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Research

Estimation of molnupiravir in bulk and capsule by Novel RP-HPLC method development and validation

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Abstract

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive RP-HPLC method for the measurement of active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form of Molnupiravir.

Methods: A simple, selective, validated isocratic RP-HPLC methodology for the quantitative determination of Molnupiravir. The chromatographic strategy utilized Phenomenex Luna ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm, using isocratic elution with a mobile phase of Phosphate Buffer (0.02M) and Acetonitrile were consists of 60:40% v/v (pH-2.80). A flow rate of 1.0 ml/min and a detector wavelength of 246 nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the two active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

Keywords: Molnupiravir, RP-HPLC, Method Development, Validation, Accuracy, Robustness.

INTRODUCTION

The IUPAC name of Molnupiravir is [(2R,3S,4R,5R)-3,4-dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-yl]methyl-2-methylpropanoate.The molecular formula and weight was $C_{13}H_{19}N_3O_7$,329.309 g·mol^{-1} and Chemical Structure shown below. [1]

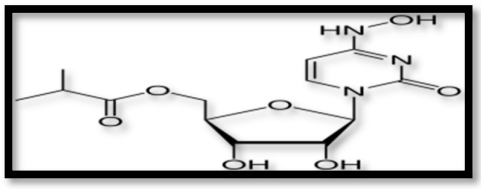

Fig1: Chemical Structure of Molnupiravir

Molnupiravir (EIDD-2801, MK-4482) is the isopropylesterprodrug of N4-hydroxycytidine.1,2with improved oral bioavailability in non-human primates, it is hydrolyzed in vivo, and distributes into tissues where it becomes the active 5'-triphosphate form. The active drug incorporates into the genome of RNA viruses, leading to an accumulation of mutations known as viral error catastrophe [2]. Recent studies have shown Molnupiravir inhibits replication of human and bat coronaviruses, including SARS-CoV-2, in mice and human airway epithelial cells. A Remdesivir resistant mutant mouse hepatitis virus has also been shown to have increased sensitivity to N4-hydroxycytidine. Molnupiravir was granted approval by the UK's Medicines and Health products Regulatory Agency (MHRA) on 4 November 2021 to prevent severe outcomes such as hospitalization and death due to COVID-19 in adults. Molnupiravir was also granted emergency use authorization by the FDA on December 23, 2021, and February 11, 2022 Letters of Authorization (LOA) referred to the authorized drug as "molnupiravir,"; however, Merck subsequently requested, and FDA concurred, that the Fact Sheets be revised to add references to molnupiravir's trade name, "LAGEVRIO." "LAGEVRIO" has been used since the March 23, 2022 reissuance of this letter. LAGEVRIO capsules contain molnupiravir; a nucleoside analogue that inhibits SARS-CoV-2 replication by viral mutagenesis. LAGEVRIO is not FDA-approved for any uses, including use as treatment for COVID-19. Patients requiring hospitalization after starting treatment with molnupiravir may complete the full 5-day treatment course per the healthcare provider's discretion [3].The method was chosen to develop with the existing literature where a dosage form can be estimated with improved chromatographic conditions. [9-10]

MATERIALS AND METHODS

Chemical and Reagents

Pharmaceutical grade Molnupiravir was obtained as a gift samples from Jai Lara Drugs Private Limited., Hyderabad. Methanol used in analysis was of HPLC grade and potassium *di*-hydrogen *ortho*-phosphate and *ortho*-phosphoric acid used were of analytical reagent grade. All chemicals were purchased from SD fine-Chem ltd; Mumbai, andRankem® Laboratory Chemicals. CapsuleMolflu® Dr. Reddy's Laboratories (200 mg) was purchased in local pharmacy.

Instrumentation and Chromatographic Conditions

Waters HPLCwith Empower2 Software with Isocratic with UV-Visible Detector. The sample introduction was performed with 20 mL sample loop injector (Rheodyne 7725*i*). ELICO SL-159 UV – Vis spectrophotometer, Electronic Balance (SHIMADZU ATY224), Ultra Sonicator(Wensar wuc-2L), P^H Analyzer (ELICO). All glassware used wereBOROSIL. All separations were performed on Phenomenex Luna ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm at ambient temperature using mobile phase consisting of Phosphate Buffer (pH-2.80) 0.02M: Acetonitrile 60:40% v/v in isocratic mode at flow rate of 1 mL/min. The detection was performed at optimum wavelength of 246 nm.

Method Development and Its Validation for Molnupiravirby RP-HPLC[3-4]

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the lambda maxima of Molnupiravir, so that the same wave number can be utilized in HPLC UV detector for estimating the Molnupiravir. While scanning the Molnupiravir solution we observed the maxima at 246 nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450 shown in Figure 2.

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 removal of air completely and make volume up to the mark with the same Methanol.Further pipette 0.5ml of the above Molnupiravir stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines [7].

Preparation of Sample solution

Weight twenty tablets and the average was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Molnupiravirwas weighed equivalent to 10mg, transferred to clean and dry 10ml standard volumetric flask, add 7ml of HPLC grade methanol and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. Pipette out 0.5 ml of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Preparation of 0.02M Potassium Dihydrogen Orthophosphate Solution

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted orthophosphoric acid Solution.

Preparation of Mobile Phase

600mL (60%) of above Phosphate buffer solution and 400mL of HPLC Grade Acetonitrile (40%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

Method Validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice [6].

ANALYTICAL METHOD VALIDATION [7]

System suitability: A standard solution was prepared by using Molnupiravir working standard as per test method and was injected 5 times into the HPLC system. The system suitability parameters were evaluated from the Resolution, USP tailing and USP plate count values obtained from standard chromatograms as shown in Table 5 & 6.

Selectivity/Specificity: Selectivity is measured accurately by an analyte in the presence of interferences that might be expected to be present in the sample matrix. It is checked by examining chromatographic blanks in the anticipated time window of the analyte peak.

Precision: Precision is the degree of agreement between individual test results after the procedure is applied repeatedly to multiple samplings under similar conditions. It is measured by injecting a series of standards or a series of analyzing samples from multiple samplings from a homogeneous batch of material. It aims to demonstrate the random error that could occur in a method.

Accuracy: "Accuracy is that the degree of agreement among the value which is accepted whichever as a conventional true value or an accepted reference value and therefore the value found". It is measured with a known concentration of analyte standard and analyzing the sample by spiking the sample matrix of interest.

Linearity: Linearity is expressed by injecting a series of standards of stock solution or diluted stock solution using the solvent or mobile phase, at a minimum of 5 different concentrations in the range of 30–70% of the anticipated working range. It is simple between Concentrations vs. Peak Area Response.

Range: The range (the interval between the upper and lower levels) of an analytical method has been demonstrated to determine precision, accuracy, and linearity using the set method. This range is defined by the concentration range in which the Linearity test was completed.

LOD and LOQ: Limit of detection (LOD) is defined as the lowest concentration at which point the instrument can identify but not quantify, and the noise/signal ratio for LOD must be 1:3.Limit of quantitation (LOQ) is

defined as the lowest concentration at which point the instrument can detect and quantify, and the noise/signal ratio for LOQ should be 1:10.0.

Robustness (or Ruggedness): The procedure to provide analytical results of acceptable accuracy and precision under a variety of conditions such as reagents (e.g. different suppliers), different columns (e.g. different lots and/or suppliers), extraction time, Variations of pH of a mobile phase, variations in mobile phase composition, temperature, and flow rate.

RESULTS AND DISCUSSION

Selection of Wavelength: The scanned UV spectrum is attached in the following,

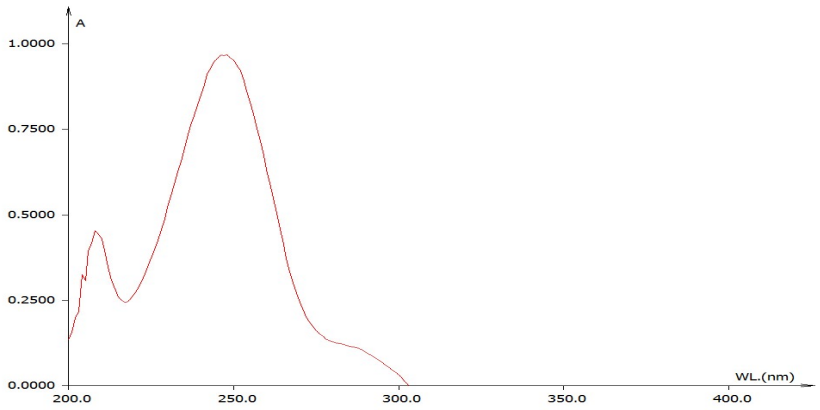

Fig2: UV Spectrum for Molnupiravir (246nm)

Method Development
Summary of Optimized Chromatographic Conditions
The Optimum conditions obtained from experiments can be summarized as below:

Table 1: Summary of Optimised Chromatographic Conditions

Mobile phase	Phosphate Buffer (0.02M): Acetonitrile = 60:40 (pH-2.80)
Column	Phenomenex Luna ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	246 nm
Flow rate	1.0 ml/ min.
Run time	08 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Mode of Elution	Isocratic
Retention time	3.688 minutes

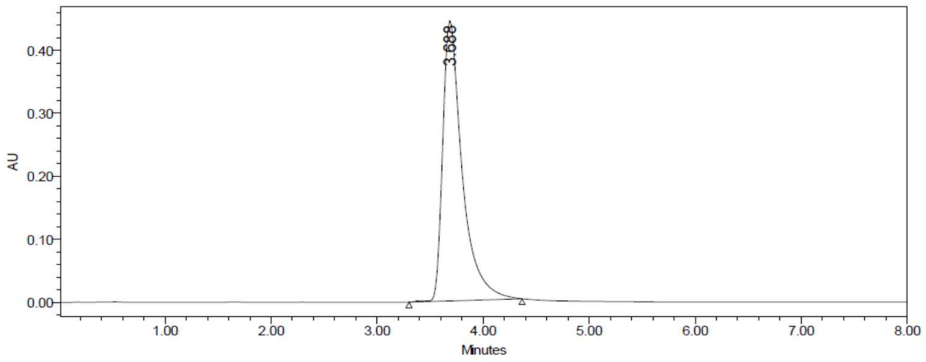

Fig 3: Chromatogram of Molnupiravir in Optimized Chromatographic Condition

Table 2: Peak Results of Optimised Chromatogram

S.No.	Drug Name	Rt	Peak Area	Tailing Factor	Plate Count
1	Molnupiravir	3.688	584624	1.42	4765

VALIDATION OF ANALYTICAL METHOD

The optimized chromatographic condition is applied for quantitative determination and the method was validated by considering some parameters linearity, precision, accuracy (% recovery), robustness, system precision, method precision, and ruggedness [7].

System Suitability Parameter:

System quality testing is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system that may be evaluated intrinsically. Following system quality check parameters were established. The information square measured shown in Table- 5 and 6.

Table3: Knowledge of System Quality Parameter

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Molnupiravir =0.98
2	Theoretical plate	N > 2000	Molnupiravir =4782
3	Tailing Factor	T<2	Molnupiravir =1.49

Table 4: Results of System Suitability for Molnupiravir

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Molnupiravir	3.644	584635	65847	4857	1.48
2	Molnupiravir	3.645	582695	65421	4955	1.42
3	Molnupiravir	3.644	587432	65369	4875	1.47
4	Molnupiravir	3.662	589687	65748	4796	1.46
5	Molnupiravir	3.660	582547	65398	4952	1.49
6	Molnupiravir	3.660	589656	652418	4896	1.47
Mean			**586108.7**			
Std.Dev.			**3275.654**			
%RSD			**0.558882**			

Specificity

Specificity can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing one drug was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the

proposed method was specific. The chromatograms representing the peaks of blank, Molnupiravir and the sample containing the one drug was shown in following figures respectively.

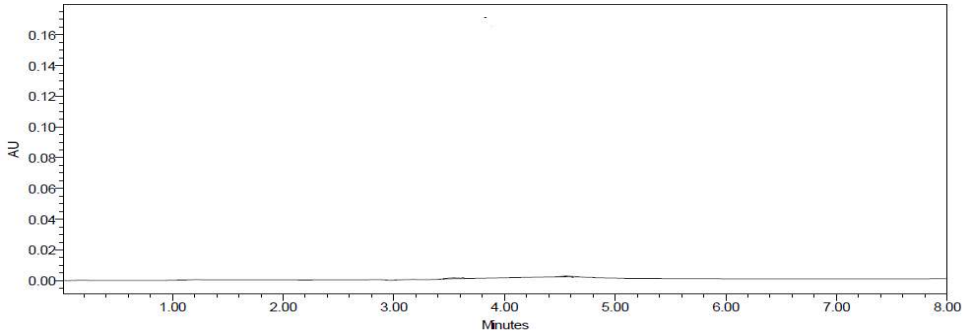

Fig 4: Chromatogram for Blank Solution

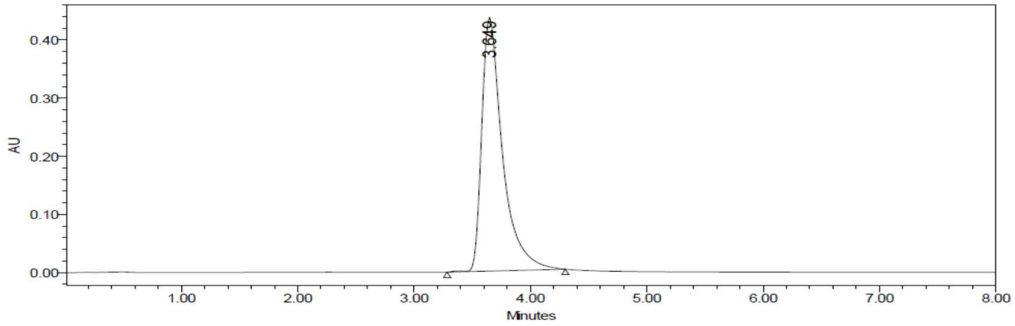

Fig 5: Chromatogram of Molnupiravir Standard Solution

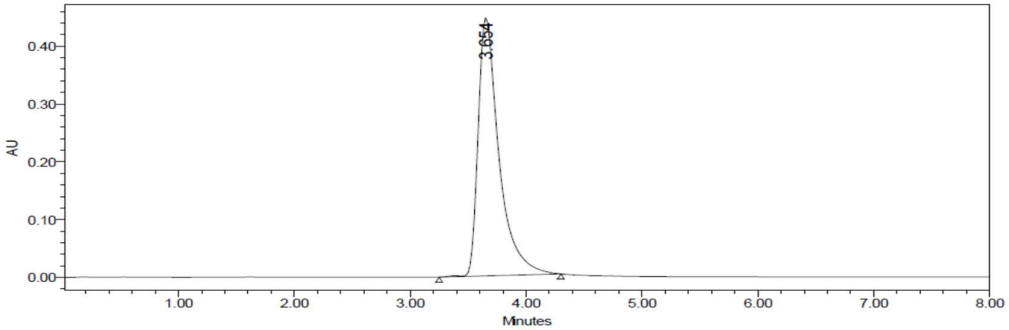

Fig 6: Chromatogram of Molnupiravir Sample Solution

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Accuracy: From the prepared stock solution the following concentrated solutions were prepared
For Preparation of 80% Standard Stock Solution: Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
For Preparation of 100% Standard Stock Solution: Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.
For Preparation of 120% Standard Stock Solution: Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Recovery Study

To determine the accuracy of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Molnupiravir were taken and extra to the

pre-analyzed formulation of concentration 50µg/ml. From that proportion recovery values were calculated. The results were shown in table-7.

Table 5: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	40	40.141	502647	100.352	Mean= 100.3947% S.D. = 0.071319 % R.S.D.=0.071038
S_2 : 80 %	40	40.191	503214	100.477	
S_3 : 80 %	40	40.142	502656	100.355	
S_4 : 100 %	50	50.044	614215	100.088	Mean= 99.98533% S.D. = 0.183045 % R.S.D.=0.183071
S_5 : 100 %	50	49.887	612451	99.774	
S_6 : 100 %	50	50.047	614254	100.094	
S_7 : 120 %	60	60.192	728547	100.32	Mean= 100.311% S.D. = 0.408574 % R.S.D.=0.407308
S_8 : 120 %	60	59.939	725698	99.898	
S_9 : 120 %	60	60.429	731211	100.715	

Precision

Repeatability

Preparation of Molnupiravir Product Solution for Precision: Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.The exactitude of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Molnupiravir (API). The % relative variance was calculated for Molnupiravir square measure bestowed within the table-8.

Table 6: Repeatability Readings

HPLC Injection Replicates of Molnupiravir	Retention Time (Minutes)	Peak Area
Replicate – 1	3.649	5674158
Replicate – 2	3.684	5654715
Replicate – 3	3.687	5665841
Replicate – 4	3.688	5654578
Replicate – 5	3.688	5652284
Replicate – 6	3.687	5641487
Average		**5657177**
Standard Deviation		**11369.72**
% RSD		**0.200979**

Intermediate Precision/Ruggedness

Intra-Day & Inter-Day

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Molnupiravir revealed that the proposed method is precise.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst-1/Intra Day/Day-1

Table 7: Results of Ruggedness for Molnupiravir Analyst 1

S.No.	Peak	Rt	Area (µV*sec)	Height (µV)	USP	Plate Count	USP
1	Molnupiravir	3.687	584968	65982		4985	1.42
2	Molnupiravir	3.688	582479	66354		4876	1.46
3	Molnupiravir	3.688	586236	67425		4896	1.48
4	Molnupiravir	3.687	586985	65982		4986	1.47
5	Molnupiravir	3.684	582679	65932		5016	1.45
6	Molnupiravir	3.649	583989	65874		4987	1.43
Mean			**584556**				
Std.Dev.			**1846.658**				
%RSD			**0.315908**				

Analyst 2/Inter Day/Day-2

Table 8: 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	3.649	598698	66985		5265	1.49
2	Molnupiravir	3.684	596847	67458		5168	1.47
3	Molnupiravir	3.687	596354	66985		5436	1.46
4	Molnupiravir	3.688	598676	67854		5369	1.45
5	Molnupiravir	3.688	596874	68521		5247	1.48
6	Molnupiravir	3.687	598989	67898		5375	1.42
Mean			**597739.7**				
Std.Dev.			**1168.098**				
%RSD			**0.195419**				

Linearity & Range

Preparation of DrugSolutions for Linearity: Pipette 0.5ml of the above Molnupiravir stock solutions into a 10ml volumetric flask and dilute up to the mark with Mobile Phase.

Preparation of Level – I (30ppm of Molnupiravir): Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – II (40ppm of Molnupiravir): Take 0.4ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – III (50ppm of Molnupiravir): Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – IV (60ppm of Molnupiravir): Take 0.6ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Preparation of Level – V (70ppm of Molnupiravir): Take 0.7ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

The calibration curve showed good linearity in the range of 0-70µg/ml, for Molnupiravir (API) with correlation coefficient (r^2) of 0.999 (Fig-7). A typical calibration curve has the regression equation of y = 11266.x + 50416 for Molnupiravir.

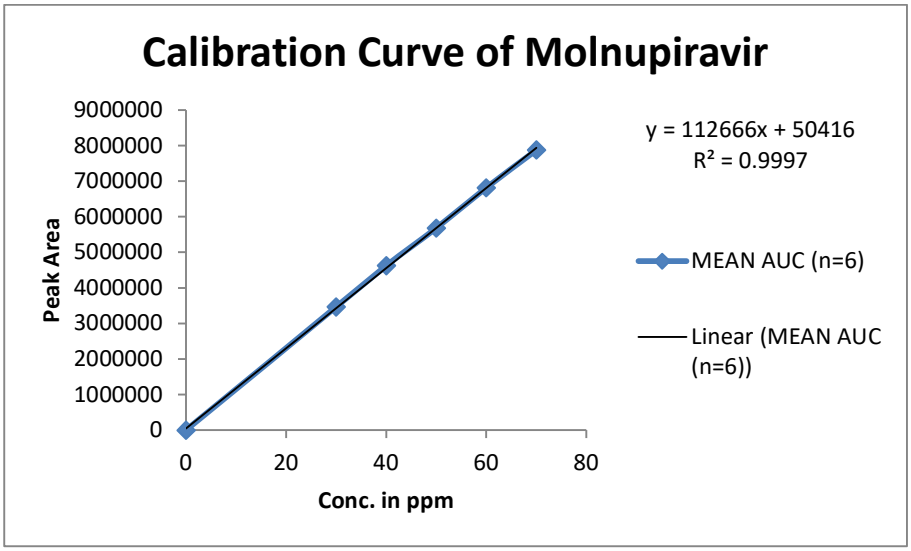

Fig7:Calibration Curve of Molnupiravir (API)

Table 9: Linearity Results

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
30	3465974
40	4626478
50	5682284
60	6815478
70	7878721

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Molnupiravir is a straight line.

Y = mx + c. Where: Slope (m) = 112666; Intercept (c) = 50416

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 50416. These values meet the validation criteria.

Method Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Molnupiravir. The method is robust only in less flow condition and the method is robust even by 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.

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

For Preparation of Standard Solution

Take 0.5ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

Effect of Variation of Flow Conditions

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Phosphate Buffer was taken in the ratio and 40:60, 30:70 instead of 35:65, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

LOD & LOQ

LOD: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where: σ = Standard deviation of the response; S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10 × σ/S

Where: σ = Standard deviation of the response; S = Slope of the calibration curve

Table10: Results of LOD & LOQ

SE of Intercept	48846.22527
SD of Intercept	109223.4801
LOD	3.199168
LOQ	9.694449

Observation: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 3.19 & 9.69 µg/ml respectively.

Estimation of Molnupiravir in Pharmaceutical Dosage Form (Assay)

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Above weighed tablets/Capsules were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-13.

ASSAY: % Assay=AT/AS×WS/DS×DT/WT×P/100×AW/LC×100

Where:AT = Peak Area of Molnupiravir obtained with test preparation

AS = Peak Area of Molnupiravir obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Results obtained are tabulated below:

Table 11: Recovery Data for Estimation Molnupiravir

Brand Name of Molnupiravir	Labelled Amount	Mean (± SD) Amount (mg) found	Assay % (±
Molflu Capsule (200mg) (Dr.	200mg	199.356 (± 0.478)	99.475

The amount of drugs in MolnupiravirCapsulewas found to be 199.356 (± 0.478) mg/Cap for Molnupiravir& % assay was 99.475 (± 0.582).

SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 246nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Phenomenex Luna ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm particle size because it was giving good peak.Ambient temperatures were found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Phosphate Buffer (0.02M) and Acetonitrile were taken in the ratio of 60:40 % v/v (pH-2.80)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 8.0 min because analyze gave peak around 3.688min and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear

and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range of 30-70ppm of the Molnupiravir target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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