Section A-Research paper

Estimation of Molnupiravir in bulk and formulation using green UV- Spectrophotometric method

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ABSTRACT

Introduction

Patients with COVID-19 are treated with the antiviral drug molnupiravir, which is in phase III trials. It is an isopropyl ester prodrug that the host's esterases in the plasma transform into the active nucleoside analogue D-N4-hydroxycytidine (NHC). Positive- and negative-sense RNA viruses are both susceptible to NHC's antiviral action. The creation of Molnupiravir for the treatment of influenza and coronavirus infections has been the focus of recent study. Molnupiravir is a pyrimidine ribonucleoside analogue which has a chemical name of ((2R, 3S, 4R, 5R)-3, 4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin-1-(2H)-yl)-tetrahydrofuran-2-yl)methyl isobutyrate.

Aim and Objectives

Our aim is to develop and validate an easy, green, sensitive, accurate and cost-effective UV-spectrophotometric method for the estimation of Molnupiravir from active pharmaceutical ingredient and its pharmaceutical formulation. Our Objectives are procurement of raw material samples with certified purity, selection of ecofriendly solvents / chemicals for solubility studies and to develop a validated novel green UV-spectrophotometric method.

Method

The drug was precisely weighed 100 mg and then put in volumetric flask of 100 ml. The volume was then adjusted accordingly with distilled water to produce the desired final strength of 1000 µg/ml. Then further diluted to get 6 µg/ml. The solution we got is being scanned in UV range

(200 nm — 400 nm)[6]. In the spectrum Molnupiravir showed a maximum absorbance at 235 nm. The λmax of Molnupiravir in distilled water was found to be 235 nm.

Results

In the concentration range of 2–10 g g/ml, the substance displays linearity with a correlation coefficient of 0.999. Pharmaceutical formulations are subjected to the suggested procedure, and it was discovered that the amount of the medication at 99.99% was in good accord with the label claim. To assess the method's accuracy, recovery trials at three different levels—80%, 100%, and 120% w/w—were carried out. A range of 98.85% to 99.56% is recorded for the recovery percentage. The low readings of% R.S.D. demonstrate the method's accuracy and reproducibility. Both intra-day and inter-day variations of the precision are investigated. When the % R.S.D. number is less than 2, it means that the procedure is accurate. With the aid of two analysts, the proposed method's ruggedness is examined.

Conclusion

The approach described above uses UV spectrophotometry, which is an efficient, simple, reliable, repeatable, and sensitive technique. This UV method was created for Molnupiravir quantification, and the validation approach demonstrates that it is a suitable, quick, and affordable quality-control tool for regular analysis of Molnupiravir both in bulk and in pharmaceutical dose forms of the drug.

Keywords: Molnupiravir, COVID-19, Green Method, UV-Visible Spectroscopy, Validation

1. Introduction

Coronaviruses are RNA viruses that resemble solar coronas under an electron microscope, hence their name. SARS-CoV-2, the coronavirus that started the COVID-19 worldwide pandemic and has since become a persistent pandemic, was discovered for the first time in China in December 2019. Currently in phase III trials for COVID-19 patients is the antiviral drug molnupiravir [1, 2, 3]. The isopropyl ester is transformed into D-N4-hydroxycytidine (NHC) or EIDD-1931 in the plasma by the host's esterases. This exhibits antiviral action [4] in both the positive and negative senses against different RNA viruses. The goal of recent research is to create Molnupiravir, which will be utilised to treat influenza and coronavirus infections. A pyrimidine ribonucleoside is molnupiravir. The formula for them is ((2R, 3S, 4R, and 5R)Tetrahydrofuran-2-yl-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin1-(2H)-yl)-methyl isobutyrate. The literature review shows that only a few spectrophotometric methods, such as UV, HPLC, and LCMS/MS approaches, are available for the detection and quantification of molnupiravir [8]. Therefore, the authors' goal was to create and validate a brand-new green UV-Spectrophotometric approach for Molnupiravir dosage calculation in both bulk and pharmaceutical formulation in accordance with ICH recommendations.

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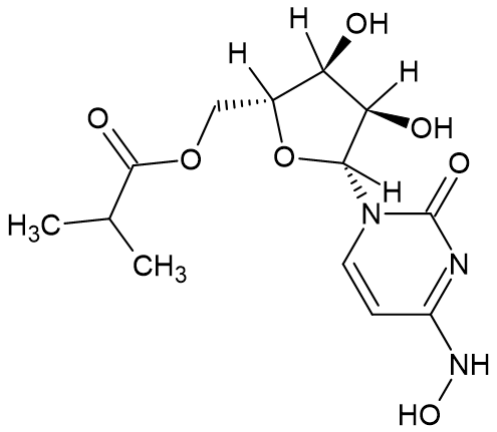

Fig. 1: Molnupiravir chemical structure

2. Materials and Methods

2.1. Materials

Molnupiravir is procured from MSN laboratories private limited, Telangana, Andhra Pradesh. Distilled water was chosen as a solvent because Molnupiravir is readily soluble.

2.2. Preparation of standard stock solution

To dissolve the drug, 10 mg of Molnupiravir is placed to a 100 ml standard flask along with 50 ml of distilled water. The volume is adjusted with the same up to the mark to give 100 µg/ml as a final strength

2.3. Molnupiravir wavelength selection for analysis

Using distilled water, 1 ml of the Molnupiravir stock solution standard drug stock was produced up to the desired concentration of 10 g/ml before being transferred to the 10 ml standard flask. The resulting solution is subjected to a UV scan between 200 and 400 nanometers. Figure 2 illustrates the medication Molnupiravir's highest absorbance at 235 nm.

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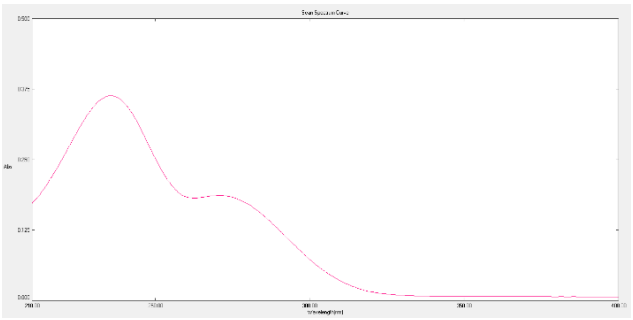

Fig. 2: Molnupiravir UV spectrum at 235 nm

2.4. Validation of the method

The method was validated for the following validation parameters

2.4.1. Linearity study

Various aliquots of the drug were taken in the range of 0.4 - 2 ml (50 µg/ml) was transferred to a series of five 10 ml standard flasks. The volume is made up with distilled water to mark to achieve concentrations 2, 4, 6, 8 and 10 µg/ml, respectively. The resultant solutions are scanned in the range of 200 - 400 nm in UV-Spectrophotometer. The spectrum is measured at around 235 nm. The plot for calibration has been plotted as concentration vs absorbance as shown in Figure 3.

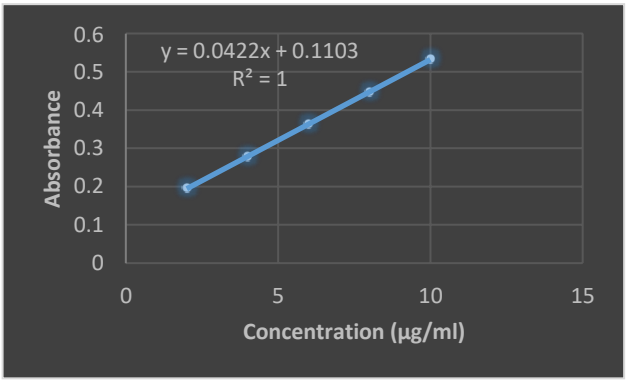

Fig. 3: Molnupiravir calibration curve at 235 nm

2.4.2. Accuracy

A known quantity of the standard solution was added to the previously examined sample solutions at various concentrations, such as 80%, 100%, and 120%. The suggested method was utilized to reevaluate the answers.

2.4.3. Precision

Analyzing three drug concentrations—4, 6, and 8 g/ml—about three times in the same day allows for intra-day precision. Precision Inter-day was performed by examining the drug solutions at doses of 4, 6, and 8 g/ml every day for roughly three days in a week.

2.4.4. Sensitivity

The Limit of Quantification (LOQ) and Limit of Detection (LOD) are used to calculate the Molnupiravir sensitivity when utilising the suggested approach. The LOQ and LOD were calculated using the formulas LOD = 3.3 x N/B and LOQ = 10 x N/B, respectively, where 'N' stands for the standard deviation of the peak areas of the provided medicines (n = 3), which is used as a measure of noise, and 'B' for the slope of the related calibration curve.

2.4.5. Repeatability

Molnupiravir solution at a concentration of 6 µg/ml was used six times to test the repeatability.

2.4.6. Ruggedness

By adopting the identical operational parameters and environmental conditions for two analysts to analyse a sample from the same lot, the suggested method's robustness was tested for a concentration of 6 µg/ml Molnupiravir.

2.5. Determination of Molnupiravir in bulk

10 mg of the drug Molnupiravir was weighed and was transferred into a 100 ml standard flask. Add 50 ml of the mobile phase to make up the volume using the same mobile phase (water). A 10 ml volumetric flask is filled to the appropriate level with distilled water after 1 ml of the aforementioned solution was transferred there. The resultant solution was scanned using a spectrophotometer in the UV range 200 – 400 nm. The concentrations of the drug was calculated using the linear regression equations.

2.6. Applying the proposed method to a Pharmaceutical formulation

Around 20 capsules were collected. The total drug powder was weighed. Average quantity of the drug present in each capsule was calculated. A 100 ml volumetric flask was filled to the appropriate level with the same solvent after 10 mg of powdered drug was weighed and deposited there. 1 ml was pipetted out from this solution and is transferred to 10 ml standard flask and the volume is made up using distilled water to produce a 6 µg/ml concentration soultion. A spectrophotometer was used to scan it in the UV spectrum between 200 and 400 nm. At 235 nm, the spectrum was captured. The equation called linear regression was used to calculate the different aliquots of the drug.

3. Results and Discussion

3.1. Method validation

The method is validated according to the guidelines given by ICH. Solutions of Molnupiravir were prepared as per the method developed. The method has been validated for the following parameters.

3.1.1. Linearity studies

Over the full range of the concentrations 2- 10 µg/ml for Molnupiravir, the data from the calibration curves demonstrated a satisfactory linearity relationship. The results were as shown in the Table 1. The calibration plot are shown as in figure 3.

Table 1: Molnupiravir linearity study

S. No.	Concentration (*µ*g/ml)	Mean Abs± SD	%RSD
1.	2	0.195±0.002	1.43
2.	4	0.278±0.006	1.74
3.	6	0.364±0.021	1.62
4.	8	0.447±0.030	1.22
5.	10	0.532±0.010	1.91

3.1.2. Accuracy

The solution concentrations were detected using the method of our study. Recovery study results were tabulated as in Table No. 2. The table shows the percentage of the amount of the drug which was found to be around 99.16% to 99.95% with a less than 2% of percentage RSD.

Table 2: Study of recovery of the drug Molnupiravir

Name of the drug	Amount of the drug present initially (*µ*g/ml)	Amount of the drug added (*µ*g/ml)	Amount of the drug recovered (*µ*g/ml)	% of the drug recovered	RSD %
Molnupiravir	6	5	10.24	99.38	0.22
	6	6	11.90	99.95	0.24
	6	7	12.74	99.16	0.18

3.1.3. Precision

The validation parameter, precision of the proposed method is given in terms of the percentage relative standard deviation. This gives reproducible values for the assay. The percentage R.S.D. values was less than 2 which indicates that the method of our study is precise enough for the analysis of Molnupiravir and its formulation. Values are as tabulated in Table No. 3.

Table 3: Study of precision of the drug Molnupiravir

Std. Concentration (*µ*g/ml)	**Amount of the drug found (*µ*g/ml)**	**%Amount of the drug found**	**RSD %**
Intra - day Precision			
4	3.6154	101.14	1.83
6	6.4421	98.41	3.44
8	7.9926	99.95	0.46
Inter - day Precision			
4	3.4154	97.05	1.32
6	5.9421	100.04	1.68
8	7.8926	95.88	0.63

3.1.4. Sensitivity

The LOQ as well as LOD of the drug Molnupiravir was 1.5 µg and 1 µg, respectively.

3.1.5. Repeatability

By analysing a 6 µg /ml Molnupiravir solution concentration at least six times, repeatability is determined. 99.32% to 100.5% of the medication was discovered, and the percentage R.S.D. value was less than two. The values are as shown in Table no. 4.

Table 4: Study of repeatability of the drug Molnupiravir

Name of the drug	**Amount of the drug taken (*µ*g/ml) (n=6)**	**Amount of the drug found*(%) ± SD**	**R.S.D %**

Molnupiravir	6	99.96 ± 0.10	0.1061

*average of 6 estimations

3.1.6. Ruggedness

Peak area is measured for the same solutions of same concentrations for six times. The results were found to be the within the acceptable range for the drug. The results are as shown in Table no. 5. The results show that the percentage R.S.D. was found to be less than 2%

Table 5: Study of ruggedness of the drug Molnupiravir

Name of the drug	**Amount of the drug taken (*µ*g/ml) (n=3)**	**Amount of the drug found (%) ***	
		Analyst One ± S.D.	Analyst Two ± S.D.
Molnupiravir	6	99.22 ± 0.81	99.24 ± 0.82

*average of 6 estimations

3.1.6.1. Molnupiravir determination in bulk.

From linear regression equations, the drugs concentrations were determined. The percentage amount of the drug found was 99.32 % to 100.21 %. The values are as shown in Table no. 6.

Table 6: Evaluation of Molnupiravir in API

S. No.	**Amount of the drug taken (*µ*g/ml)**	**% Amount of the drug found**
1.	6	99.32
2.	6	99.62
3.	6	100.12
4.	6	100.21
5.	6	99.41
	Mean ± SD	99.71±0.36
	%RSD	0.516

3.1.6.2. Applying the proposed method to a Pharmaceutical formulation.

The absorbance spectrum is been recorded at around 235 nm. The drug concentrations have been calculated using the equation called linear regression. The percentage amount of the drug

found is 99.84% to 100.2% with % RSD less than 2%. The values are given in Table no. 7.

Table 7: Analysis of Molnupiravir formulation in a capsule dosage form

S. No.	Amount of the drug taken (*µ*g/ml)	% Amount of the drug found
1.	6	99.84
2.	6	100.12
3.	6	100
4.	6	99.87
5.	6	100.2
	Mean ± SD	99.99±0.2
	%RSD	0.108

4. Conclusion

This method is very basic, exact, accurate, consistent and sensitive. This UV methodology was developed for Molnupiravir quantification in a formulation. The validation of this procedure proves that the developed method is a very appropriate one for the drug quantification in the bulk as well as formulations[7]. It can be also used in the regular quality control testing of the raw materials and their formulations having this compound.

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6. Source of Funding

None.

7. Conflict of Interest

None.

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