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Development and validation for the quantitative determination of Molnupiravir in bulk and marketed pharmaceutical dosage form by RP-HPLC Method

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Abstract

A simple, accurate, precise and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method has been developed, which can separate and quantitatively estimate Molnupiravir in bulk and marketed pharmaceutical dosage form. The chromatographic separation for Molnupiravir was achieved with mobile phase containing Acetonitrile and Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70% v/v and Symmetry C18 ODS (4.6mm × 250mm) 5µm particle size column in isocratic mode at room temperature and UV detection at 246nm. The compound was eluted at a flow rate of 1.0ml/min. The retention time of Molnupiravir were found to be 5.493min. The above method was validated in terms of system suitability, linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) in accordance with ICH guidelines. The method was rapid, simple, economical and suitable for routine quality control analysis.

Keywords: Molnupiravir, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines

Introduction

Molnupiravir is a nucleoside analogue that is N (4)-hydroxycytidine in which the 5'-hydroxy group is replaced by a (2-methylpropanoyl) oxy group. It is the prodrug of the active antiviral ribonucleoside analog N (4)-hydroxycytidine (EIDD-1931), has activity against a number of RNA viruses including SARS-CoV-2, MERS-CoV, and seasonal and pandemic influenza viruses. It is currently in phase III trials for the treatment of patients with COVID-19. It has a role as a prodrug, an anticoronaviral agent and an antiviral drug [1]. It is a nucleoside analogue, an isopropyl ester and a ketoxime. It is functionally related to an N (4)-hydroxycytidine. Molnupiravir is a ribonucleoside analogue and antiviral agent that is used in the therapy the severe acute respiratory syndrome (SARS) coronavirus 2 (CoV-2) infection, the cause of the novel coronavirus disease, 2019 (COVID-19). Molnupiravir therapy is given orally for 5 days early in the course of SARS-CoV-2 infection and has not been linked to serum aminotransferase elevations or to clinically apparent liver injury [2]. Molnupiravir is an orally bioavailable prodrug of EIDD-1931, the synthetic ribonucleoside derivative N4-hydroxycytidine and ribonucleoside analog, with potential antiviral activity against a variety of RNA viruses. Upon oral administration, Molnupiravir, being a prodrug, is metabolized into its active form EIDD-1931 and converted into its triphosphate (TP) form. The TP form of EIDD-1931 is incorporated into RNA and inhibits the action of viral RNA-dependent RNA polymerase [3]. This results in the termination of RNA transcription and decreases viral RNA production, and viral RNA replication. The IUPAC Name of Molnupiravir is [(2R, 3S, 4R, 5R)-3, 4-dihydroxy-5-[4-(hydroxy amino)-2-oxopyrimidin-1-yl] oxolan-2-yl] methyl 2-methyl propanoate. The Chemical Structure of Molnupiravir is shown in follows

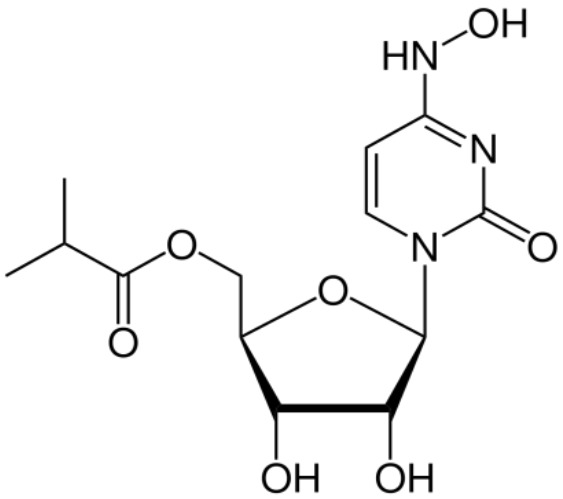

Fig 1: Chemical Structure of Molnupiravir

Materials and Methods

Materials and Instruments

The following are the list of instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Molnupiravir [4].

Equipment's

Table 1: List of Equipments

S. No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C_{18} Column, 250 mm x 4.6 mm and 5µm particle size
7.	P^H Analyser (ELICO)
8.	Vaccum Filtration Kit (Labindia)

Chemicals and Reagents

Table 2: List of Chemicals used

S. No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

Method Development

HPLC Instrumentation & Conditions: The HPLC system employed was HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.

Standard Preparation for UV-Spectrophotometer Analysis

The Standard Stock Solutions – 10 mg of Molnupiravir standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration.

It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Molnupiravir, so that the same wave number can be utilized in HPLC UV detector for estimating the Molnupiravir.

Selection of Chromatographic Methods

The proper selection depends upon the nature of the sample, (ionic or ion stable or neutral molecule) its molecular weight and stability. The drugs selected are polar, ionic and hence reversed phase chromatography was selected [5].

Optimization of Column

The method was performed with various columns like Hypersil C_{18} column, X- bridge column and X-terra (4.6 ×150mm, 5µm particle size), Symmetry C18 ODS (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow [6].

Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and Water: Acetonitrile and Methanol with TEA Buffer with

varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate buffer (0.01M, pH-3.2) in the ratio of 30:70 respectively.

Estimation of Molnupiravir in Bulk and Pharmaceutical Dosage Form

Procedure

- **Preparation of Mobile Phase:** Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter.
- **Preparation of 0.01M Potassium Dihydrogen Orthophosphate Buffer Solution:** About 1.36086grams 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 3.20 with diluted orthophosphoric acid.
- **Diluent Preparation:** Accurately measured 300 ml (300%) of HPLC Grade Acetonitrile and 700 ml of Phosphate buffer (70%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filter [7].

Assay

Preparation of the Molnupiravir Standard Solution

Preparation of Standard Solution: (Molnupiravir)

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

Further pipette 0.1ml of Molnupiravir from stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

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 [8].

Preparation of Sample Solution: Take average weight of Tablet and crush in a mortar by using pestle and taken weight 10 mg equivalent weight of Molnupiravir sample into a 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.

Procedure: Further pipette 0.1ml of Molnupiravir from above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Analytical Method Validation

Validation is a process of establishing documented evidence which provide a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specification and quality characteristics [9-11].

System Suitability

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standards required by a method. A system suitability evaluation usually contains its own set of parameters. For chromatographic assays, these may include tailing factor, resolution, precision, capacity factor time and theoretical plates [12].

Accuracy

- **For Preparation of 50% Standard Stock Solution:** Further pipette 0.05 ml of Molnupiravir from stock solutions in to a 10 ml volumetric flask and dilute up to the mark with diluent.
- **For Preparation of 100% Standard Stock Solution:** Further pipette 0.1 ml of Molnupiravir from stock solutions in to a 10 ml volumetric flask and dilute up to the mark with diluent.
- **For Preparation of 150% Standard Stock Solution:** Further pipette 0.15 ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
- **Procedure:** Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Molnupiravir and calculate the individual recovery and mean recovery values [13].
- **Acceptance Criteria:** The % RSD for each level should not be more than 2.

Precision

Repeatability

Preparation of Molnupiravir for Precision: Further pipette 0.1 ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent

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

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different days by maintaining same conditions.

Procedure

- **Day 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.
- **Day 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.
- The % RSD for the area of five standard injections results should be not more than 2%.

Linearity

- **Preparation of Level – I (6 µg/ml of Molnupiravir):** Further pipette 0.06 ml of Molnupiravir from stock solutions in to a 10 ml volumetric flask and dilute up to the mark with diluent.
- **Preparation of Level – II (8 µg/ml of Molnupiravir**):

Further pipette 0.08 ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.

- **Preparation of Level – III (10 µg/ml of Molnupiravir):** Further pipette 0.1ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
- **Preparation of Level – IV (12 µg/ml of Molnupiravir):** Further pipette 0.12ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the mark with diluent.
- **Preparation of Level – V (14 µg/ml of Molnupiravir):**
- Further pipette 0.14ml of Molnupiravir from stock solutions in to a 10ml volumetric flask and dilute up to the 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 [14].

- **Acceptance Criteria:** Correlation coefficient should be not less than 0.999.
- **Limit of Detection:** The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected.

Limit of Quantitation

The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision [15].

- **Robustness:** The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .
- **Effect of Variation of Flow Rate:** The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl 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 70:30, 75:25 instead of 65:35, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.
- **Forced Degradation Studies:** The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient [16].
- **Acid Degradation Studies:** To 1 ml of Molnupiravir stock, 1 ml of 2N HCl was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N NaOH and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.
- **Alkali Degradation Studies:** To 1 ml of stock solution of Molnupiravir 1 ml of 2N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml 2N HCl and makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. The sample of 20µl was injected into the system, and the chromatograms were recorded to an assessment of sample stability.
- **Oxidation Degradation Studies:** To 1 ml of stock solution of Molnupiravir 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 30 min at 60ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded to assess the stability of the sample.
- **Dry Heat Degradation Studies:** The 1 ml of standard drug solution was placed in the oven at 60ºC for 6h to study dry heat degradation. For HPLC study, the resultant solution was makeup to final volume to obtain (10µg/ml) solution. Cool the solution to room temperature and filtered through a 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.
- **Photo Degradation Studies:** The photo stability of the drug was studied by exposing the stock solution to UV light for 1day or 200Watt-hours/m2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) solution and filtered with 0.45µm membrane filter. A sample of 20µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.
- **Water Degradation Studies:** To 1 ml of stock solution of Molnupiravir, 1 ml of distilled water was added. The solution was kept aside for 30 min at 60 ºC. For HPLC study, the resultant solution was diluted to obtain (10µg/ml) cool the solution to room temperature and filtered with 0.45µm membrane filter. A sample of 20µl was injected into the HPLC system, and the chromatograms were recorded for the assessment of sample stability.

Results and Discussion

Analytical Method Development

Selection of Wavelength: The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum of Molnupiravir was obtained and the Molnupiravir showed absorbance's maxima at 246nm [17]. The UV spectra of drug are follows:

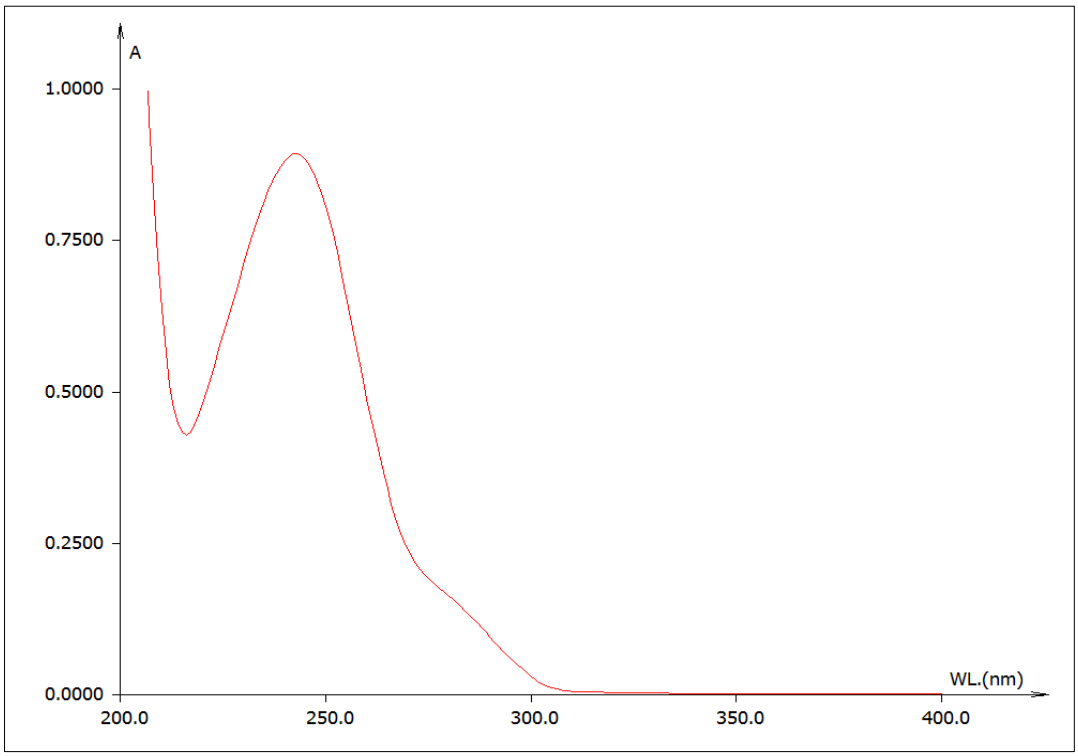

Fig 2: UV Spectrum of Molnupiravir (246 nm)

Observation: While scanning the Molnupiravir solution we observed the maxima at 246nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Optimized Chromatographic Conditions

Mobile phase : Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70v/v)
Column : Symmetry C18 ODS (4.6mm×250mm) 5µm particle size
Flow rate : 1 ml/min
Wavelength : 246 nm
Column temp : Ambient
Injection Volume : 20 µl
Run time : 10 minutes

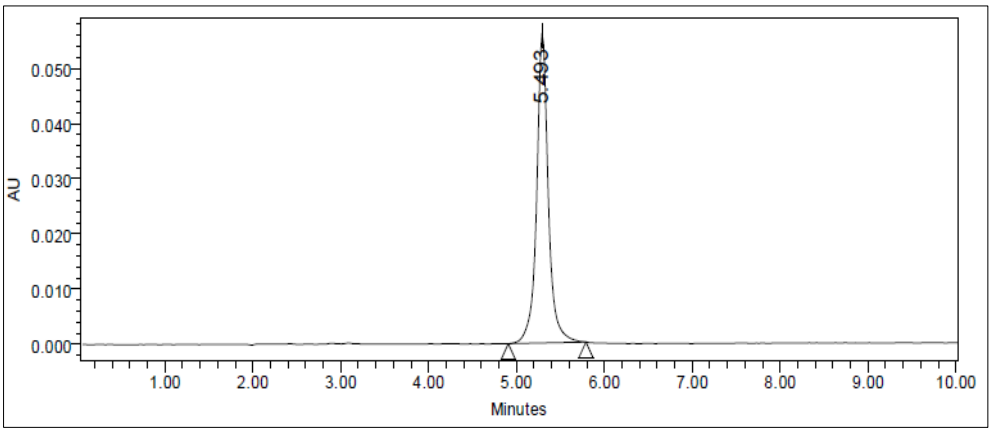

Fig 3: Optimized Chromatographic Condition

Method Validation

The validation has been done according to ICH guidelines. The proposed method has validated with respect to specificity, linearity, the limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and robustness [18].

System Suitability

The system suitability parameters were found to be within the specified limits for the proposed method.

Table 3: Observation of System Suitability Parameters

S. No.	Parameter	Molnupiravir
1.	Retention Time (min)	5.453
2.	Theoretical Plates	6967
3.	Tailing factor	1.12
4.	Peak Area (AUC)	647856

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components [19].

Analytical method was tested for specificity to measure accurately quantitates Molnupiravir in drug product.

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Molnupiravir in present in the marketed pharmaceutical dosage form was found to be 99.85%.

Linearity

Table 4: Chromatographic Data for Linearity Study of Molnupiravir

Concentration µg/ml	Average Peak Area
6	468784
8	615798
10	768759
12	925748
14	1078765

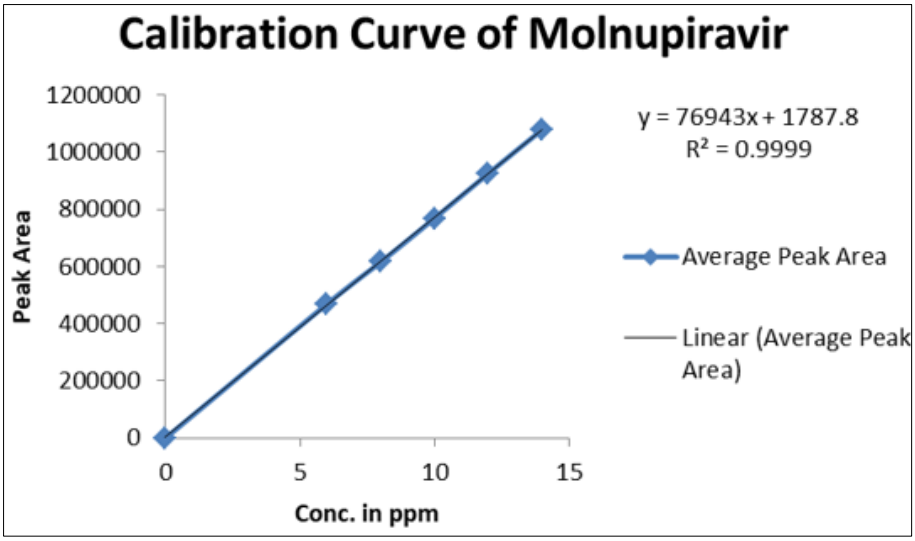

Fig 4: Calibration Curve of Molnupiravir

Linearity Plot: The plot of Concentration (x) versus the Average Peak Area (y) data of Molnupiravir is a straight line.
Y = mx + c
Slope (m) = 76943
Intercept (c) = 1787
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 76943. These values meet the validation criteria.
- **Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [20].
- **Repeatability**: Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table 5: Results of Repeatability for Molnupiravir

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Molnupiravir	5.419	645784	83685	6825	1.05
2	Molnupiravir	5.405	642589	84932	6849	1.09
3	Molnupiravir	5.478	643658	85847	6845	1.08
4	Molnupiravir	5.466	648759	86295	6839	1.09
5	Molnupiravir	5.493	649657	86587	6895	1.07
6	Molnupiravir	5.466	647854	87853	6874	1.10
Mean			646383.5			
Std. Dev			2853.319			
% RSD			0.441428			

Intermediate Precision/Ruggedness

Analyst 1

Table 6: Results of Intermediate Precision for Molnupiravir

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USPT ailing
1	Molnupiravir	5.484	636854	84863	6758	1.09
2	Molnupiravir	5.493	637489	84759	6726	1.08
3	Molnupiravir	5.406	635762	84685	6749	1.09
4	Molnupiravir	5.419	636984	84697	6698	1.07
5	Molnupiravir	5.446	634856	84258	6728	1.08
6	Molnupiravir	5.452	639689	84753	6699	1.08
Mean			636939			
Std. Dev.			1649.149			
% RSD			0.258918			

Analyst 2

Table 7: Results of Intermediate Precision Analyst 2 for Molnupiravir

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USPPlate Count	USPTailing
1	Molnupiravir	5.491	628985	85698	6985	1.09
2	Molnupiravir	5.482	624879	85479	6899	1.07
3	Molnupiravir	5.416	625846	85748	6928	1.06
4	Molnupiravir	5.482	623568	85647	6874	1.09
5	Molnupiravir	5.495	628985	85246	6984	1.07
6	Molnupiravir	5.427	628473	85924	6872	1.08
Mean			626789.3			
Std. Dev.			2340.636			
% RSD			0.373433			

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated [21].

Table 8: The Accuracy Results for Molnupiravir

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	386559	5	5.00	100.000%	100.130%
100%	768536	10	9.965	99.650%	
150%	1164522	15	15.111	100.740%	

Limit of Detection for Molnupiravir

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 [22].

$LOD = 3.3 \times \sigma / s$

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

Result: = 0.487µg/ml

Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined [23].

$LOQ = 10 \times \sigma / S$

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

Result: = 1.477µg/ml

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. The standard of Molnupiravir was injected by changing the conditions of chromatography [24]. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 9: Results for Robustness

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	648759	5.484	6845	1.08
Less Flow rate of 0.9 mL/min	635248	5.599	6786	1.09
More Flow rate of 1.1 mL/min	659865	4.576	6528	1.05
Less organic phase	625986	7.415	6689	1.03
More organic phase	615869	3.827	6354	1.01

Forced Degradation Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, thermal, UV, water degradations. The sample was exposed to these conditions the main peak of the drug was studied for peak purity that indicating the method effectively separated the degradation products from the pure active ingredient [25].

Fig 10: Results of Forced Degradation Studies for Molnupiravir

S. No.	Stress Condition	Peak Area	% of Degraded Amount	% of Active Amount	Total % of Amount
1	Standard	648759	0	100%	100%
2	Acidic	539378.232	16.86	83.14	100%
3	Basic	603540.497	6.97	93.03	100%
4	Oxidative	545217.063	15.96	84.04	100%
5	Thermal	616450.801	4.98	95.02	100%
6	Photolytic	533344.773	17.79	82.21	100%
7	Water	625079.296	3.65	96.35	100%

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 Symmetry C18 ODS (4.6 mm×250 mm) 5µm particle size because it was giving good peak. Ambient temperature was 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 Acetonitrile: Phosphate buffer (0.01M, pH-3.2) (30:70 v/v) 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 10min because analyze gave peak around 5.453min 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 6-14ppm of the Molnupiravir target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

The % RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was 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 drug and in Pharmaceutical dosage forms.

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