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COMPARATIVE ASSAY SCREENING OF MOLNUPIRAVIR BY UV SPECTROSCOPY AND LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple, rapid and sensitive validated UV-Spectrophotometry and RP-liquid chromatographic method have been developed for the assay of Molnupiravir in Bulk and Pharmaceutical dosage form. Chromatographic separation achieved on a C18 SpolarG 250×4.6mm column in Isocratic elution with mobile phase containing water methanol, acetonitrile in ratio (75 : 15 : 10 v/v). The flow rate was 1 mL/min and effluents were monitored at 235nm. The elution/retention time of molnupiravir at 6.01 min. LOD and LOQ for HPLC method was 0.125µg/mL and 0.380 µg/mL respectively. U.V absorption spectra were screened in the range in between 200-400 nm and the assay for molnupiravir absorption set at λ maximum 235nm, the developed method was validated according to ICH guidelines. The linearity data of molnupiravir are2 to 12 µg/mL, LOD and LOQ are 0.230µg/mL and 0.699µg/mL respectively. The comparative assay studies were performed using UV and liquid chromatography. The developed method was successfully applied to bulk drugs as well as in Pharmaceutical dosage form.

Keywords: UV Spectroscopy, RP -HPLC, method development, Molnupiravir

INTRODUCTION

Antiviral Drugs are used to treat infections caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Molnupiravir **(Figure 1)** is an formulated oral antiviral drug having its effect on nasopharyngeal SARS-COV-2, thus preventing the proliferation of viral RNA by targeting RNA dependent RNA polymerase of the virus **[1, 2]**. Molnupiravir is an antiviral drug approved by Food and drug administration for treating mild to moderate infections caused by SARS-CoV-2. Molnupiravir enhances the frequency of viral RNA mutations in animal models and humans **[3, 4]**. It is a white crystalline powder with molecular weight 329.31 g/mol and its IUPAC name as [(2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate **[5]**.

By an extensive literature review we found that very few studies have been reported for the determination of molnupiravir using High-Performance Liquid Chromatography **[6-7]**. Another study on, LC-MS/MS studies for determination of Molnupiravir and its metabolite in human plasma **[8-9]**. The reversed-phase technique **[10-14]** is the method of preference in analysis due to its ease of use, adaptability, and diversity of applications, which include handling compounds with a variety of polarities and molecular masses. UV spectroscopic methods for analysis of Molnupiravir in pure and pharmaceutical dosage forms have not been reported. The present study involves a comparative assay of molnupiravir by methods of UV spectroscopy and Liquid chromatography. The developed methods were successfully validated and can be used to estimate the total drug content in the commercially available formulations of Molnupiravir.

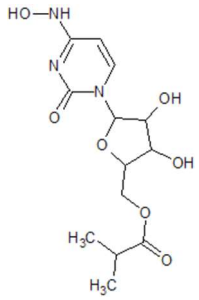

Figure 1: Chemical structure Molnupiravir

EXPERIMENTAL

Instrumentation:

All spectral measurements were made on LAB INDIA T60 UV spectrometer connected to a computer, loaded with microwave progression software. ELITE analytical balance, HPLC was shimadzu LC-20 AD HPLC with a binary pump, and a UV SPD-20A detector are used. Rheodyne injector fitted with a 20µL was used and data were recorded and analyzed using LC

solutions software. C18 Spolar column (250×4.6mm, 5µm particle) was used.

Material & Reagents:

A sample of Molnupiravir with a purity of 99.8 w/w purity was obtained. Chemicals and reagents are of Analytical grade. Molnupiravir of 300mg with a brand name MOLFLU was purchased from the pharmacy.

Preparation of standard stock solution (1000 µg/ml)

A standard drug solution of Molnupiravir was prepared by adding 100mg of the drug into a 100mlvolumetric flask and made up to the mark with mobile phase diluent to get a concentration of 1000 µg/ml.

Preparation of working standard solution (100 µg/ml)

From the above standard stock solution 10ml of the sample solution was transferred to a 100mlvolumetric flask and made up to the mark with mobile phase diluent to get concentration of 100 µg/ml.

Chromatographic Parameters:

The Reverse phaseseparation was performed using C18 SpolarG HPLC packed column, Shiseido, Japan with specifications (250 mm X 4.6 mmX 5µm) isocratic elution with mobile phase containing water, acetonitrile and methanol in ratio(75 : 15 : 10 v/v) with flow rate 1 mL/min at ambient temperature with sample injection quantity of 15µL. The analysis was performed at wavelength 235 nm. The runtime for analysis was 10 minutes.

HPLC Method Development:

Aliquots of the solution containing molnupiravir was prepared and a number of trials were performed for the optimization for separation of drug using different C18columns, different mobile phases and chromatographic conditions. The method optimized with C18 SpolarG 250×4.6mm column in isocratic separation mode. The optimized mobile phase was with mobile phase containing water, acetonitrile and methanol in ratio (75 : 15 : 10 v/v) at flow rate 1mL per minute and detector wavelength 235 nm. The injection volume was 15 µL and run time is 10 minutes at ambient temperature.

UV Method Development:

The working standard solution of Molnupiravir (8 µg/mL) was prepared in a volumetric flask by using a diluent in following ratio water, acetonitrile and methanol in ratio (75: 15: 10 v/v) and was scanned in the range 200-400nm using UV Spectrophotometer against a blank. The absorption spectra showed the most appropriate wavelength for analyzing Molnupiravir with suitable sensitivity at 235 nm **(Figure 2)**.

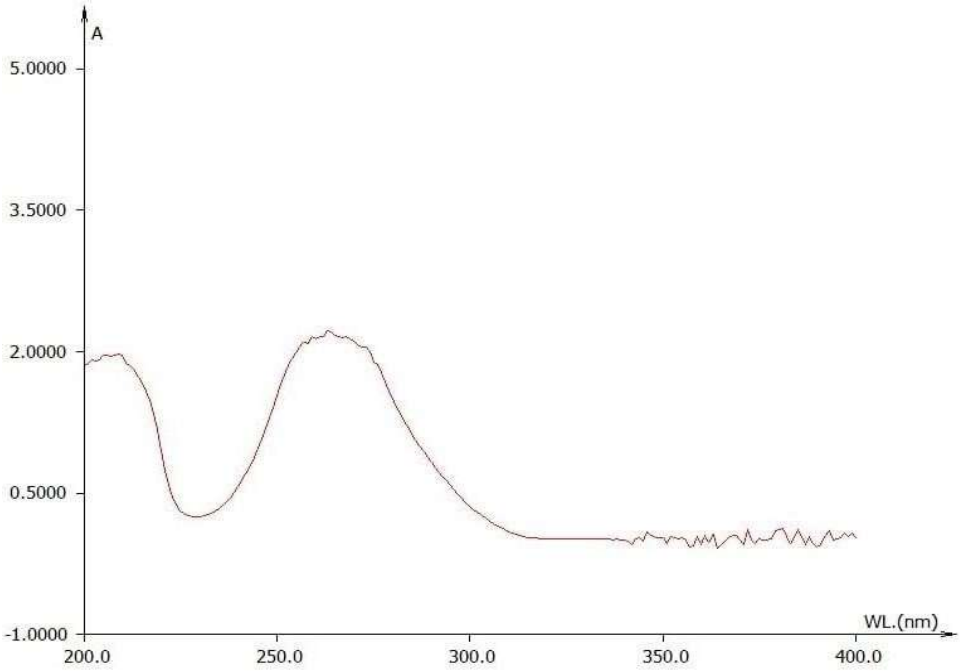

Figure 2: UV absorption Spectra of Molnupiravir

RESULTS AND DISCUSSION

Method Validation

System suitability:

The HPLC system suitability was estimated by analyzing the standard solution containing 8 µg/mL of molnupiravir. The standard solution was analyzed for six replicate times to determine the suitability of the system. The results of system suitability showed the %RSD for molnupiravir were less than 2%, tailing factor were less than 1.5 and theoretical plates column efficiency were more than 2000 indicating the system suitability for analysis.

Specificity

The individual standard analyte molnupiravir of concentration 8µg/mLwas prepared and chromatographically analyzed to know the retention time. Specificity of the method was established by comparing the chromatograms of blank versus the standard solution **(Figure 2)** and found there was no interference of the diluent at the retention time of the analyte in the standard solution.

Linearity

Various percentage aliquots of molnupiravir ranging from 25 to 150% of working standard specification were prepared and analyzed by UV spectrometry and HPLC **(Table 1)**. The calibration curve was plotted between concentration versus response to

establish the method linearity **(Figure 3)**. The correlation coefficient obtained from the curve was 0.999, indicating good method linearity.

Precision:

The precision was determined at the levels of the method, system and intermediate. The working standard at 100% level with six determinations was used for the precision study. The results obtained showed the % RSD at below 2, indicating the method precision. The results of the system precision are tabulated in the **Table 2**. The results of the intermediate precision are tabulated in **Table 3**.

Accuracy:

The method accuracy was determined by spiking known concentration of molnupiravir to the fixed formulation concentration of equivalent to 8µg/mL for UV spectroscopic and HPLC methods. The accuracy was tested at three levels (50%, 100% and 150%) of working concentration. At each accuracy level, triplicate samples were prepared and a total of nine determinations were done for each method. The % Recovery±SD by UV spectroscopic and HPLC chromatographic method for 9 determinations was 99.13 ± 0.45 and 99.09 ± 0.34 respectively. The results of % Recovery for UV spectroscopic method and HPLC methods at different levels are tabulated in **Table 4**.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

Limit of Detection and Limit of Quantification were determined from the calibration curve using Y-intercept's standard deviation and the slope of the curve. Signal of LOD should be 3 times more than the noise and the signal of LOQ should be 10 times more than the noise. LOD and LOQ for UV spectrometric method were found to be 0.230µg/mL and 0.699µg/mL respectively. LOD and LOQ for HPLC method were found to be 0.125µg/mL and 0.380µg/mL respectively.

Robustness*:*

The small variations in the method were done deliberately to prove the method robustness. The robustness study for HPLC was done by deliberate changes in mobile phase, flow rate and injection volume. The %RSD is found to be less than 2% indicating method robustness **(Table 5)**. The robustness for the UV spectroscopic method was done by small deliberate changes in the lambda maximum. The results are tabulated in **Table 6**.

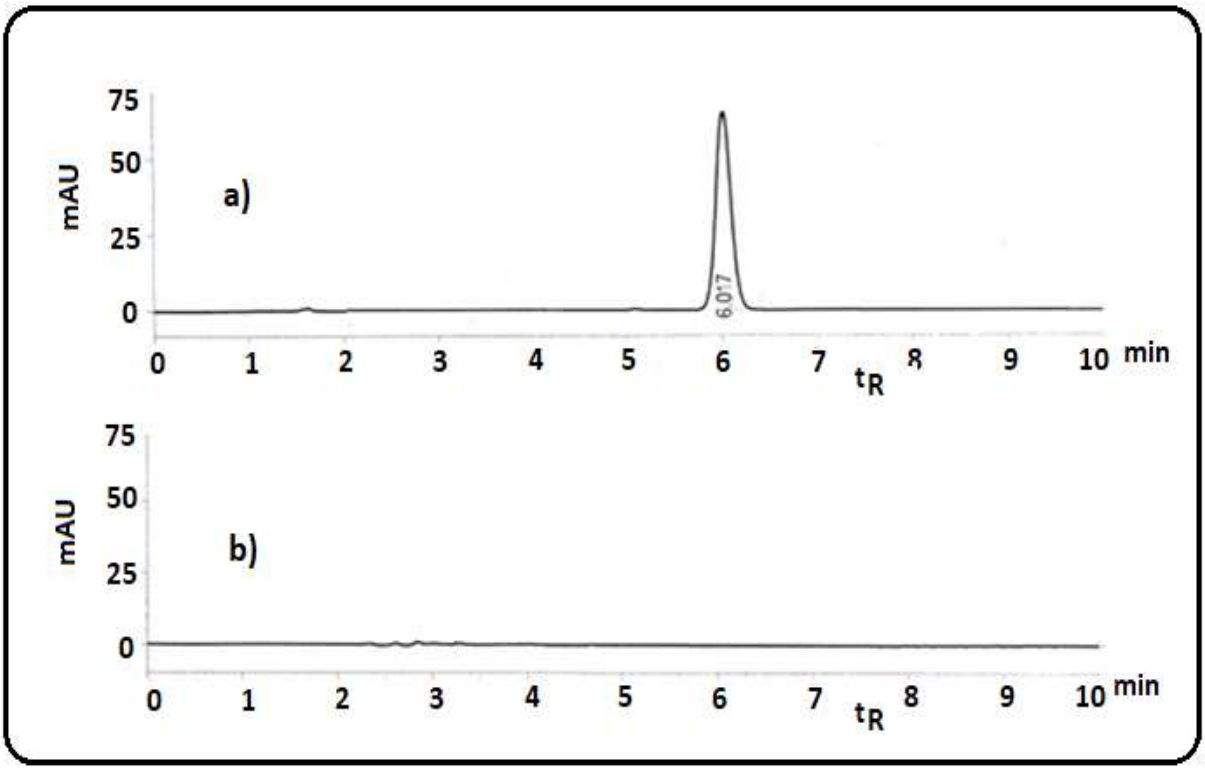

Figure 2: Chromatograms of A) Standard B) Blank

Table 1: Linearity for molnupiravir

Level	UV Spectroscopy		RP-HPLC	
	Conc. [µg/mL]	U.V Abs.	Conc. [µg/mL]	Peak area
25%	2	0.1231	2	77344
50%	4	0.1924	4	150688
75%	6	0.2687	6	231032
100%	8	0.3384	8	309177
125%	10	0.4084	10	385721
150%	12	0.4754	12	463590

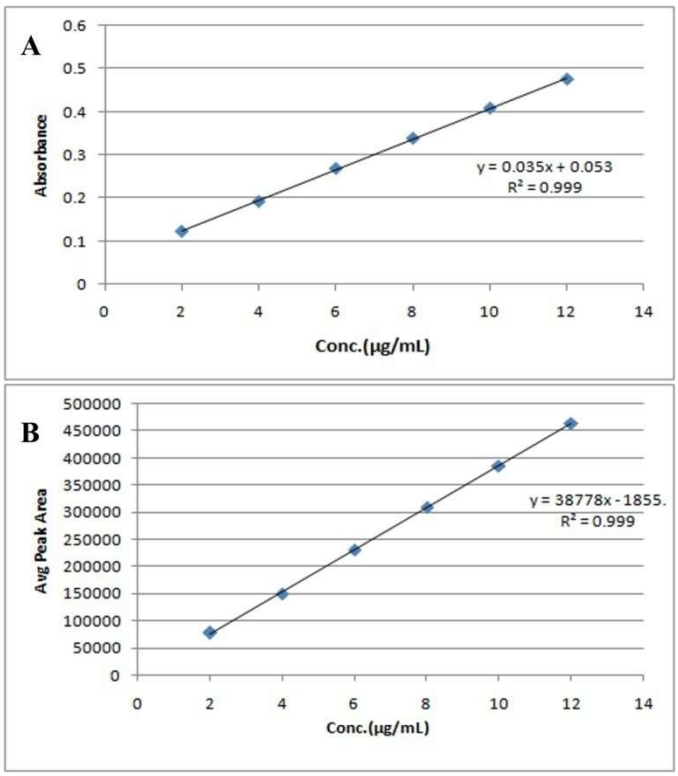

Figure 3: Calibration curves of molnupiravir A) UV Spectrometry B) HPLC

Table 2: Results of System Precision

Sample no.	UV Spectrometry	HPLC
	Absorbance	Peak Area
1	0.3312	308179
2	0.3391	308874
3	0.3312	309141
4	0.3345	309647
5	0.3387	312047
6	0.3336	312954
Mean	0.3347	310140
S.D	0.003	1910.21
%RSD	1.04	0.61

Table 3: Results for intermediate precision

Sample No.	Analyst-I /Day-I /Equip-I		Analyst-II /Day-II/ Equip-II	
	UV [Abs.]	HPLC [Area]	UV [Abs.]	HPLC [Area]
1	0.3312	308179	0.3401	310378
2	0.3391	308874	0.3431	311357
3	0.3312	309141	0.3498	311987
4	0.3345	309647	0.3424	313354
5	0.3387	312047	0.3401	313645
6	0.3336	312954	0.3461	313947
Mean	0.3347	310140	0.3436	312444.6
SD	0.0035	1910.21	0.00377	1427.4
%RSD	1.04	0.61	1.09	0.45

Table 4: Summary of %Recovery and %RSD.

Level	Actual conc. [µg/mL]	Measured conc. [µg/mL]	Recovery [%]	Avg	%RSD
Percentage recoveryby UV Spectrometry					
50.00%	6	5.93	98.83	98.83	0.67
	6	5.89	98.16		
	6	5.97	99.5		
100.00%	8	7.97	99.62	99.33	0.26
	8	7.93	99.12		
	8	7.94	99.25		
150.00%	10	9.89	98.9	99.23	0.35
	10	9.92	99.2		
	10	9.96	99.6		
Percentage recovery by HPLC					
50.0%	6	5.92	98.66	99.16	0.44
	6	5.97	99.50		
	6	5.96	99.33		
100.0%	8	7.88	98.50	98.87	0.37
	8	7.91	98.87		
	8	7.94	99.25		
150.0%	10	9.91	99.10	99.23	0.15
	10	9.94	99.40		
	10	9.92	99.20		

Table 5: Robustness Study for HPLC method

Deliberate Changes	Peak Area [%RSD]
Actual Chromatographic conditions	0.61
Change-1,(H_2O: ACN:CH_30H); 77 :14:9	0.73
Change -2, (H_2O: ACN:CH_30H); 73:16:11	0.69
Change -3, Flow Rate: 1.2 mL/min	1.04
Change -4, Flow Rate: 0.8 mL/min	1.12
Change -5, Injection volume : 10.0 µL	0.68
Change -6, Injection volume : 20.0 µL	0.58

Table 5: Robustness Study for UV Spectroscopic method

Concentration	Absorbance		
(µg/ml)	234nm	235nm	236nm
8	0.3324	0.3312	0.3391
8	0.3347	0.3391	0.3412
8	0.3369	0.3312	0.3425
8	0.3312	0.3345	0.3467
8	0.3398	0.3387	0.3487
8	0.3304	0.3336	0.3404
AVG	0.334233	0.334717	0.3431
SD	0.003621	0.003496	0.003784
%RSD	1.0835	1.044451	1.102783

CONCLUSION:

A simple, rapid and sensitive validated UV-Spectrophotometry and RP-liquid chromatographic method have been developed for the assay of Molnupiravir in Bulk and Pharmaceutical dosage form. The developed method was validated in terms of accuracy, precision, range, LOD, LOQ, robustness studies according to ICH guidelines. The comparative assay studies were performed using UV and liquid chromatography. The validated analytical method was successfully applied to bulk drugs as well as in Pharmaceutical dosage form in pharma industries.

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