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Development And Validation Of Spectrophotometric Method Using Chromogenic Reagent For The Estimation Of Molnupiravir In Bulk And Pharmaceutical Dosage Form

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ABSTRACT: An antiviral drug called Molnupiravir prevents some RNA viruses from replicating. In patients infected with SARS-CoV-2, it is used to treat COVID-19.A simple and sensitive UV visible spectrophotometric has been developed for using specific procedure for determination of Molnupiravir in bulk and pharmaceutical dosage form by using MBTH reagent. The absorbance of the chromogen was measured at absorption maxima at 602 nm gives bluish-green colored. Follows beer's law range 50-250µg/ml with coefficient was determine 0.9991.The Limit of detection and the limit of quantitation were found to be 9.54µg/ml and 28.9µg/ml. This method was validated according to ICH guidelines. The developed method are precise, linear, and accurate and this method was successfully applied for the estimation of Molnupiravir in bulk and pharmaceutical dosage form (capsules).

Keywords: MBTH, Molnupiravir, Spectrophotometric, ICH guidelines, COVID-19, SARS-CoV-2.

INTRODUCTION

Molnupiravir is chemically [(2R,3S,4R,5R)-3,4-dihydroxy-5-[(4Z)-4-(hydroxyimino)-2-oxo-1,2,3,4-tetrahydropyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate. Molnupiravir chemical formula is $C_{13}H_{19}N_3O_7$, and its molecular weight is 329.309 gm/mole. It is a white crystalline solid soluble in water, freely soluble in methanol, ethanol and DMSO, slightly soluble in Acetonitrile and has melting point 105-107°C. Molnupiravir is an antiviral drug, which prevents some RNA viruses from replicating. It is used to treat COVID 19 in those infected by SARS-CoV-2. It is a prodrug of the synthetic nucleoside derivative N4-hydroxycytidine, Molnupiravir inhibits viral RNA replication by causing copying errors.

Colorimeter, also known as visible spectrophotometry, is a method based on Beer-Lambert's law, which describes a direct relationship between the concentration and absorbance at a specific wavelength of maximum absorption, to ascertain the concentration of colored compounds in a solution. The Wavelength between 380 nm to 780 nm (400nm to 800nm) forms the visible band of light in electromagnetic spectrum. A solution or object which was colored compound and it transmits or absorbs the particular radiation in the visible spectrum. The optical characteristic of the substance is its absorption spectrum.

In this research, a colorimetric method was developed using chromogenic reagent MBTH and $Fecl_3$ and the developed methods were validated as Linearity, Accuracy, Precision, and Repeatability.

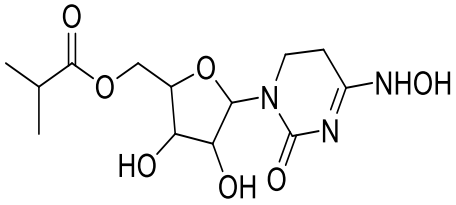

Figure 1: Structure of Molnupiravir

MBTH (3-methyl -2-benzothiazolinone hydrazone)

MBTH is a White to pale yellow crystalline powder, the oxidative coupling reagent used to determination of carbonyl compound, phenols, polyhydroxy compounds, aldehydes, aromatic amines and amino hetero aromatic compounds including indoles, carbazoles and phenothiazines to form extremely colored product.

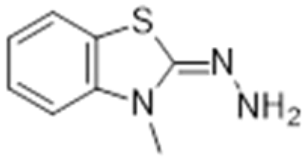

Figure 2: Structure of MBTH

Physicochemical properties:

Chemical formula: $C_8H_9N_3S.HCl$

Molar mass: 215.7g/mol

Melting point: 276-278°C

Boiling point: 342.9°C at 760 mmHg

Solubility: Soluble in water, dimethyl sulfoxide and methanol.

EXPERIMENTAL

Materials and Methods

Chemical and reagents: Molnupiravir was supplied from Dr. Reddy's laboratories. The capsules dosage form (MOVFOR) 200mg manufactured by Hetero Health Care limited was purchased from local pharmacy. The analytical grade MBTH purchased from kemphasol Mumbai and LR grade Ferric chloride anhydrous from Sd fine chemicals limited (SDFCL).

Instruments: UV-visible spectrophotometer (SCHIMADZU 1800), Digital balance (Schimadzu BL 220H), Ultrasonic bath sonicator (PCI Analytics 6.5li200H), Hot Air Oven (Tempo Equipment Private Limited).

Method Development

Preparation of MBTH (0.5%w/v): Dissolve 500mg in 100ml of Distilled water.

Preparation of 2% $Fecl_3$: Dissolve 2gm in 100ml of Distilled water.

Preparation of stock solution: 10 mg of API of Molnupiravir was weighed and transferred into 10 ml volumetric flask and dissolved in Distilled water and made flask upto the volume with distilled water to give a solution 1000µg/ml.

Calibration curve (50-250µg/ml) preparation

Fresh aliquots of Molnupiravir ranging from 0.5-2.5mL from stock solution (1000µg/mL) were transferred into a series of 10mL volumetric flasks to provide final concentration range of 50-250µg/mL. To each flask 2mL of Ferric chloride (2%) solution and 2mL of 0.5% MBTH reagent was added. Then wait for 10min for developing colour. Then solution was made up to the mark with distilled water.

The absorbance of bluish-green coloured chromogen was measured against respective blank solution (i.e. Without drug) in visible region i.e., 380-800nm which shows a maximum absorbance at 602nm.

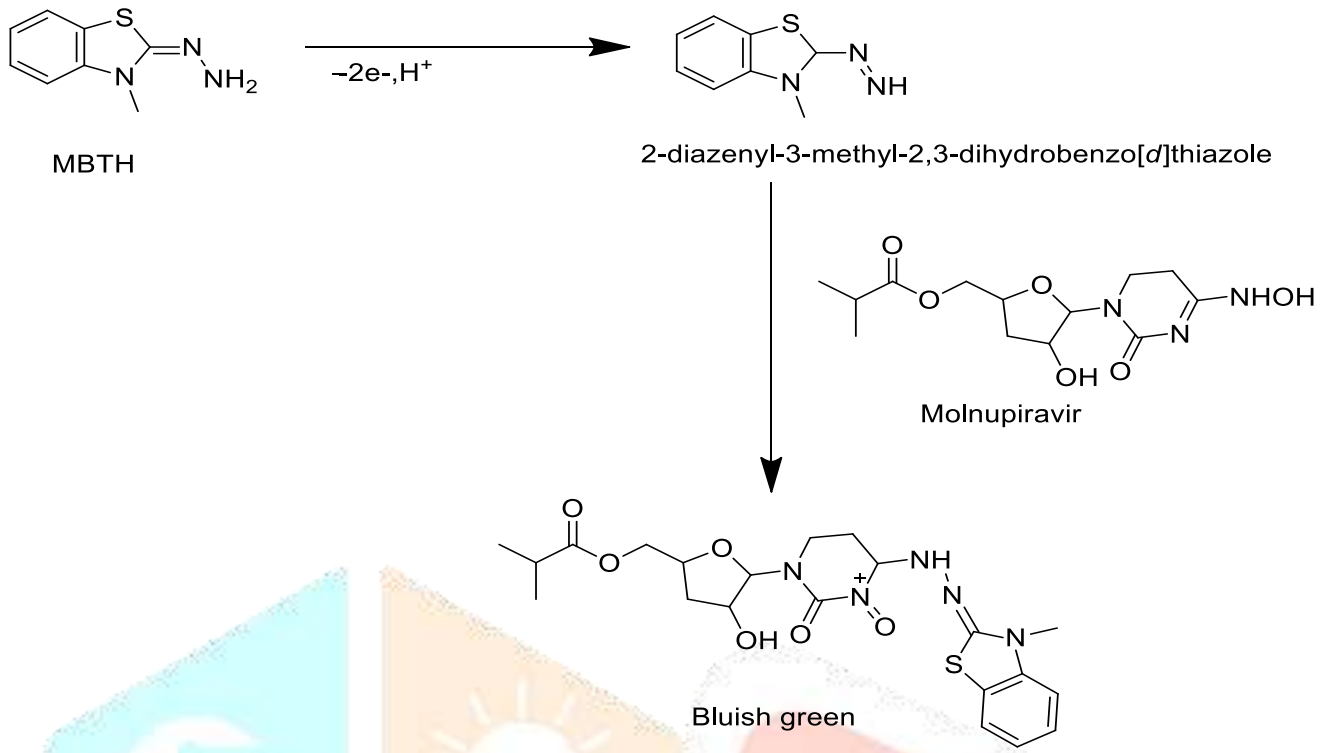

Figure 3: Scheme Reaction of Molnupiravir with MBTH

Method validation

All analytical procedures were validated according to ICH Q2 (R2) guidelines. The following parameters were performed for method validation.

Linearity: According to ICH, linearity refers to an analysis procedure's capacity to produce experiment results that are exactly proportionate to the analyte concentration in the sample. A single measurement is used to determine the linearity at various concentrations, after which the values of the slope, intercept, and coefficient of determination are ascertained. To estimate the linearity of the method, calibration samples were prepared with a suitable amount of sample and water spiking at 50, 100, 150, 200, and 250 µg/mL.

Limit of detection (LOD) and limit of quantitation (LOQ): According to the International Conference of Harmonisation (ICH) guidelines for the validation of analytical procedure, the following formulae were used as parameters to predict the sensitivity of the proposed method.

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Where, σ = standard deviation of the response (intercept) S = slope of the calibration curve

Accuracy: The degree to which test results agree when compared to the reference value or the true value. It explains how the measurement results' systematic error is determined. By examining three replicates of each sample as a batch in a single assay, the accuracy of drug substance was assessed on samples of drug solutions at different concentration levels in the range of 80%–120% (120µg/mL, 150µg/mL, and 180µg/mL).

Precision: The degree to which individual test results agree when the method is repeatedly used on several homogeneous sample samplings. It is expressed as Relative Standard Deviation (%RSD) and gives an indication of results from random errors. Analysis was done on differences in results between days (inter day) and within the same day (intraday).

Table 1: Optimum conditions and spectral data

CONDITIONS	MOLNUPIRAVIR WITH MBTH
λmax	602nm
Beer's law range	50-250µg/ml
Limit of detection (µg/mL)	9.54µg/ml
Limit of quantification (µg/mL)	28.9µg/ml
Regression equation (y)	Y=0.0037x-0.0133
Slope	0.0037
Intercept	-0.0133
Correlation coefficient (r^2)	0.9991

Y=mx+c where c is the concentration of drug in µg/mL and y is the absorbance max. The results obtained with the proposed method confirm that this method is linear and sensitive.

Result and Discussion

Linearity

The linearity of the developed method was established by performing linear regression analysis for the calibration curve constructed between concentration and absorbance. The linearity values for the drug with Molnupiravir MBTH reagent are given below in table 2.

Table 2: Linearity Data

Concentration(µg/ml)	Absorbance
50	0.177
100	0.358
150	0.531
200	0.741
250	0.915

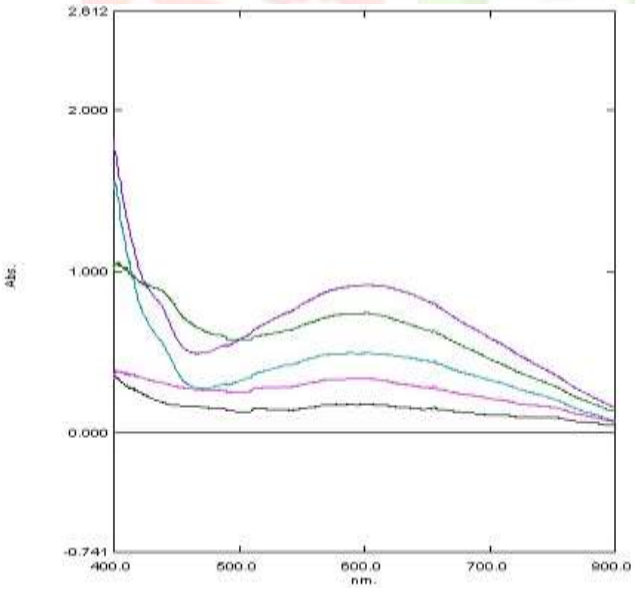

Figure 4: Overlay Spectra of Molnupiravir with MBTH At 602nm

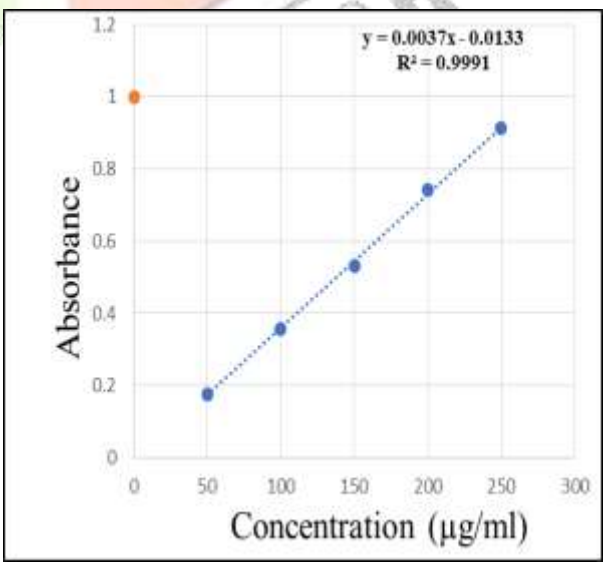

Figure 5: Calibration curve of Molnupiravir with MBTH reagent

The response of the drug was proved to be linear in the investigational range 50- 250µg/ml. The calibration curve was found to be linear with an r^2 value 0.9991 and regression equation was y =0.0037x-0.0133. For these studies obtained r^2 value was appropriate to demonstrate the linearity of the developed method.

Limit of Detection and Limit of Quantitation

The sensitivity of developed method for the measurement of Molnupiravir was estimated in terms of LOD & LOQ. The LOD and LOQ values are calculated as given below.

Table 3: LOD & LOQ data

Drug with Reagent	LOD	LOQ
Molnupiravir with MBTH	9.54µg/ml	28.9µg/ml

Precision

The precision of the developed analytical method was assessed by checking repeatability, intra-day precision and inter-day precision for Molnupiravir drug using MBTH.

Repeatability

Table 4: Repeatability Data

Sl.no	Concentration (µg/mL)	Absorbance	Mean*±standard deviation	%RSD
1	150	0.544	0.5425±0.00547	1.00794%
2	150	0.542		
3	150	0.546		
4	150	0.549		
5	150	0.541		
6	150	0.533		

The results reveal that %RSD values were within the limits

Intermediate Precision

Intra-day precision

The intraday precision of the proposed method was determined by analysing the corresponding concentration (120, 150, and 180µg/ml).Average 3 determinations on the same day and the results were reported in terms of relative standard deviation.

Table 5: Intra-day Precision Data

Sl.no	Concentration (µg/mL)	Mean Absorbance			Mean*±standard deviation	%RSD
		Mrng	AN	Evng		
1	**120**	0.320	0.318	0.310	0.316±0.00542	1.71%
2	**150**	0.422	0.417	0.409	0.416±0.00656	1.57%
3	**180**	0.535	0.522	0.518	0.525±0.0087	1.65%

Inter-day precision

The inter-day precision of the proposed method was determined by analyzing the corresponding concentrations (120, 150 and 180µg/ml) on 3 consecutive days and the results were reported in terms of relative standard deviation.

Table 6 : Inter-day Precision Data

Sl.no	Concentration (µg/mL)	Mean Absorbance			Mean*±standard deviation	%RSD
		Day1	Day2	Day3		
1	**120**	0.320	0.317	0.309	0.315±0.00564	1.78%
	150	0.473	0.468	0.458	0.466±0.0076	1.62%
	180	0.562	0.555	0.543	0.553±0.00976	1.76%

Acceptance Criteria – RSD less than or equal to 2%

Accuracy

The analytical accuracy is the nearest of the results obtained against the real values at each level of Molnupiravir concentration. The results of obtained for accuracy studies for the drug product were reported in terms of %RSD and %recovery respectively.

For drug product (recovery study)

The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the capsule powder with pure Molnupiravir drug at three different levels (80%, 100% and 120%).

Table 7 : Accuracy Data

Capsule used	Levels	Amount of sample added (µg/ml)	Amount of standard drug added (µg/ml)	Amount recovered (µg/ml)	%Recovery±standard deviation
MOVFOR	80%	120	150	270	100.237±0.00723
	100%	150	150	298	99.372±0.00208
	120%	180	150	329	99.866±0.00603

Assay

The proposed method was then applied for the determination of Molnupiravir in marketed formulations (capsules) brand name-MOVFOR capsules manufactured by (Hetero Healthcare Limited), contains 200mg. The %purity of the drug was presented in table 8.

Table 8: Assay Data

Capsule	Label claim	Amount found	%Purity
MOVFOR	200mg	196.02	98%

Acknowledgement

Finally, I would like to acknowledge the general support of the entire G.Pulla Reddy College of pharmacy community. The collaborative and supportive atmosphere significantly enriched the research experience.

Conclusion

It can be concluded that the determination of Molnupiravir was done by using MBTH as chromogenic reagent in bulk and pharmaceutical dosage form and the method was proved to be linear in the range 50-250µg/ml. The method was done according to ICH guidelines and the validation parameters were found within limits. The developed method was found to be sensitive, Linear, Precise, Accurate, reproducible.

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