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Quantitative estimation of molnupiravir by UV- Spectrophotometric method

Pritam Jain[1],*, Manali Bhamare[1], Sanjay Surana[1]

[1] *Dept. of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India=*

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ABSTRACT

A simple, rapid, accurate and economical UV-spectrophotometric method has been developed for estimation of Molnupiravir from bulk and pharmaceutical formulation. The λ_{max} of Molnupiravir in Distilled water was found to be 235 nm.
The *Method A* is for zero order of drug and *Method B* for Zero order AUC. The AUC of the drug was found to be in the range of 228.00 – 243.40 nm. The drug follows linearity in the concentration range 5-30 μg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated 99.99 % was found in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels i.e., 80%, 100% and 120 % w/w. The % recovery was found to be in the range 98.15%– 99.97% for method A and 98.85% — 99.56% for method B. The low values of % R.S.D. are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intra-day, inter-day variations and repeatability. The % R.S.D. value less than 2 indicate that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts. The above method was a rapid and cost-effective quality-control tool for routine analysis of Molnupiravir in bulk and in pharmaceutical dosage form.

1. Introduction

Coronaviruses are single-stranded, positive-sense, and enveloped RNA viruses that can cause zoonosis and are named for the appearance of a solar corona under an electron microscope. The third coronavirus, SARS-CoV-2, which is causing the global pandemic of coronavirus disease 2019 (COVID-19), was initially identified in December 2019 in China, alongside the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV).[1]

Molnupiravir is an antiviral medication candidate for the treatment of COVID-19 patients that is now in phase III trials.[2] Molnupiravir is an isopropyl ester prodrug that is converted into an active nucleoside analogue -D-N4-hydroxycytidine (NHC) or EIDD-1931 by host esterases in the plasma.[3] NHC exhibits antiviral activity against a variety of positive-and negative-sense RNA viruses. Recent research has centred on the development of molnupiravir for the treatment of influenza and coronavirus infections, respectively.[4] Molnupiravir is a pyrimidine ribonucleoside analogue having a chemical name of ((2R, 3S, 4R, 5R)-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1-(2H)-yl)-tetrahydrofuran-2-yl) methyl isobutyrate.[5] The literature survey revealed that two methods of analysis for Molnupiravir have been reported, which included LC-MS/MS for the quantification of β-D-N4-hydroxycytidine in human plasma and β-D-N4-hydroxycytidine-triphosphate in peripheral blood mononuclear cell lysates;[6] and novel LC-MS/MS method for the simultaneous quantification of

* *Corresponding author*.
E-mail address: pritash79@yahoo.com (P. Jain).

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Molnupiravir and its metabolite β -d-N4-hydroxycytidine in human plasma and saliva.[7] Accordingly, the objective of this study was to develop and validate the first order derivative method for the estimation of Molnupiravir in bulk and pharmaceutical formulation as per ICH guidelines.[8]

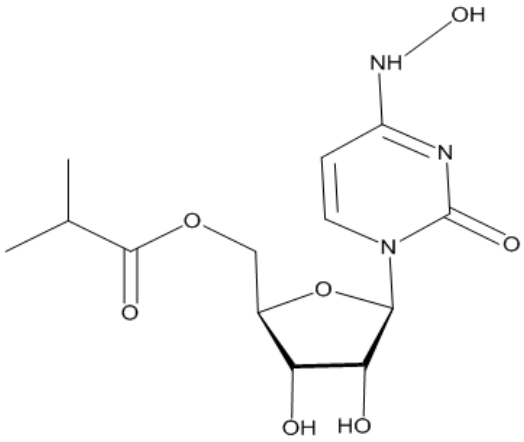

Fig. 1: Chemical structure of molnupiravir

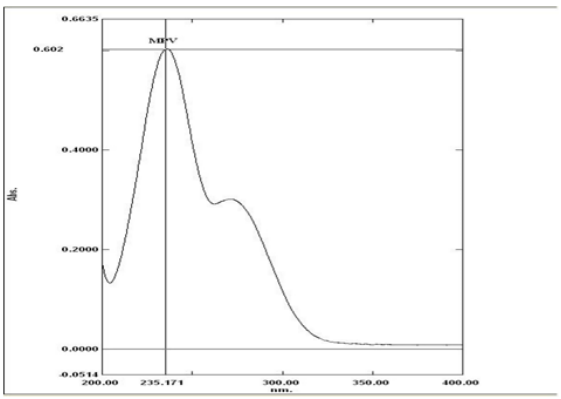

Fig. 2: UV spectrum of molnupiravir 235 nm

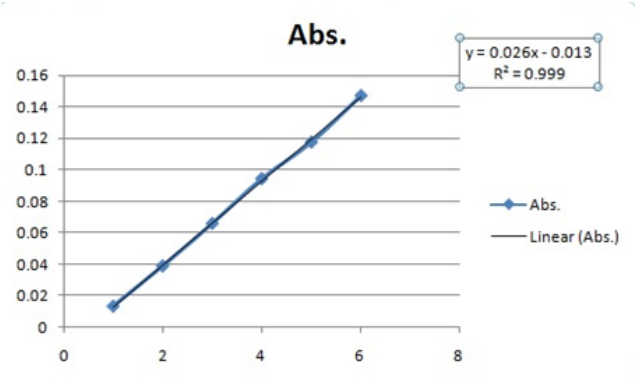

Fig. 3: Calibration curve of Molnupiravir 235 nm

2. Materials and Methods

2.1. Materials

Molnupiravir was bought from Alain Pharmaceuticals, Hyderabad, Telangana. As the solubility of drug Molnupiravir is freely soluble in water, a distilled water is used as a solvent.

2.2. Preparation of standard stock solution

Accurately weighed 10 mg of Molnupiravir was transferred to 100 ml volumetric flask and the volume was adjusted by distilled water. The volume was adjusted with the same up to the mark to give final strength of 100 μg/ml.

2.3. Selection of wavelength for analysis of Molnupiravir

Appropriate volume 1 ml of standard stock solution of Molnupiravir was transferred into 10 ml volumetric flask, diluted to mark with distilled water to give concentration of 10μg/ml. The resulting solution was scanned in UV range (200 nm — 400 nm). In spectrum Molnupiravir showed absorbance maximum at 235 nm (Figure 2).

2.4. Validation of the method

The method was validated in terms of linearity, accuracy, precision, and ruggedness.

2.4.1. Linearity study

Different aliquots of Molnupiravir in range 0.5-3 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with distilled water to get concentrations 5, 10, 15, 20, 25 and 30 mg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 235 nm. The calibration plot was constructed as Absorbance versus concentration (Figure 3).

2.4.2. Accuracy

To the pre-analyzed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method.

2.4.3. Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 10, 15 and 20 μg/ml of Molnupiravir solutions for three times in the same day. Inter-day precision was determined by analyzing the 10, 15 and 20 μg/ml of Molnupiravir solutions daily for three days over the period of week.

Table 1: Linearity study of molnupiravir

Sr. No.	Conc. (μg/ml)	Method A Abs. Mean ± SD	% RSD	Method B Area Mean ± SD	% RSD
1.	5	0.2034 ± 0.003	1.4395	0.2376 ± 0.001	0.42
2.	10	0.4037 ± 0.007	1.73	0.4810 ± 0.003	0.62
3.	15	0.6179 ± 0.01	1.61	0.7056 ± 0.009	1.27
4.	20	0.8287 ± 0.01	1.20	0.9661 ± 0.006	0.62
5.	25	1.0377 ± 0.02	1.92	1.1849 ± 0.007	0.59
6.	30	1.2617 ± 0.02	1.58	1.4122 ± 0.006	0.42

Table 2: Recovery studies (Method A)

Drug	Initial Amount (μg/ml)	Amount Added (μg/ml)	Amount Recovered (μg/ml)	% Recovery	% RSD
	15	12	11.99	99.97	0.23
MPV	15	15	14.90	99.34	0.23
	15	18	17.84	99.15	0.17

Table 3: Recovery studies (Method B)

Drug	Initial Amount (μg/ml)	Amount Added (μg/ml)	Amount Recovered (μg/ml)	% Recovery	% RSD
	15	12	11.94	99.50	0.14
MPV	15	15	14.93	99.56	0.13
	15	18	17.93	98.85	0.37

Table 4: Precision studies

	Method A			Method B		
Std. Conc. (μg/ml)	Amt. found (μg/ml)	% Amt. found	% RSD	Amt. found (μg/ml)	% Amt. found	% RSD
Intra-day Precision						
10	10.1154	101.15	1.84	10.0432	100.43	0.073
15	14.7642	98.42	3.43	14.8212	98.80	1.30
20	19.9926	99.96	0.45	19.7255	98.62	1.068
Inter-day Precision						
10	9.7073	97.07	1.31	9.8297	98.29	1.69
15	15.0081	100.05	1.67	14.8921	99.28	0.71
20	19.1780	95.89	0.64	19.30	96.5	1.09

Table 5: Repeatability studies

		Method A		Method B	
Component	Amount taken (μg/ml) (n=6)	Amount found*(%) ± SD	%R.S.D.	Amount found*(%)	%R.S.D.
Molnupiravir	15	99.98 ± 0.10	0.1062	99.96 ± 0.39	0.3971

*average of six estimations

Table 6: Ruggedness studies (Method A)

Component	Amount taken (μg/ml) (n=3)	Amount Found (%) *	
		Analyst I ±S.D.	Analyst II ±S.D.
Molnupiravir	15	99.23 ± 0.82	98.92 ± 0.95

*average of six estimations

Table 7: Ruggedness studies (Method B)

Component	Amount taken (μg/ml) (n=3)	Amount Found (%) *	
		Analyst I ±S.D.	Analyst II ±S.D.
Molnupiravir	15	99.27 ± 0.65	98.89 ± 0.81

Table 8: Analysis of molnupiravir in bulk

Sr. No.	Amount taken (μg/ml)	Method A Amount found	% Amount found	Method B Amount found	% Amount found
1.	15	14.90	99.33	14.99	99.97
2.	15	14.94	99.60	14.93	99.5
3.	15	15.02	100.1	15.04	100.2
4.	15	15.04	100.2	15.14	100.9
5.	15	14.92	99.4	14.94	99.64
6.	15	14.98	99.8	14.99	99.9
	Mean ± SD	14.9 ± 0.05	99.7 ± 0.36	15.01 ± 0.07	100.07 ± 0.51
	% RSD	0.517	0.517	0.37	0.36

Table 9: Analysis of formulation of molnupiravir capsule

Sr. No.	Amount taken (μg/ml)	Method AAmount found	% Amount found	Method BAmount found	% Amount found
1.	15	14.97	99.85	14.95	99.68
2.	15	15.02	100.1	15.04	100.2
3.	15	15	100	15.12	100.8
4.	15	14.98	99.88	14.97	99.80
5.	15	15.02	100.1	15.03	100.2
6.	15	15	100.01	14.99	99.98
	Mean ± SD	14.99 ± 0.02	99.99 ± 0.1	15.01 ± 0.06	100.1 ± 0.39
	% RSD	0.136	0.106	0.407	0.3971

2.4.4. Sensitivity

The sensitivity of measurements of Molnupiravir by the use of the proposed method was estimated in terms of the Limit of Quanfication (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

2.4.5. Repeatability

Repeatability was determined by analyzing 15 μg/ml concentration of Molnupiravir solution for six times.

2.4.6. Ruggedness

Ruggedness of the proposed method is determined for 15 μg/ml concentration of Molnupiravir by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions.

2.5. Determination of molnupiravir bulk

Accurately weighed 10 mg of Molnupiravir was transferred into 100 ml volumetric flask containing distilled water and volume was made up to the mark using same. Appropriate volume 1.5 ml of this solution was transferred to 10 ml volumetric flask and volume was adjusted to mark using distilled water. The resulting solution was scanned on spectrophotometer in the UV range 200 – 400 nm. The concentrations of the drug were calculated from linear regression equations.

2.6. Application of proposed method for pharmaceutical formulation

About 10 Molnupiravir in house capsules were taken and average quantity of the drug was determined. A quantity of powdered drug equivalent to 10 mg was transferred into a 100 ml volumetric flask containing distilled water and the volume was adjusted upto the mark using same solvent. From this 1.5 ml was taken and transferred to 10 ml volumetric flask and volume was made up to the mark with distilled water to give 15 μg/ml concentration. It was scanned on spectrophotometer in the UV range 200 – 400 nm. The spectrum was recorded at 235 nm. The concentrations of the drug were calculated from linear regression equation.

3. Results and Discussion

3.1. Method validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

3.1.1. Linearity studies

The linear regression data for the calibration curves showed good linear relationship over the concentration range 5-30 μg/ml for Molnupiravir. Linear regression equation was found to be Y = 0.041 X + 0.017 (r^2 = 0.999). The result is expressed in Table 1.

3.1.2. Accuracy

The solutions were reanalyzed by proposed method; results of recovery studies are reported in Table 2 which showed that the % amount found was between 98.54% to 99.98% with %R.S.D. >2.

3.1.3. Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, so that indicate this method precise for the determination of both the drugs in formulation (Table 3).

3.1.4. Sensitivity

The linearity equation was found to be Y = 0.009 X + 0.164. The LOQ and LOD for Molnupiravir were found to be 3.3 μg and 1.1 μg, respectively.

3.1.5. Repeatability

Repeatability was determined by analyzing 15 μg/ml concentration of Molnupiravir solution for six times and the % amount found was between 99.33% to 101% of Method A and 99.64% to 100.9% of Method B with % R.S.D. less than 2 (Table 4).

3.1.6. Ruggedness

Peak area was measured for same concentration solutions, six times. The results are in the acceptable range for both the drugs. The results are given in Table 5**.** The result showed that the % R.S.D. was less than 2%

3.1.6.1. Determination of molnupiravir in bulk. The concentrations of the drug were calculated from linear regression equations. The % amount found was between 99.17 % to 100.43 % (Table 6).

3.1.6.2. Application of proposed method for pharmaceutical formulation. The spectrum was recorded at 235 nm. The concentrations of the drug were calculated from linear regression equation. The % amount found was between 99.85% to 100.1% of Method A and 99.68% to 100.2% for Method B with % RSD less than 2%. (Table 7).

4. Conclusion

This UV-spectrophotometric technique is quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of Molnupiravir in capsule formulation. The validation procedure confirms that this is an appropriate method for their quantification in the bulk material and formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound.

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6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Pritam Jain, Professor

Manali Bhamare, Student

Sanjay Surana, Principal

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