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STABILITY INDICATING METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF MOLNUPIRAVIR IN BULK AND PHARMACEUTICAL DOSAGE PREPARATIONS BY RP-UPLC

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Abstract:

Stability indicating UPLC method has been developed for the estimation of Molnupiravir in bulk and capsule dosage form. Estimation of Molnupiravir by this method is rapid and reliable. This RP-UPLC method was developed and validated by Acquity C18 BEH (100mm x 2.1mm,)1.7µm particle size in isocratic mode, and the sample was analyzed using a ratio of Acetonitrile and 10mM Phosphate Buffer with pH 3.5 (25:75% v/v) as mobile phase at a flow rate of 0.5 ml/min and detection was carried out at 237 nm. Molnupiravir was eluted at the retention time of 1.9min with good efficiency and less tailing (<1.5). The capsule assay method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with ICH Validation guidelines. Method has shown Precise, Specific, Linear sensitive Accurate, Robust and Rugged results. For Linearity Parameter, Calibration plots in the concentration range of 50-150µg/ml for Molnupiravir and shown correlation coefficient >0.999, and recoveries from capsule dosage form were between 98.0 and 102.0 %. The % assay in Method Precision was shown to be 99.3%. This method can be used for routine of the quality control in pharmaceuticals. The RP-UPLC method was found to be simple, economical and rapid as compared to previous methods. Previous methods were not focused on Forced degradation studies. Those methods may not be used to analyze the stability samples. This method is simple and reliable and as we have performed forced degradation studies, this method is also stability indicating.

Key words: *UPLC, Molnupiravir, Waters Acquity BEH C18, Method validation, Forced degradation studies.*

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INTRODUCTION:

Molnupiravir is the isopropyl ester pro-drug of N4-hydroxycytidine.[1,2] With 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.[3] Recent studies have shown Molnupiravir inhibits replication of human and bat coronaviruses, including SARS-CoV-2, in mice and human airway epithelial cells[1].

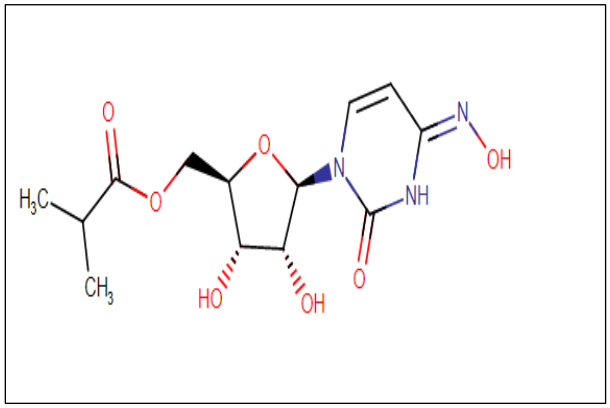


Fig No-01: Structure of MOLNUPIRAVIR[1]

Molnupiravir is hydrolyzed in vivo to N4-hydroxycytidine, which is phosphorylated in tissue to the active 5'-triphosphate form, and incorporated into the genome of new variants, resulting in the accumulation of inactivating mutations, known as viral error catastrophe[1].

Mechanism of Action: Antiviral drugs often target viral polymerases and function as nucleoside analogs that terminate RNA chain elongation. However, such chain-terminating antivirals are generally not effective against SARS-CoV-2 because coronaviruses carry an exonucleolytic proofreading activity that can remove misincorporated nucleotides from the nascent RNA 3′ end.

The nucleoside analog remdesivir can circumvent proofreading because its incorporation does not terminate elongation but only stalls RdRp after the addition of three more nucleotides. Remdesivir was the first FDA-approved drug for the treatment of patients with COVID-19, but its effectiveness is disputed, emphasizing the need to develop new antiviral drugs[1].

About Method: Stability indicating UPLC method has been developed for the estimation of Molnupiravir in bulk and capsule dosage form. Estimation of Molnupiravir by this method is rapid and reliable. Previous methods were not focused on Forced degradation studies. Such methods may not be used to analyze the stability samples. This method is simple and reliable and as we performed forced degradation studies, this method is also stability indicating.

Experimental Methodology:

Equipment: Water Acquity UPLC with PDA Detector, Model Number:2487 with empower software, quaternary pump, auto sampler with thermostat, Analytical column: Waters Acquity BEH C18 (100mmx2.1mm ID) 1.7µm, UV-Visible spectrophotometer, make: Shimadzu model: 1700UV with vision pro software, pH meter, make: Thermo Fisher and model: Orion star, Ultra sonic cleaner, Shimadzu balance (analytical)AY-0220.

Materials: Molnupiravir is obtained as gift sample from Chandra labs, Prashanthi nagar, Kukatpally, Molulife capsules formulation was obtained in Local pharmacy, Acetonitrile, Methanol, Milli-Q water is used of HPLC-grade, Potassium di hydrogen phosphate purchased from Rankem and Merck and India.

Optimized Chromatographic conditions: Analytical separation was carried out with column Waters Acquity BEH C18 (100mmx2.1mm ID) 1.7µm by using Isocratic mode with a mixture of Acetonitrile: 10mM Phosphate Buffer with pH 3.5 in 25:75% v/v. Column Oven Temperature: 35 ±0.2°C, 0.5 mL/min was maintained as flow rate and 5µL maintained as sample injection volume with detection at 237nm with UV detector.

Selection of working wavelength (λmax):

Accurately weighed and transferred 10mg of Molnupiravir into a 100ml volumetric flask and dissolved in 60ml of methanol. Volume was made up to the mark with water and mixed well. From the above stock solution 3µg/ml of Molnupiravir was prepared by diluting 3ml to 100ml with water respectively.

UV spectrum of 3µg/mL solution of Molnupiravir in water was recorded by scanning in the range of 200nm to 400nm, by using water as a blank. From the UV spectrum, the wavelength maxima was identified to be 237nm. The spectrum was shown in Figure No: 2.

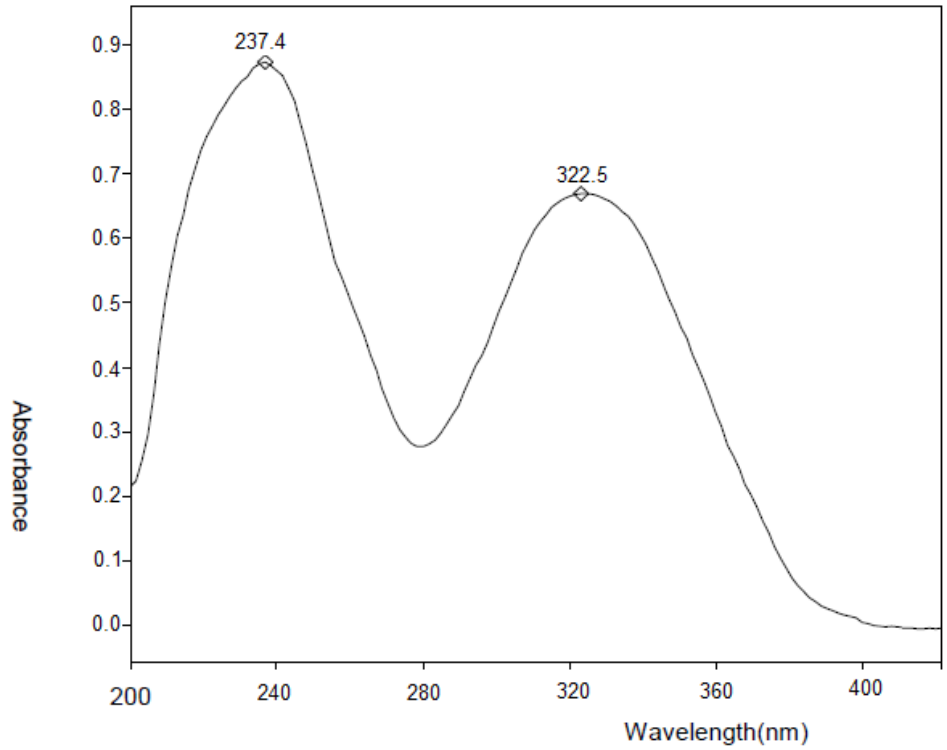


Fig No: 02 UV Spectrum of Molnupiravir showing maximum absorbance at 237nm.

Preparation of standard solution
Accurately weighed and transferred 50mg of Molnupiravir into a 50ml volumetric flask and dissolved in 35ml of mobile phase. Volume was made up to the mark with mobile phase and mixed well. From above stock solution 100µg/ml of Molnupiravir was prepared by diluting 5ml to 50ml with mobile phase respectively.

Preparation of sample solution:
20 Capsules were weighed as it is, then collected the powder from the 20 capsules accurately in poly bag, the weighed empty capsule shells. The calculated average weight of the filled powder by using below formula:

Weight of the 20 Capsules powder = (20 Capsules weight with filled powder - weight of the capsule shells without filled powder)

After getting the average weight, powder equivalent to 200 mg of Molnupiravir was accurately weighed and transferred into a 200ml volumetric flask and dissolved in 160ml of mobile phase, sonicated for 20min, then volume was made up to the mark with mobile phase and mixed well. The sample solution was centrifuged at 10000rpm for 10min. Prepared 100µg/mL sample Solution by further diluting 5mL of the above sample stock solution to 50mL with mobile phase. Then the sample was filtered with PVDF or Nylon 0.45µm syringe filter by discarding the 5mL of filtrate.

Analytical method validation[3]:
System suitability and System Precision[3]
Six standard solutions of the same concentration (100%) were prepared and injected into the UPLC system as per test procedure.

Acceptance Criteria:
The % RSD of the area response of Standard peak obtained from the six injections of standard solution should not be more than 2.0. The Theoretical plates for 1st injection should be NLT 2000 for Molnupiravir. The Tailing factor for 1st injection should be NMT 2.0 for Molnupiravir peak.

Specificity[3]
Preparation of Placebo solution:
Weighed Placebo powder equivalent to 200 mg of Molnupiravir in 200 ml of volumetric flask and dissolve in 350ml of mobile phase by 30min of sonication and make up the volume with mobile phase. Centrifuged sample at 10000rpm for 10min.

Prepared Placebo solution by further diluted 5mL above sample stock solution to 50mL with mobile phase and mixed well. Then the placebo solution was filtered with PVDF or Nylon 0.45µm syringe filter by discarding the 5mL of filtrate.

Blank, Placebo solution, standard solutions were injected into UPLC system

Acceptance Criteria:
No interference should be observed at the retention time of Molnupiravir due to blank and Placebo.

Linearity[3]:
Calibration curve was constructed at five linear concentrations of Molnupiravir (50, 80, 100, 120& 150µg/mL) (50% to 150%, 5 Levels). The solutions were injected in to the chromatographic system, the results were plotted into a graph taking concentration versus area to evaluate correlation coefficient.
Acceptance criteria: Correlation coefficient should Not be less than 0.99.

Method Precision[3]:
Six sample solutions of the same concentration (100%) were prepared individually and injected into the UPLC system as per test procedure.
Acceptance Criteria: % Assay should be 95.0 to 105 & %RSD for six preparations assay should be ≤ 2.0

Accuracy & Recovery[3]:
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. recovery studies were carried out by addition of standard drug solution to the placebo solution at three different levels 50%, 100% & 150%.

Acceptance criteria of %Recovery should be 98.0 to 102.0 & Acceptance criteria of % RSD for nine preparations recover values should be ≤ 2.0.

Robustness[3]:
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The mobile phase flow rate was changed to 0.4mL/min & 0.6mL/min, the Column oven temperature was changed to 30°C & 40°C. Acceptance Criteria: System suitability should be within the limit.

Intermediate precision (also called Ruggedness) Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory & from analyst to analyst. Six preparations were injected individually in to chromatographic system by the analyst-II & Calculated % Assay of individual samples.

Acceptance criteria of %Assay should be 95.0 to 105.0, % RSD for six preparations assay values should be ≤ 2.0 & % RSD for Method precision and Intermediate Precision assay mean values should be ≤ 2.0.

RESULT AND DISCUSSION:

System suitability and System Precision[3]:

Table No.:01 Results for System suitability and System Precision

Parameter	Results
Retention time(min)	1.89
Theoretical Plates	6065
Tailing factor	1.1
Resolution	NA
%RSD for Six replicated injection of Standard	0.7

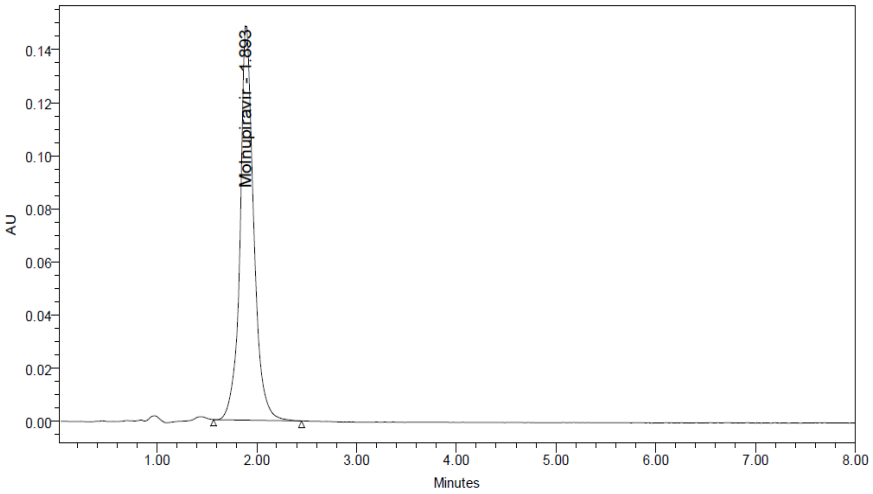
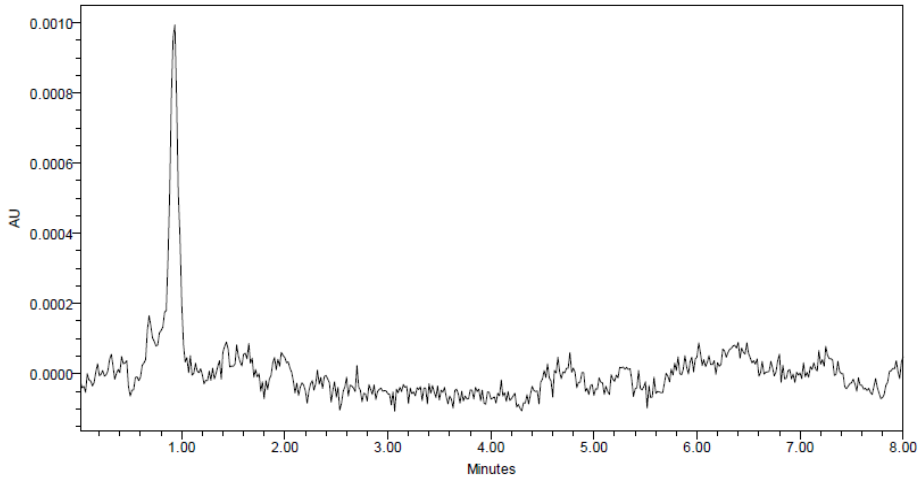


Fig No-03: Chromatogram of Standard

Observation:

System suitability and System Precision results were met with acceptance criteria, hence system is precise.



Specificity[3]:

Fig No-04: Chromatogram of Blank

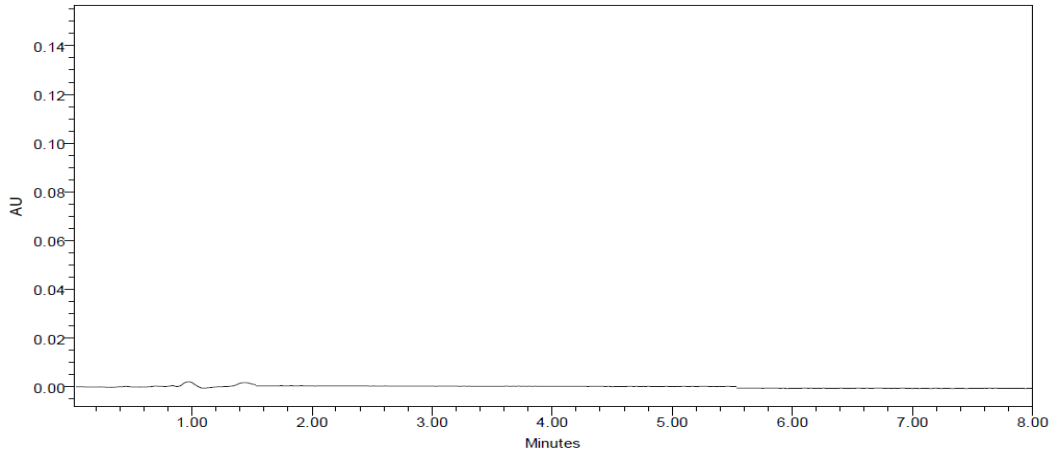


Fig No-05: Chromatogram of Placebo

Observation:
There was no interference observed at the retention time of MOLNUPIRAVIR peak due to blank and Placebo. Hence System is specific.

Forced Degradation studies[6]:

Acceptance Criteria: Main analyte Peak purity should be Pass, peak purity value should be in positive

Table No.:02 Forced Degradation results for MOLNUPIRAVIR

Name of the Degradation	Condition	Peak purity threshold	Peak purity Angle	Purity flag	%Assay
Photolytic degradation	1.2mill/LUX Hours	201.3	158.6	Absent	100.1
Thermal Degradation	60°C/7Days	207.8	147.5	Absent	99.6
Acid Degradation	5mL of 0.1N HCl/4Hrs at 80°C	189.7	120.3	Absent	94.5
Base Degradation	5mL of 0.1N NaOH Solution/4Hrs at 80°C	206.6	140.3	Absent	99.1
Peroxide Degradation	5mL of 1% H_2O_2/4Hrs at Bench top	208.6	157.3	Absent	99.4
Control Sample	NA	201.6	157.6	Absent	99.2

Observation:

From the above results purity flag was absent and Purity threshold is greater than Purity angle.

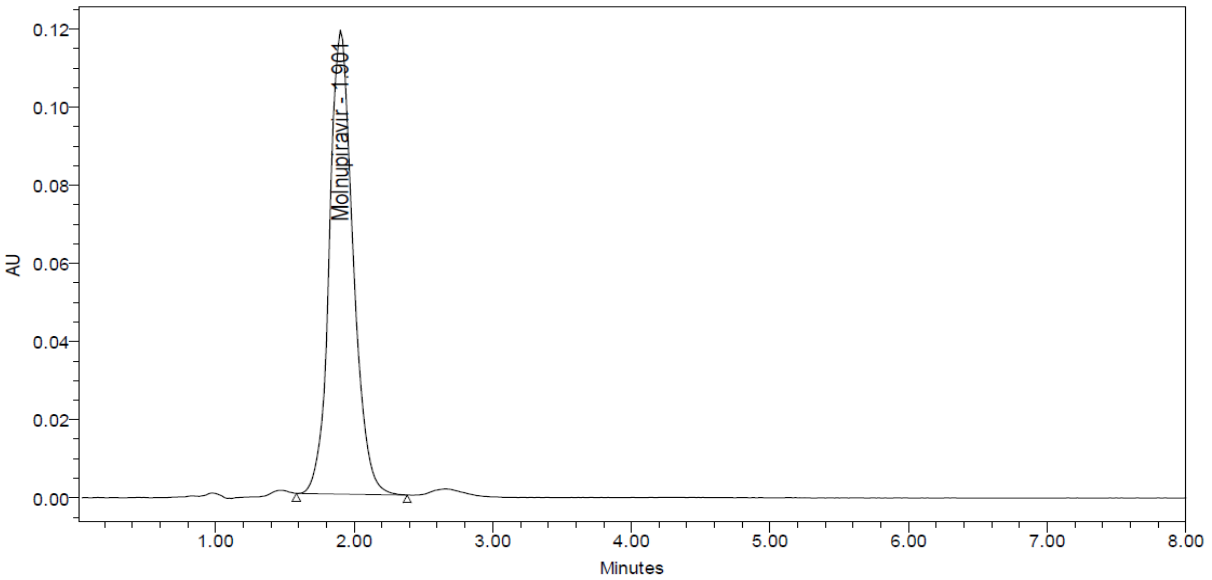


Fig No.:06 Chromatogram of acid degradation

Linearity[3]:

Table No.:03

Parameter	Result
Concentration range in µg/mL	50µg/mL to 150µg/mL
Correlation coefficient	0.9998
Intercept	868.8
Slope	14436

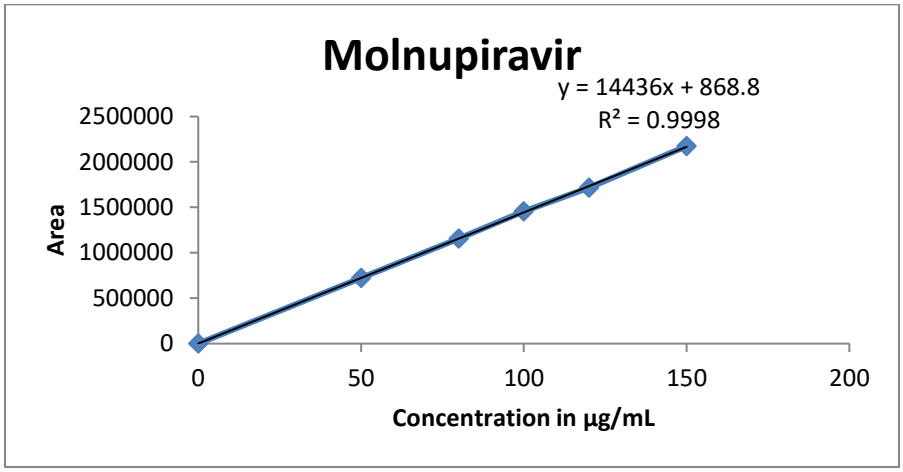


Fig No-07: Calibration curve for Molnupiravir

Observation: The correlation coefficient value obtained to be 0.9998 for MOLNUPIRAVIR

Method Precision[3]:

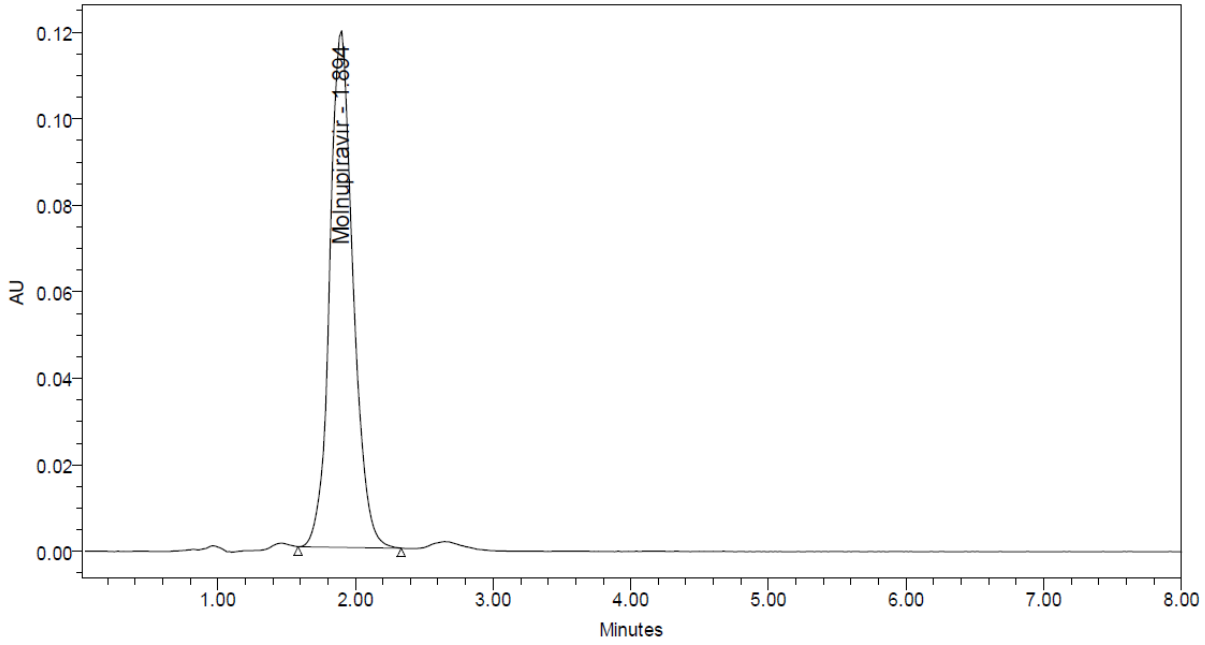


Fig No-08: Test solution Preparation-01 chromatogram.

Table No-04: Method Precision results of MOLNUPIRAVIR

S.No.	Solution details	%Assay of Molnupiravir
1	Test solution preparation-1	98.9
2	Test solution preparation-2	98.8
3	Test solution preparation-3	98.8
4	Test solution preparation-4	99.1
5	Test solution preparation-5	98.8
6	Test solution preparation-6	98.7
Average		98.7
Std Dev		98.8
%RSD		0.1

Observation: Mean %Assay was obtained between 95.0 to 105.0% for MOLNUPIRAVIR and the % RSD of % Assay results obtained from Test solution was obtained to be less than 2.0% for MOLNUPIRAVIR

Accuracy and Recovery[3]:

Table No-05

Parameter	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery
50% Recovery	**50**	**49.35**	**99.7**
100% Recovery	**100**	**100.1**	**100.1**
150% Recovery	**150**	**148.95**	**99.3**
Mean	-	-	**99.3**
%RSD	-	-	**0.8**

Observation:

- The % Recovery was obtained between 98.0 to 102.0%, Mean % Recovery was obtained between 98.0 to 102.0% for MOLNUPIRAVIR and %RSD obtained for all %recoveries was less than 2.0%

Intermediate Precision[3]:

Table No-05 Anlyst-01 vs. Analyst-02

S.No.	Solution details	%Assay
Analyst-01	%Assay mean from method Precision_ Analyst-01	98.8
Analyst-02	%Assay mean from intermediate Precision_ Analyst-02	98.9
Average		**98.9**
Std Dev		**0.1**
%RSD		**0.1**

Observation: Cumulative % RSD of % Assay results was obtained less than 2.0 for both analysts-I & II of MOLNUPIRAVIR and Cumulative Mean of %Assay for both analysts-I & analysts-II obtained to be between 90.0 to 110.0% for MOLNUPIRAVIR

Robustness[3]:

Table No-06

Name of the Parameter	%RSD	Theoretical Plates	Tailing factor
Low Column Oven Temperature(30°C)	0.36	6589	1.09
Low Column Oven Temperature(40°C)	0.85	6047	1.07
Lower Flow rate(0.4mL)	0.48	7085	1.09
Higher Flow rate(0.6mL)	0.31	6022	1.01

Observation: System suitability met the acceptance criteria in Robustness parameters hence method is Robust.

DISCUSSION:

The proposed method for the estimation of Molnupiravir in bulk and capsule dosage forms was found to be sensitive, specific, simple, accurate, economical, and rapid. The method was validated as per the current ICH Q2 (R1) guidelines. Linearity of standard calibration yielded a correlation coefficient (r^2) of 0.999 for Molnupiravir at 237nm. The values of % RSD are within the prescribed limit of 2% in system precision, showing high precision of methods, and recovery was close to 100% for Molnupiravir. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their estimation with virtual interference of any additive present in pharmaceutical formulations. In Forced Degradation study, purity flag was absent and Purity threshold is greater than Purity angle. Hence, the above methods can be applied successfully for estimation of Molnupiravir and method is stability indicating.

CONCLUSION:

Method was found to Rapid reliable, sensitive, accurate, specific, robust and rugged method was described for the estimation of pharmaceutical dosage form (Capsules) consisting of Molnupiravir. Active ingredient was successfully resolved and quantified using UPLC with Waters Acquity BEH C18(100mmx2.1mm ID) 1.7µm column in a relatively short run time of 8 minutes with 0.5mL/min flow in Isocratic mode of the chromatographic system. The developed and validated method in that Accuracy, linearity, range, precision, and robustness were found to be more accurate, Linear, precise, and reproducible. The method was found to be simple and time and cost saving. The proposed method can be applicable for the routine analysis in quality control laboratories.

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