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UV VISIBLE SPECTROSCOPY METHOD DEVLOPMENT AND VALIDATION FOR ESTIMATION OF MOLNUPIRAVIR IN SOLID DOSAGE FORM.

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ABSTRACT: Molnupiravir is an antiviral medication that inhibits the replication of certain rna viruses. It is used to treat covid-19 in those who infected by Sars-cov-2. The main intent of this investigation was to devlop and validate an uncomlicated, forehanded, parsimonious (time-saving), and canny (efficient) for the resolution (will ower) of molnupiravir capsule in the solid dosage from. Standard and sample solutions for molnupiravir were prepared in distilled water. Absorbance correction method for molnupiravir 280nm was measured. The method followed linearity in range of 0.2-1 µg/ml with correlation value of 0.9998 for molnupiravir. This method was validated for various parameters according to ich guidelines. Satisfactory values of percent relative standard deviation for the intra-day and inter-day precision indicated that method was precises.the mean percentage recovery studies was found to be 100.17% for molnupiravir. Lod and loq were calculated as 0.175503 µg/ml and 0.531827 µg/ml for molnupiravir.

KEY WORDS: Molnupiravir, antiviral medication, RNA-viruses, Sars-cov-2, parsimonious, canny,

INTRODUCTION :

Novellus coronavirus disease 2019 (COVID-19) was identified as the source of a cluster of pneumonia cases in Wuhan, China, in December 2019. 1,2 It has quickly spread over the world. 2–4 COVID-19 was declared a global pandemic by the World Health Organization (WHO) in March 2020. 5 There have been 276 436 619 confirmed cases of COVID-19 reported to WHO as of December 23, 2021, with 5 374 744 deaths. The two main modes of transmission of COVID-19 infection to people are person-to-person contact and respiratory droplets. COVID-19 typically takes 14 days to incubate. The real-time reverse transcriptase-polymerase chain reaction approach is used to determine the final diagnosis of COVID-19. Fever, dry cough, sore throat, shortness of breath, exhaustion, headache, loss of taste or smell, diarrhoea, and nausea are all symptoms of COVID-19. COVID-19 has a lower fatality rate and a greater infectivity altitude than the Severe Acute Respiratory Syndrome coronavirus and the Middle East Respiratory Syndrome coronavirus, according to epidemiologic data. However, in COVID-19 patients, underlying disease (such as hypertension, diabetes, and cancer) may increase mortality. The coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2) is an enclosed, single-stranded RNA virus that belongs to the Coronaviridae family. COVID-19 vaccines have been developed for the prevention of COVID-19, including mRNA-based vaccines and viral vector vaccines. The vaccine is the best way for the protection against COVID-19 but disadvantages of COVID-19 vaccines include:

- Need for booster doses due to short-term immunisation
- Severe allergic reactions such as anaphylaxis (rarely)
- Long-term side effects are unknown.
- Vaccination rates are also low in several poor- and lower-middle-income nations. Patients, on the other hand, prefer oral agents since they are easier to use. [1]

In numerous low- and lower-middle-income countries, vaccination rates are likewise low. Oral agents, on the other hand, are preferred by patients because they are easier to administer. [1] The RNA-dependent RNA polymerase (RdRp) is a critical enzyme in SARS-CoV-2 replication and has a key role in COVID-19 pathogenesis. [(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-4-(hydroxyimino)-2-oxo-1,2,3,4-tetrahydropyrimidin-1yl] Molnupiravir ([(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-4-(hydroxyimino)-2-oxo-1,2,3,4- ′B-D-N4-hydroxycytidine (known, EIDD-1931 or NHC), an oral ribonucleoside analogue with broad-spectrum antiviral action (EIDD-2801, MK-4482), targets RdRp and is an isopropyl ester prodrug of ′B-D-N4-hydroxycytidine (known, EIDD-1931 or NHC). It has been proposed as a possible treatment for COVID-19 because it inhibits SARS-CoV-2 replication in cell lines, animal infected models, and culture media containing airway epithelial cells.16 One of the benefits of this therapeutic target is that there is no human analogue of the RNA-dependent polymerase. [1]

This drug is currently under review by the United States Food and Drug Administration.[1]

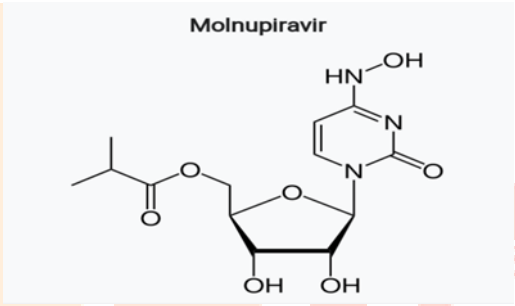

Figure 1 Chemical structure of molnupiravir

MECHANISM OF ACTION OF MOLNUPIRAVIR IN COVID-19

- Host esterases proselyte molnupiravir to the active nucleoside analogue (EIDD-1931) in plasma. Chikungunya virus, Venezuelan equine encephalitis virus, Respiratory Syncytial virus, Norovirus, Influenza A and B viruses, Ebola virus, and human Coronaviruses have all been reported to be resistant to EIDD-1931. EIDD-1931 diffuses throughout the body and converts to a triphosphate form in the proselyte. Instead of cytidine-triphosphate and uridine-triphosphate, RdRp uses NHC triphosphate as a substrate, which is more conducive to the generation of altered RNA. Molnupiravir is a more desirable electron donor, which vary the conditions obliged for infectivity.
- Molnupiravir inhibits SARS-RdRp CoV-2's enzyme, causing numerous lapses in RNA virus replication.
- In other words, molnupiravir-like remdesivir can inhibit coronavirus pathogenesis and replication. According to the findings of the docking investigation, the limited space of mutations in the drug structure can induce molnupiravir's inhibitory effects on the formation of drug resistance-related mutations. Molnupiravir can thus be used to treat patients who have developed resistance to remdesivir. [2]

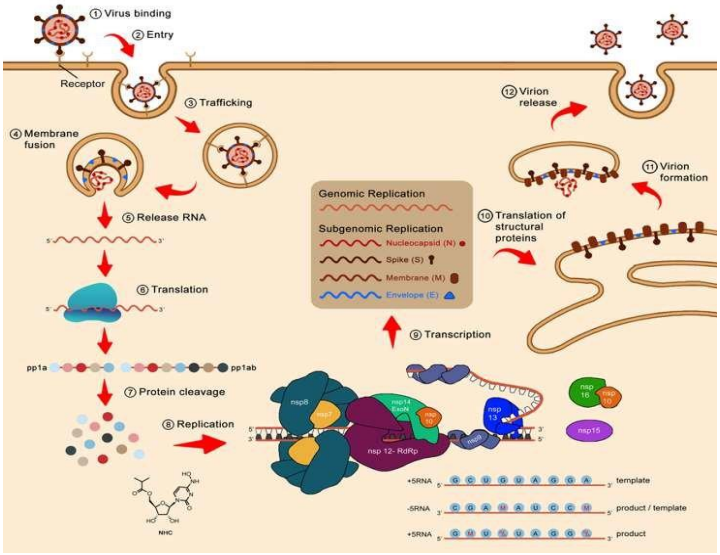

Figure : 2 Mechanism of molnupiravir and its pharmacological active form after oral administration [10]

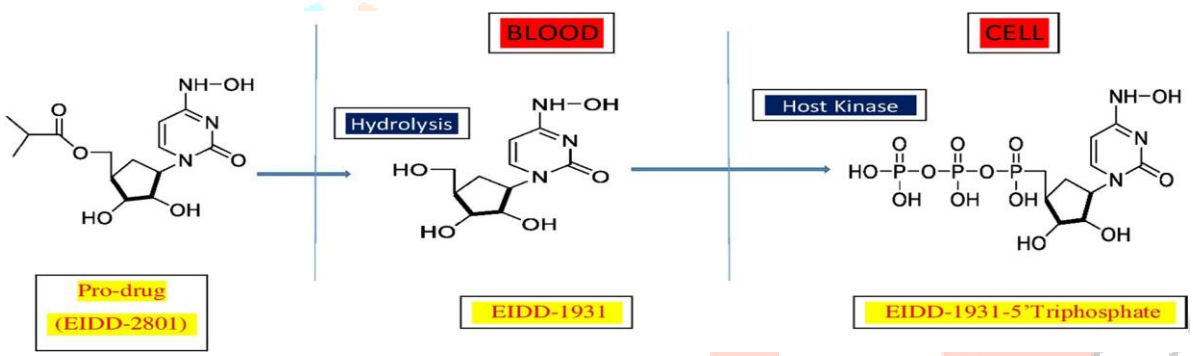

Figure : 3 Molnupiravir converted into EIDD-1931 in blood then in cell by host kinase converted into EIDD-1931-5'Triphosphate (active form) [3]

ABSORPTION: The active component N-4 hydroxycytidine reaches a Cmax of 1.5 hours and an AUC 0-12h of 8360 h*ng/ml after an 800 mg oral dose of molnupiravir every 12 hours.

METABOLISM: In vivo, molnupiravir is hydrolyzed to N-4 hydroxycytidine, which is dispersed throughout the body. N-4 hydroxycytidine is phosphorylated to the 5'triphosphate form once within cells.

ELIMINATION STRUCTURE: The active metabolite N-4 hydroxycytidine excretes 3% of an oral molnupiravir dosage in the urine.[8]

INTRODUCTION TO SPECTROSCOPY: Spectroscopy is the study of how an electromagnetic field interacts with matter. It began as a study of the dispersion of visible light by a prism based on wavelength. The concept was broadened to include all interactions involving the fluctuation of radiative energy with wavelength or frequency. The emission spectrum of a wavelength or frequency dependent response is used to represent spectroscopic data. Spectroscopy can be divided into two categories: (a) techniques based on photon-to-sample energy transfer, and (b) reflections, refraction, diffraction, dispersion, or scattering from the sample that alter the amplitude, phase angle, polarisation, or propagation direction of electromagnetic radiation.[11]

Principle of UV-visible spectroscopy: The wavelength of light reaching the detector is measured when a light beam passes through an object. The wavelength that is measured provides crucial information about the chemical structure and quantity of molecules present (present in intensity of the measured signal). As a result, both quantitative and qualitative data can be collected. In the wavelength range of 160 to 3500 nm, information can be collected as transmittance, absorbance, or reflectance of light [2,3]. Electrons are promoted to excited states or anti-bonding orbitals as a result of incident energy absorption. Photon energy must match the energy required by the

electron to go to the next higher energy state for this transfer to take place. The basic operating principle of absorption spectroscopy is this mechanism. [11]

When a given wavelength and energy of light is focused onto a sample, it absorbs some of the incident wave's energy. A photodetector detects the sample's absorbance and measures the energy of transmitted light from the sample. The wavelength is used to generate the absorption or transmission spectrum of the light absorbed or transmitted by the sample. [11]

The Lambert-Beer rule states that the absorbance of a solution scales directly with analyte concentration, and it is a fundamental principle of quantitative analysis. The absorbance (unit less) A is the molar absorptivity of the absorbing species (M-1 cm-1), b is the route length of the sample holder (usually 1 cm), and c is the concentration of the solution for a specific wavelength (M).

A = a . b . c (1)

In the UV – visible wavelength region, a UVVisNIR spectrometer can measure absorbance or transmittance. The following is a description of the relationship between incident light of intensity 'Io' and transmitted light of intensity 'I'. [11]

Figure lambert's−Beer Rule.

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

log (1/*T*) = *log*(*Io*/*I*). T = I/Io = 10−kcl (2)

abs = log (1/T) = log(Io/I) = −kcl (3)

The constant of proportionality, k, is used here. While transmittance is unaffected by sample concentration, absorbance is proportional to both sample concentration (Beer's law) and optical path (Lambert's law). Furthermore, when the optical path is 1 cm and the concentration of the targeted substance is 1mol/l, k is denoted as " and is characterised as molar absorption coefficient. The material's molar absorption coefficient is representative of the material under specified conditions.

Any stray, produced, scattered, or reflected light is assumed to be absent in the Lambert-Beer rule. [11]

MATERIAL ANS METHOD :

Chemical & Reagents:

- Molnupiravir 200mg capsules.
- Molnupiravir API was received as gift sample from century pharmaceutical limited, sayajiganj, vadodra.
- Granule: hydroxypropyl cellulose, microcrystalline cellulose, croscarmellose sodium, magnesium stearate.
- Distilled water for development purpose & 0.1N HCL, O.1N NAOH, Methanol to check solubility of drug.

Instruments:

- Spectroscopic Analysis was carried out on a UV/VISIBLE 2080. double beam UV-Visible spectrophotometer. The zero order absorption spectra were recorded over the wavelength range of 200-400 nm, against solvent blank, in quartz cuvettes with 1 cm diameter.
- An Semi micro analytical balance (prompt) was used for weighing purpose.
- All volumetric glassware used were calibrated

METHOD DEVELOPMENT :

Solvent selection in uv visible spectroscopy:

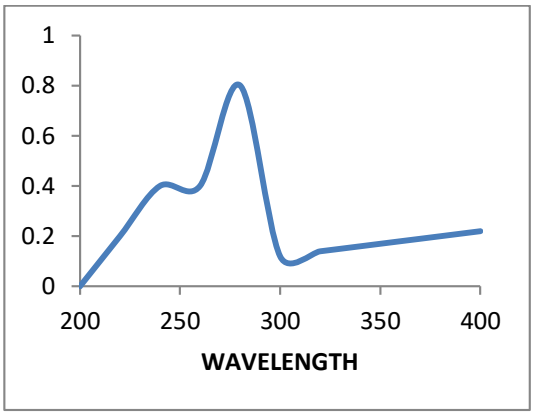

Figure : 4 Wavelength selection for molnupiravir

Spectra in water :

The spectra showed sharp peak at 280 nm when n=1 and linearity was measured at 280nm (Fig wavelength). The absorbance difference at n=1 (dA/d) is calculated which was directly proportional to the concentration of the standard solution.

Preparation of working standard drug solution :

Stock 1: The standard molnupiravir API (200 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with water to obtain final concentration of 1000 mcg/ml and the resulting solution was used as working standard solution.

Stock 2: Take 1ml from stock 1 solution and transferred to volumetric flask (10 ml) and diluted up to the mark with water to obtain final concentration of 100 mcg/ml and the resulting solution was used as working standard solution.

Stock 3: Take series consisted of five concentrations of standard molnupiravir solution (100 mcg/ml) ranging from 0.2 to 1 µg/ml. The solutions were prepared by pipetting out Standard solution of molnupiravir solution (0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1ml) was transferred into a series of 100 ml volumetric flask and volume was adjusted up to 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 10 mg of molnupiravir was weighed and transfer into 100 ml 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 100 mcg/ml.

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

Spectroscopic method:

The spectra showed sharp peak at 280 nm when n=1 and linearity was measured at 280nm (Fig wavelength). The absorbance difference at n=1 (dA/d) is calculated which was directly proportional to the concentration of the standard solution. The standard drug solution was diluted so as to get the final concentration in the range of 0.2-1 mcg/ml and scanned in the spectra. The calibration curve of dA/d against concentration of the drug showed linearity.

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

RESULT AND DISCUSSION:

Linearitry and range :

The linearity response was determined by analysing 4 independent level of concentration in range of 0.2-1 mcg/ml for molnupiravir

Calibration curve for molnupiravir :

- This series consisted of five concentrations of standard molnupiravir solution ranging from 0.2 to 1 µg/ml. The solutions were prepared by pipetting out Standard solution of molnupiravir& stock solution (0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1ml) was transferred into a series of 100 ml volumetric flask and volume was adjusted up to mark with water.
- A zero order derivative spectrum of the resulting solution was recorded, measured the absorbance at 280 nm against a reagent blank solution (water). Calibration curve was prepared by plotting absorbance versus respective concentration of molnupiravir.

Table: 4 Result of calibration

molnupiravir (µg/ml)	**280 nm**		
	ABSORBANCE	**SD**	**% RSD**
0.2	0.059	0.000707	1.198486
0.4	0.11	0.0007071	6.428243
0.6	0.159	0,000707	0.444721
0.8	0.213	0.000707	0.331975
1	0.265	0.000707	0.266833

CALIBRATION CURVE :

The spectra showed sharp peak at 280 nm when n=1 and linearity was measured at 280 nm. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 0.2-1 mcg/ml with r2= 0.9998 and given in table.

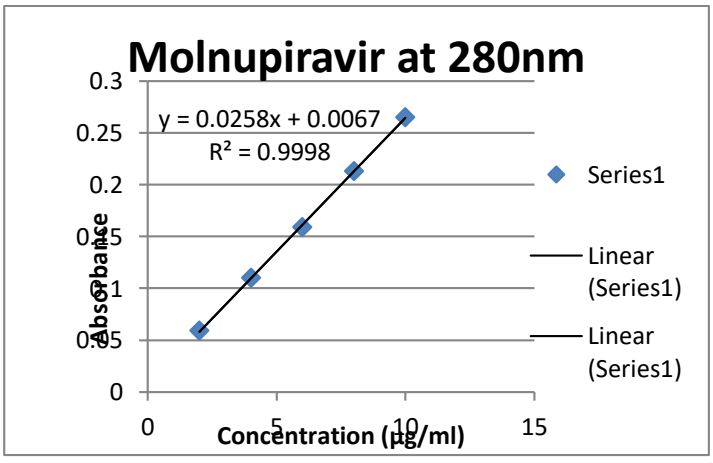

Figure 5 : calibration curve for molnupiravir

Precision :

From stock solution (1000mg/ml) take 10ml and make up the dilution to 10ml with water. The precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision results were expressed as standard deviation or relative standard deviation.

Table 5 : Intraday precision result

Conc (µg/ml)	molmupiravir Abs. ±% RSD
0.2	0.214 ±0.700
0.4	0.215 ±0.700
0.6	0.216 ±0.693
0.8	0.217 ±0.593
1	0.218 ±0.685

Table 6 : Intraday precision

Conc (µg/ml)	molmupiravir Abs. ±% RSD
0.2	0112. ±0.513
0.4	0.113 ±0.845
0.6	0.114 ±0.873
0.8	0.116 ±0.703
1	0.117 ±0.697

Acceptance criteria:

% RSD of the six replicate injections should not more than 2.0%.

Recovery Studies:

Recovery studies were carried out at three different levels i.e. 80%, 100% and 120% by adding the pure drug to the previously analysed drug powder sample and shown in Table 7.

The percentage recovery value indicates non-interference of the excipients used in formulation.

Table 7 : Result of recovery study:

Initial conc. (µg/ml)	Level of recovery	Quantity of Std. Added (µg/ml)	Total Amount (µg/ml)	Result of recovery study	
				Total Quantity Found* (µg/ml)± %RSD	% recovery ± %RSD
10	0%	-	10	10.00±0.161	100.16±0.16
10	80 %	8	18	18.04±0.160	100.16±0.16
10	100%	10	20	20.02±0.159	100.15±0.15
10	120 %	12	22	22.10±0.158	100.15±0.15
				Mean of 3 Determination	**100.16%**

LOD & LOQ:

The Limit of detection and Quantitation of the developed method was assessed by analyzing 10 replicates of standard solutions containing concentrations 5µg/ml for molnupiravir.

The LOD and LOQ were calculated as LOD = 3.3*σ/S, and LOQ = 10*σ/S, where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

% RSD was calculated.

Table 9 : Result of LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Molnupiravir	0.175503	0.531827

ASSAY :

Composition of capsule :

- 200 mg of molnupiravir active substance.
- Hydroxypropyl cellulose, microcrystalline cellulose, croscarmellose sodium, magnesiumstearate as excipients (Quntity sufecient).
- All the excipients were mixed in 100ml volumetric flask and. make up the volume with water up to 25 ml. sonicated for 15min. The solution was filtered through Whatman filter paper. and make up the volume up to 100 ml with water.
- In all the solution had concentration 100µg/ml for molnupiravir.

Table 8 : Result of assay

Molnupiravir (µg/ml)	ABSORBANCE	% Assay	% RSD(n=3)
10	0.609	100.16	0.164474

SUMMARY OF VALIDATION PARAMETER :

PARAMETERS	Absorbance correction method
	MOLNUPIRAVIR
Concentration range(µg/ml)	0.2-1
Regression equation	Y=0.00258x + 0.0067
Correlation Coefficient(r2)	0.9998
Accuracy(%Recovery) (n=3)	100.16
Intra-day Precision (%RSD) (n=3)	0.112-0.845
Inter-day precision (%RSD) (n=3)	0.214-700
LOD(µg/ml)	0.175503
LOQ(µg/ml)	0.531827
% Assay	100.16%

Conclusion :

A spectrophotometric method for quantifying molnupiravir-200mg in formulation samples has 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 lablel claim formulations.

ACKNOWLEDGEMENT

“IF YOU CAN DREAM IT.

YOU CAN DO IT.....”

My own thought for Life…….

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