assay of commercial tablets containing Molnupiravir. Sample was analyzed five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation indicate very good repeatability of the measurement. Thus, it is shown that the equipment used for the study was correct, and hence the developed analytical method is highly repetitive. For the intermediate precision, a study carried out by the same analyst working on the same day, on three consecutive days indicated a RSD of 1.5. This indicates good method precision.

3.1.3. Stability

The stability of Molnupiravir in standard and sample solutions containing different solutions was determined by storing the solutions at an ambient temperature (25 ± 10◦C). The solutions were checked in triplicate after three successive days of storage, and the data were compared with freshly prepared samples. In each case, it is noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 99%. This denotes that Molnupiravir is stable and standard for at least 2 days at ambient temperature. Results are furnished in Table 3.

3.1.4. System Suitability

A feasibility study was also conducted on the new monopiravir preparation solution, and the results were calculated and recorded by determining the standard deviation of the monopiravir standard by injecting five samples repeated at six-minute intervals. System compliance parameters such as capacity, asymmetry factor, queuing factor and theoretical number of plates are calculated. All values were found to be within the limits (Table 3). Evaluation of this model demonstrated its good linearity, repeatability and applicability to variables and concluded that this method can be used for the rapid and reliable determination of monopyravir in tablet formulations. The results are depicted in Table 3.

Table 4: Assay results of tablet formulation

Formulation	Labelled claim	% Molnupiravir of tablet
MOVFOR 200mg	200mg	99.57%

4. CONCLUSION

An RPHPLC method was developed and validated for the determination of monopiravir in tablet dosage forms. This method is simple, fast, accurate, clear and very sensitive. Chromatographic run time is 10 minutes, allowing large samples to be analyzed in a short time. Therefore, daily analysis of monopyravir in quality pharmaceutical is analysis. (Figure 3, Table 4).

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Corresponding author *Email address:
cchinthasrinivas@gmail.com