International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 2359-2363 www.ijprajournal.com ISSN: 2249-7781

Method Development and Validation for the Estimation of Molnupiravir in Bulk and Pharmaceutical Dosage form by UV Spectroscopy

[1]Lydiasmily S*, [2]Sindhumathi periyasamy*, [2]Surya k, [2]Sivakanishkar kasiyanand, [2]Naveen kumar R, [2]Abish S.

[1]*Associate Prefessor, PPG College of Pharmacy, Coimbatore, Tamilnadu.*
[2]*Student, PPG College of Pharmacy, Coimbatore, Tamilnadu.*
Corresponding author: Lydiasmily S,

Submitted: 15-15-2023 Accepted: 25-12-2023

ABSTRACT: A simple, rapid, accurate and economical UV -spectrophotometric method has been developed for estimation of Molnupiravir from bulk and pharmaceutical formulation. Molnupiravir is an antiviral medication that inhibits the replication of certain RNA viruses. It is used to treat Covid-19 those who infected by Sars-cov-2. The λmax of Molnupiravir in distilled water was found to be 236nm.The drug follows linearity in the concentration range 2.5 - 12.5µg/ml with correlation coefficient value 0.999. The proposed method was applied to pharmaceutical formulation. The accuracy of the method was checked by recovery experiment performed at two levels i.e., 50% & 100% w/w. The recovery was found to be in the range 99.11% - 99.85%. 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 Suitable for the analysis of commercial samples. This method was Validated for various parameters according to ICH guidelines.

KEYWORDS: Molnupiravir, antiviral medication, UV Spectroscopy,Validation.

I. INTRODUCTION :

Molnupiravir is an antiviral medication candidate for the treatment of COVID-19 patients that is now in phase III trialsThe main mode modes of transmission of COVID-19 infection to people are person to person contact and respiratory droplets. Fever, headache, shortness of breath, loss of taste or smell, diarrhoea, nausea are the symptoms of COVID-19 patients Single-stranded RNA virus that belongs to the corona virus family is the cause of Severe acute respiratory syndrome (SARS-CoV-2). Vaccine is the best way for the protection of
COVID-19 that have been developed for the prevention of COVID-19 . 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 NHC exhibits antiviral activity against a variety of positive-and negative-sense RNA viruses. Recent research has centered on the development of molnupiravir for the treatment of influenza and coronavirus infections. respectively. 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. The literature survey revealed that two methods of analysis for Molnupiravir have been reported, which included LC- MS/MS for the quantification of B-D-N4-hydroxycytidine in human plasma and B-D-N4-hydroxycytidine-triphosphate peripheral blood mononuclear cell lysates; and novel LC-MS/MS method for the simultaneous quantification of

N4-hydroxycytidine-triphosphate

peripheral blood mononuclear cell lysates and novel LC-MS/MS method for the simultaneous quantification of Molnupiravir and its metabolite β-d-N4-hydroxycytidine in human plasma and saliva. Accordingly ,the objective of this study was to develop and validate the method for the estimation of Molnupiravir in bulk and pharmaceutical formulation by UV spectroscopy as per ICH guidelines.

UV spectroscopy :

Ultraviolet spectroscopy deals with the study of absorption of UV radiation whose wavelength ranges from 200nm-400nm.Coloured compounds absorb radiation from 400nm-800nm(wavelength region) and colourless

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 2359-2363 www.ijprajournal.com ISSN: 2249-7781

compounds absorb radiation in UV region. In UV spectroscopy, valence electrons absorb the energy and the molecules or atoms move from ground state to the higher energy or excited state.

Principle :

Any molecule has either n, o or a or a combination of these electrons. These bonding (σ & π) and non-bonding (n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks, the nature ofthe electrons present and hence the molecular structure can be elucidated.

lambert's Beer Rule:

Transmittance (T) is given by lo and (1/10)*100 gives transmission rate (T%). Absorbance (abs) is the inverse of transmittance and given by,

log(1/T) = log(lo/I). TI/Mo 10-kel (2)

abs=log (1/T)= log(lo/I)= -kcl (3)

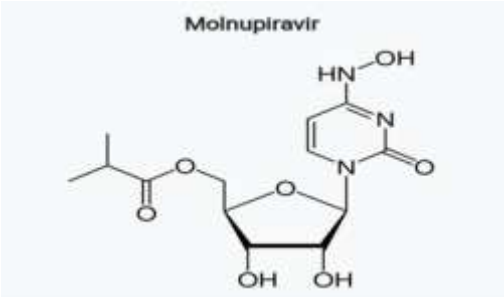

Figure 1: Chemical structure of Molnupiravir

II. MATERIALS AND METHOD :

2.1. Materials

Molnupiravir was collected from Microlabs, Bangalore. The drug is freely soluble in water, distilled water is used as solvent.

2.2. Instrument :

Spectroscopic Analysis was carried out on a UV-1780 double beam UV spectrophotometer. The spectra were recorded over the wavelength range of 200-400nm, against solvent blank, in quartz cuvettes with 1 cm diameter. All volumetric glassware used were calibrated.

METHOD DEVELPOMENT :

Selection of wavelength:

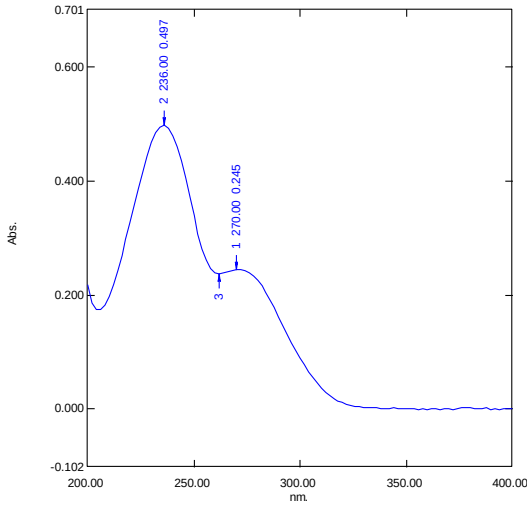

Figure 2:Wavelength selection forMolnupiravir

Preparation of standard stock solution:

Stock 1: Accurately weigh 0.05 of standard Molnupiravir API and transferred to 50ml volumetric flask and the volume was adjusted with methanol to obtain final concentration of 1000mcg/ml.

Stock 2: Appropriate volume 5ml of standard stock solution of Molnupiravir was transferred to 100ml volumetric flask and the volume was adjusted by distilled water to obtain final concentration of 50mcg/ml.

Stock 3: Take series consisted of five concentrations of standard molnupiravir solution ranging from 2.5 to 12.5µg/ml. The solutions were prepared by pipetting out Standard solution of molnupiravir solution (0.5ml, 1ml, 1.5ml, 2ml, and 2.5ml) was transferred into a series of 10ml volumetric flask and volume was adjusted upto mark with water.

Analysis of marketed formulation :

For the estimation of molnupiravir in capsule formulations by this method. 6 branded capsules were weighed and collect to fine powder. Drug powder equivalent to 50mg of molnupiravir was weighed and transfer into 50ml volumetric flask than dissolved with water and further diluted with water.

It was kept for ultrasonication for 30 min; this was filtered through Whatman filter paper No. 41 and then final dilution was made with water/ Methanol to get the final stock solution of 50 mcg/ml.

From this stock solution, various dilutions of the sample solution were prepared and analysed.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 2359-2363 www.ijprajournal.com ISSN: 2249-7781

Spectroscopic method:

The spectra showed sharp peak at 236nm. The standard drug solution was diluted so as to get the final concentration in the range of 2.5-12.5mcg/ml and scanned in the spectra. The calibration curve of Absorbance against concentration of the drug showed linearity.

Similarly absorbance of sample solution was measured and amount of molnupiravir was determined from standard calibration curve.

III. RESULT AND DISCUSSION :

Linearity and Range:

Different aliquotes of Molnupiravir in range 0.5-2.5ml were transferred into series of 10ml Volumetric flasks and the volume was made upto the mark with distilled water to get concentrations 2.5, 5, 7.5, 10 and 12.5mcg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400nm. The spectrum was recorded as 236nm.

Table 1: Result of calibration

Molnupiravir (mcg/ml)	236nm		
	Absorbance	SD	%RSD
2.5µg/ml	0.236	0.00173	0.73%
5µg/ml	0.447	0.00158	0.35%
7.5µg/ml	0.680	0.001	0.14%
10µg/ml	0.882	0.00122	0.13%
12.5µg/ml	1.081	0.001	0.09%

Calibration Curve:

The spectra showed sharp peak at 236 nm when n=1 and linearity was measured at 236 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 2.5-12.5 mcg/ml with R^2 of 0.999 and given in table,

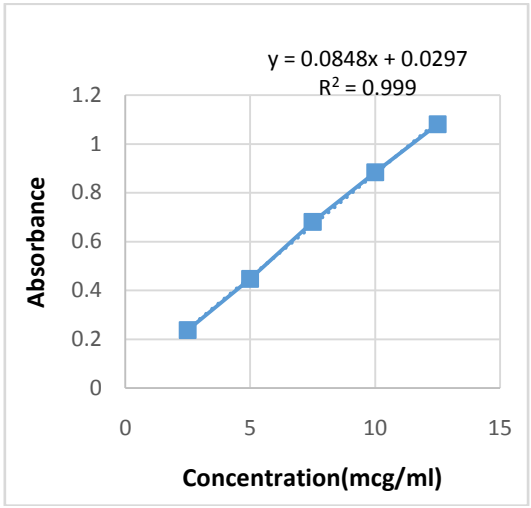

Figure 3:CalibrationCurve of Molnupiravir236nm

Accuracy:

To the pre-analyzed sample solution, a known amount of standard stock solution was added at different levels i.e, 50% and 100%. The solution was reanalyzed by proposed method.

%Recovery:

Table 2: Result of calibration

Drug name	Level of recovery	Amount present (µg/ml)	Amount added (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% recovery	S.D	%RSD
MPV	50%	1.5	0.75	8.24	0.748	99.85	O.0038	0.507%
	100%	1.5	1.5	8.98	1.48	99.11%	0.0026	0.319%

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 2359-2363 www.ijprajournal.com ISSN: 2249-7781

Precision:

Inter-day and Intra-day precision were studied. Intra-day precision was determined by analysing the 2.5, 7.5, 12.5 mcg/ml, of Molnupiravir solutions for three times in the same day. Inter-day precision was determined by analysing2.5, 7.5, 12.5 mcg/ml of Molnupiravir solutions daily for three days over the period of week.

Table 3: Intra-day Precision

S.NO	Conc(µg/ml)	Mean absorbance	S.D	%RSD
1	2.5µg/ml	0.116	0.0015	1.29%
2	7.5µg/ml	0.463	0.0017	0.36%
3	12.5µg/ml	0.784	0.0015	0.19%

Table 4: Intra-day Precision

S.NO	Conc(µg/ml)	Mean absorbance	S.D	%RSD
1	2.5µg/ml	0.116	0.0015	1.29%
2	7.5µg/ml	0.464	0.0021	0.45%
3	12.5µg/ml	0.794	0.0125	1.57%

Assay:

Composition of capsule:

200mg of molnupiravir active substance. Hydroxypropyl cellulose, microcrystalline cellulose, croscarmellose sodium, magnesiumstearate as excipients (quantity sufficient). All the excipients were mixed in 100ml volumetric flask and make up the volume with water, sonicated for 15min. The solution was filtered through Whatman filter paper and make up the volume up to 100ml with water.

Tabel 5: Result of assay

Molnupiravir(mcg/ml)	**Mean absorbance**	**%Assay**	**%RSD**
10mcg/ml	0.622	100.24%	0.32%

SUMMARY OF VALIDATION PARAMETER :

Parameters	Absorbance correction method
	MOLNUPIRAVIR
Concentration range	2.5-12.5mcg/ml
Correlation coefficient(R^2)	0.999
Regression equation	Y=0.0848x+0.0297
Accuracy(% Recovery)	99.85%
Intra-day precision(%RSD)	1.29%
Inter-day precision(%RSD)	1.29%
%Assay	100.24%

IV. CONCLUSION :

A spectrophotometric method for or quantifying molnupiravir-200mg in formulation samples has s been developed and validated. From the results, the method described in this report is precise, accurate and reproducible. The proposed method can be prosperous applied for validation, and can be extended to the analysis of molnupiravir

in a good agreement with bulk and label claim formulations.

ACKNOWLEDGEMENT :

The authors are thankful to the Principal and the management, PPG College of Pharmacy, coimbatore, India for providing the required facilities to carry out this research work.

REFERANCE :

[1]. NourahZoman Al-Zoman1, Hadir Mohamed Maher1.2 and Amal Al-Subaiel, "Simultaneous. determination of newly developed antiviral agents in pharmaceutical formulations by HPLC-DAD" Journal of chemistry central; Year 2017, DOL: 10.1186/%13065-016-0232-6

[2]. Fariba Pourkarim1,2 Samira Pourtaghi-Anvarian1,2 Halch Rezace2,3," Molnupiravir. A new candidate for COVID-19 treatment" wileyonlinelibrary.com/journal/prp2: Year 2021, June 25 10.1002/prp2.909. DOL

[3]. Kabinger F, Stiller C, Schmitzová J, Dienemann C, Kokic G, Hillen HS, et al. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. Nat Struct Mol Biol. 2021;28(9):740-6.

[4]. Imran M, Arora MK, Asdaq SMB, Khan SA, Alaqel SI, Alshammari MK. Discovery, development, and patent trends on Molnupiravir: A prospective oral treatment for COVID-19. Molecules.

[5]. Essam ezzeldin1,2, nisreenf.talib 24, marwar h. tammam 2, yousif alasiri3,abd, el-galil e amr1.4. "Validation reversed-phase liquid chromatographic method with gradient elution for simultaneous determination of antiviral agent: sofobuvir, ledipasvir, daclatasvir, &simeprevir in their dosage forms" Journal mdpi, year 2020 october 10, DOI: 10.3390/molecules25204611.

[6]. Gordon CJ, Tehesnokov EP, Schinazi RF, Götte M. Molnupiravir promotes SARS-CoV-2 mutagenesis via the RNA template. J Biol Chem. 2021;297(1):100770.doi:10.1016/j.jbc.2021.100770.

[7]. Fariba Pourkarim1,2 Samira Pourtaghi-Anvarian1,2 Halch Rezace2,3," Molnupiravir. A new candidate for COVID-19 treatment" wileyonlinelibrary.com/journal/prp2: Year 2021, June 25 10.1002/prp2.909. DOL

[8]. Cokorda Agung Wahyu Purnamasidhi, GiovancaVerentziaPurnama, Molnupiravir: a novel efficacious antiviral candidate to COVID-19, Molnupiravir, um novo candidatoa antiviral eficaz para COVID-19, Molnupiravir, un nuevo antiviral eficazcandidato a COVID-19, Research, Society and

[9]. Imran M, Arora MK, Asdaq SMB, Khan SA, Alaqel SI, Alshammari MK. Discovery, development, and patent trends on Molnupiravir: A prospective oral treatment for COVID-19. Molecules.2021;26(19):5795.oi:10.3390/ molecules26195795.