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Original Article

New analytical method development and validation for estimation of molnupiravir in bulk and tablet dosage form by RP-HPLC method

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ABSTRACT

A new simple, selective, rapid, precise reversed-phase high-performance liquid chromatography method has been developed and validated for the estimation of Molnupiravir in bulk and its pharmaceutical dosage form. The separation was made using Symmetry ODS C18 (4.6×150mm, 5µm) column. The mobile phase used contained Methanol. Phosphate Buffer pH-4.2 adjusted with Orthophosphoric acid solution in the ratio of 35:65% v/v in an isocratic mode at a wavelength of 236nm. The mobile-phase flow rate and the sample volume injected were 1 ml/min and 10 µL, respectively. The retention time of Molnupiravir was found to be 2.8 ±0.2mins. A good linear relationship of Molnupiravir r =0.999) was observed over a concentration range of 20 to 100µg/ml of Molnupiravir. The limit of detection (LOD) and limit of quantification (LOQ) for Molnupiravir was found to be 2.6µg/ml and 6.35µg/ml. The recovery percentage was observed in the range of 98-102%. The relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for the estimation of Molnupiravir in bulk and marketed pharmaceutical dosage form. It was concluded that in the present developed RP- HPLC method is simple, rapid, and accurate, hence can be used for routine quality control analysis in the Pharmaceutical industry.

1. Introduction

Molnupiravir [1, 2] is a prodrug of the synthetic nucleoside derivative N4-hydroxyl cytidine and exerts its antiviral action through the introduction of copying errors during viral RNA replication. 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 chemically [(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-4-(hydroxyimino)-2-oxo-1,2,3,4-tetrahydropyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate (Figure 1). The molecular formula of Molnupiravir $C_{13}H_{19}N_3O_7$ and has a molecular weight of 329.309 gm/mole. It is a white crystalline solid soluble in water, freely soluble in methanol, ethanol and DMSO [5-7].

Molnupiravir is available in the market with the brand name of MOVFOR 200mg manufactured by Hetero. Review of the literature [8, 9] on Molnupiravir gave information regarding its physical and chemical properties and various analytical methods that were conducted alone and in combination with other Molnupiravir. Literature survey [9-11] reveals that certain chromatographic methods were reported for the estimation of Molnupiravir and single

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method is available for such estimation by RP-HPLC[12, 13]. In view of the need for a suitable RP-HPLC method for routine analysis of Molnupiravir in formulations, attempts were made to develop a simple, precise and accurate analytical method for simultaneous estimation of Molnupiravir and extended it for their determination in the formulation.

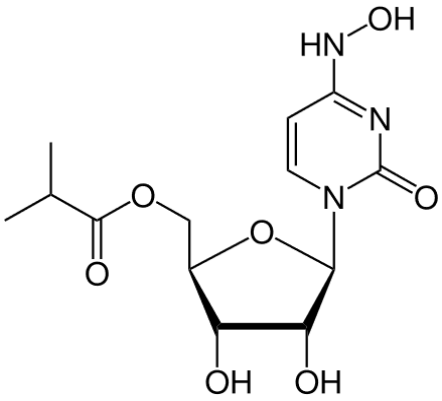

Fig.1. Chemical Structure of Molnupiravir

2. Material and methods

2.1. Instrumentation

HPLC was equipped with Quaternary pump, Autosampler, PDA Detector and Empower-2 software. A double beam UV-Visible spectrophotometer WATERS Alliance 2695 series separation module and 1cm quartz cell. The separation was made using Symmetry ODS C_{18} (4.6×150mm, 5µm) column [13]

2.2. Chemicals and Reagents

The solvents used were of HPLC/AR grade.

2.3. Chromatographic conditions

2.3.1. The Mobile phase consisted of Methanol

Phosphate Buffer pH-4.2 adjusted with Orthophosphoric acid solution in the ratio of 35:65% v/v in an isocratic mode. The mobile phase was pumped with 1.0 ml/min flow rate from the solvent reservoir to the column. The injection volume was 10µl. The eluents were detected at 236nm [13].

2.4. Preparation of Potassium Dihydrogen Phosphate Buffer PH – 4.2

Dissolve 6.8043 grams of potassium dihydrogen phosphate in 1000ml HPLC grade water and adjust the pH4.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra-sonication [13, 14].

2.5. Preparation of Mobile Phase

Accurately measured 350ml of methanol, 650ml of phosphate buffer were mixed and degassed in a digital ultra sonicator for 15 minutes and then filter through 0.45 µ filter under vacuum filtration[12].

2.6. Preparation of standard solution

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

Further pipette 0.6ml of the above Molnupiravir stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

2.7. Preparation of sample solution

The average weight of tablets was determined by weighing 10 tablets and powder. Weigh 10mg equivalent weight of Molnupiravir sample into 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 [6, 14]. Further pipette 0.6ml of Molnupiravir above stock solution into 10ml volumetric flask and dilute up to the mark with diluent.

3. Result

The solution of10µg/ml of Molnupiravir in diluent was prepared and the solution was scanned between 200-400nm. The drug showed an absorption maximum of 236 nm. Hence this was selected as the detection wavelength. After considering all system suitability parameters, Methanol: Phosphate Buffer pH-4.2 adjusted with Orthophosphoric acid solution in the ratio of 35:65% v/v in an isocratic mode. The mobile-phase flow rate and the sample volume injected were 1 ml/min and 10 µL, respectively. The retention time of Molnupiravir was found to be 2.8 ±0.2mins (Figure 2).

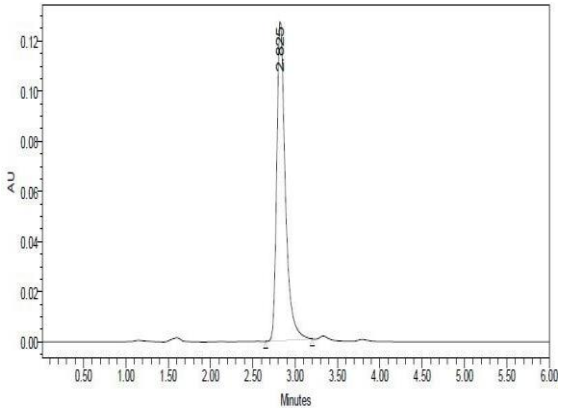

Fig. 2. Chromatogram showing the retention time of Molnupiravir

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 methanol and various concentrations have been prepared in the range of 20-100µg/ml of Molnupiravir in the diluent. 10µl of each solution was injected individually and the corresponding chromatogram was recorded at 236nm. The calibration curve has been plotted using concentration against peak area. The procedure has repeated three times. The R^2 was found to be 0.999 indicating the concentration of Molnupiravir has good linearity. The calibration graph was shown in Table 1.

3.1.2. Precision

The precision of the method was confirmed by repeatable injection of the standard solution 5 times. The% RSD value was found to be 0.248032. It shows that the method has good intra and inter-day precision (Table 2, 3, and 4).

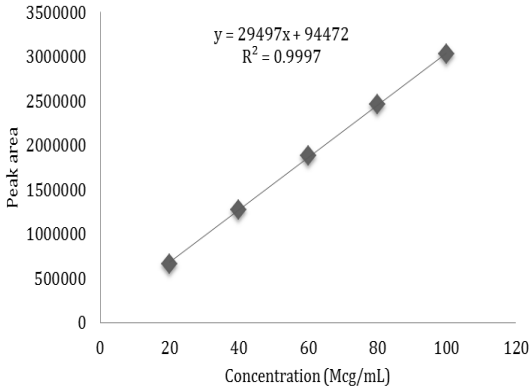

Fig. 3. Calibration curve of Molnupiravir

Table 1. Linearity of Molnupiravir

Concentration (µg/ml)	Average (Peak Area)
20	668748
40	1278875
60	1886598
80	2458644
100	3028547
R^2= 0.999	

Table 2. Results of Repeatability for Molnupiravir

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Molnupiravir	2.824	1825463	133526	5426	1.6
2	Molnupiravir	2.827	1825685	132564	5369	1.7
3	Molnupiravir	2.833	1825426	133254	5428	1.6
4	Molnupiravir	2.833	1835687	132546	5385	1.6
5	Molnupiravir	2.836	1825642	132658	5364	1.6
Mean			1827581			
Std.dev			4532.982			
%RSD			0.248032			

Table 3. Results of Intermediate precision for Molnupiravir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Molnupiravir	2.823	1836524	133658	5469	1.6
2	Molnupiravir	2.827	1836875	133695	5487	1.7
3	Molnupiravir	2.828	1836958	133693	5436	1.6
4	Molnupiravir	2.828	1836597	134568	5498	1.6
5	Molnupiravir	2.825	1845689	134598	5426	1.6
6	Molnupiravir	2.822	1845784	133659	5468	1.7
Mean			1839737.833			
Std. Dev.			4649.5024			
% RSD			0.248032			

Table 4. Results of Intermediate precision Analyst 2 for Molnupiravir

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Molnupiravir	2.833	1325684	134568	5568	1.7
2	Molnupiravir	2.836	1332658	134579	5489	1.6
3	Molnupiravir	2.827	1334526	133659	5598	1.6
4	Molnupiravir	2.827	1325487	134526	5458	1.7
5	Molnupiravir	2.823	1336598	134758	5587	1.6
6	Molnupiravir	2.827	1326587	134569	5569	1.6
Mean			1330257			
Std. Dev.			4926.085			
% RSD			0.370311			

3.1.3. ACCURACY

Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated (Figure 8; Table 5, 6, 7, and 8).

Table 5. Results of Accuracy for concentration-50%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Molnupiravir	2.836	952654	131265	1.2	4896	1
2	Molnupiravir	2.838	951658	130269	1.3	4798	2
3	Molnupiravir	2.853	952364	131258	1.2	4674	3

Table 6. Results of Accuracy for concentration-100%

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Molnupiravir	2.826	1862587	132658	1.7	5469	1
2	Molnupiravir	2.830	1860598	133265	1.6	5396	2
3	Molnupiravir	2.822	1865984	132698	1.7	5475	3

Table.7. Results of Accuracy for concentration-150%

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Molnupiravir	2.831	2765847	165325	1.9	6125	1
2	Molnupiravir	2.835	2768542	166532	1.8	6239	2
3	Molnupiravir	2.839	2759898	165878	1.9	6126	3

Table 8. Accuracy results of Molnupiravir

% Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	952225.3	30	30.068	100.226%	
100%	1863056	60	60.256	100.426%	100.27%
150%	2764762	90	90.142	100.157%	

3.1.4. ASSAY

The tablet formulation was selected for analysis. The nominal concentration 20µg/ml from the calibration curve has been prepared by using a diluent.10µl of the formulation was injected and the chromatogram has been recorded in Figure 4. The amount of Molnupiravir present in the formulation was found to be 100.6

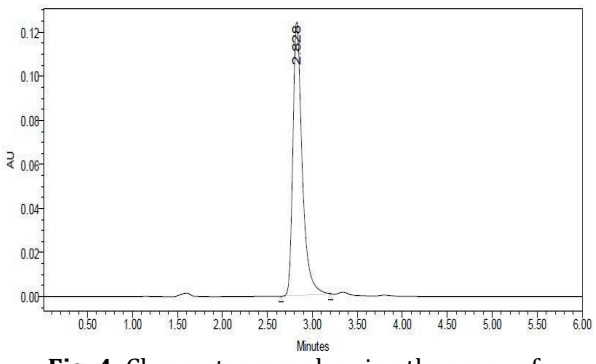

Fig. 4. Chromatogram showing the assay of standard injection -1

3.2. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) Of the developed method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The detection limit (LOD) was found to be 2.6µg/ml and the quantitation limit (LOQ) was found to be 6.35µg/ml.

3.3. Robustness

The robustness was performed for the flow rate variations from 0.8ml/min to 1.0ml/min and mobile phase ratio variation from the more organic phase to less organic phase ratio for Embramine HCL. The method is robust only in less flow conditions and the method is robust even by the change in the Mobile phase ±5%. The standard and samples of Molnupiravir 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.

4. Discussion

Symmetry ODS C18 (4.6×150mm, 5µm) column with a dimension of 4.6 x 75mm was used and the mobile phase consisted of Mobile phase used contained Methanol: Phosphate Buffer pH-4.2 adjusted with Orthophosphoric acid solution in the ratio of 35:65% v/v in an isocratic mode at a wavelength of 236nm. The mobile-phase flow rate and the sample volume injected were 1 ml/min and 10 µL, respectively. The retention time of Molnupiravir was found to be 2.8 ±0.2mins.

A good linear relationship of Molnupiravir (r =0.999) was observed over a concentration range of 20 to 100µg/ml of Molnupiravir. The limit of detection (LOD) and limit of quantification (LOQ) for Molnupiravir was found to be 2.6µg/ml and 6.35µg/ml. % recovery was observed in the range of 98-102%.w/v.

The retention time of molnupravir was found to be fast enough for rapid analysis (4.5 minutes). Different columns did not give variable results and it was accepted as suitable (relative standard deviation < 1%, tailing factor < 1.5, and theoretical plate number > 5000). The method was found to be specific for Molnupiravir [15, 16].

Mobile phase and other solutions did not give any peak at or around the retention time of the active substance and all these did not significantly affect the analysis. The calibration curve was linear with six consecutive concentrations of molnupravir and the correlation coefficient r^2 was higher than 0.995. The RSD% values of the areas were less than 1.0 Recovery values were between 98.0% -102.0% and RSD values were not more than 1.0. Intermediate precision and repeatability values were found to be suitable [17-19]. The proposed method was determined to be robust and stable for more than 24 hours. Molnupiravir can be analyzed using this simple “Inverse gradient” HPLC method in the literature. This is offered as an orthogonal approach for a Reversed Phased Method and will show various polar impurities not detected by Reversed Phase HPLC.

5. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Molnupiravir in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Molnupiravir was slightly soluble in methanol, DMSO, water. Methanol: Phosphate Buffer pH-4.2 (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise. The results expressed in the Tables for the RP-HPLC method were promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Molnupiravir in bulk drugs and in Pharmaceutical dosage forms.

Conflict of Interest

All authors state that they are free of any conflicts of interest related to this paper.

Author's contributions

All authors confirm that they have read and approved this manuscript.

Consent for publications

All authors have read and approved the final manuscript for publication.

Availability of data and material

The authors have embedded all data in the manuscript.

Ethics approval and consent to participate

The authors did not use human or animals in the research.

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