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Spectrophotometric Method Development and Validation for the Determination of Molnupiravir in Bulk Powder and Pharmaceutical Formulation

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Abstract

A simple fast ecofriendly spectrophotometric method was developed and applied successfully for accurate and sensitive assessment of Molnupiravir (MOL) in pure bulk powder and pharmaceutical formulation. The solvent used during measurements was a mixture of ethanol and water in ratio (1:1, v/v) where the λ_{max} of MOL was observed at 230.0 nm. Linearity for MOL was displayed within the concentration range of 2.5-20.0 µg/mL exhibiting 0.9997 as the correlation coefficient. The method validation was performed in accordance to ICH guidelines while the limits of detection and quantification were calculated and found to be 0.37 and 1.11 µg/mL; respectively. The obtained results assured method specificity where MOL can be determined in the presence of common pharmaceutical excipients.

Keywords: Molnupiravir; ICH Validation; Spectrophotometry; Covid-19

1. Introduction

The Immediate intervention with confirmed COVID-19 patients with mild symptoms can effectively diminish the progress to severe symptoms and hospitalization. Among the suggested promising treatments is Molnupiravir (MOL) which is considered one of the portentous antiviral agents that could minimize the risk of hospitalization and death rates in non-hospitalized COVID-19 patients. MOL proved to be most effective against infections with existing and potential emerging SARS-CoV-2 variants of concern (VoCs) (**~~1-4~~**). The effectiveness data have been widely described in COVID-19 patients (**~~5-10~~**). MOL, N-Hydroxy-5'-O-isobutyryl-3,4-dihydrocytidine [(2R,3S,4R,5R)-3,4-Dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl] oxolan-2-yl] methyl 2-methylpropanoate, is a prodrug metabolized to the ribonucleoside analogue N-hydroxycytidine (NHC) which circulates into cells, then phosphorylated to generate the pharmacologically active ribonucleoside triphosphate (NHC-TP). Its mechanism of action is recognized through a viral error catastrophe where the NHC-TP inclusion into viral RNA by the viral RNA polymerase, results in a buildup of errors in the viral genome that will lead to the discontinuation of replication (**~~11-15~~**). MOL could be counted as the first oral, direct-acting antiviral with a pronounced efficiency in decreasing the nasopharyngeal SARS-CoV-2 infectious virus and viral RNA while exhibiting a promising safety and tolerability reports (**~~16-19~~**). The chemical structure of MOL is shown in Figure 1.

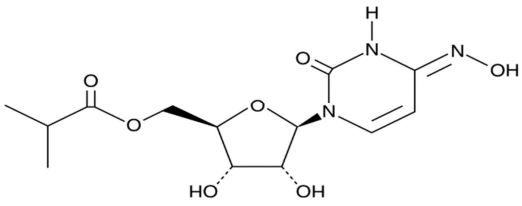

Figure 1: Chemical structure of MOL

Few analytical methods for MOL determination have been found in the literature review. Levels of MOL in human plasma have been measured using liquid chromatography coupled with tandem mass

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spectrometry (LC-MS/MS) **(17)** and in hamster model (**20**). However, the validation details have not been fully defined or reported except for the LC-MS/MS determination of MOL and its metabolite in human plasma and saliva (21). To the best of our knowledge, there is no simple and direct ultraviolet (UV) spectrophotometric method was found for analysis of MOL. Hence, the aim of our work was to construct a fully validated simple UV method for the assessment of MOL in bulk powder and solid pharmaceutical formulation in accordance with the ICH guidelines.

Table 1

A comparison between literature and the developed UV method

Method	Maximum wavelength (nm)	Linear range (µg/mL)	Ref.
RP-HPLC-PDA	240	0.1–60	(22)
RP-HPLC-UV	230	0.2-80	(23)
UV	230	2.5-20	The developed method

II. Experimental

II.1. Materials and reagents

MOL, purity (98%), was obtained from Optimus Drugs Pvt LTD, India. (MOL 200 mg per capsule, R&D prepared samples) was used as pharmaceutical formulation dosage form. American Society for Testing and Materials (ASTM) grade I water, was daily obtained from the central laboratory. Acetonitrile, ethanol and methanol were supplied by Scharlau, Barcelona, Spain.

II.2. Apparatus

For the spectrophotometric method development, the electronic balance, pH meter (Mettler Toledo, 235, MA, USA), UV–visible spectrophotometer Shimadzu UV JAPAN 1801, with 2.0 nm slit width and 10.0 mm path length, and Sonicator were utilized.

II.3. Preparation of standard solutions

20.0 mg of MOL was transferred into a 100-mL volumetric flask, dissolved with ethanol and the volume was completed with the same solvent to finally obtain a stock solution with a concentration of 200.0 µg/mL (Solution A). Then 5.0 mL of solution A was transferred into another calibrated 50-mL volumetric flask and the volume was completed with a mixture of ethanol: water in ratio (1:1, *v/v*) (Solution B).

Then, different volumes of Solution B were accurately transferred into a set of 10-mL volumetric flasks and diluted with a mixture of ethanol and water in ratio (1:1, v/v) as needed to prepare different standard solutions over the concentration range of 2.5-20 µg/mL for the MOL as a calibration set.

II.4. Preparation of Test solution

The content of ten capsules (MOL 200 mg per capsule, R&D prepared samples) was grinded to fine powder and weight equivalent to 20.0 mg MOL was dissolved into a 100 mL volumetric flask enclosing 80 mL ethanol. Afterward, the solution was sonicated for 15 min and diluted to final volume was completed with ethanol. 5.0 mL of this solution was diluted in a 100 mL volumetric flask using the diluent to obtain a final nominal concentration of 0.01 µg/mL.

II.5. Procedures

Zero-order absorption spectra of Molnupiravir

The previously prepared standard solutions in the calibration set as previously mentioned under II.3, were scanned and the absorption spectra were stored. In addition, the test solution prepared as mentioned in details under II.4 was also scanned in wavelength range from 200-400 nm using a mixture of ethanol and water in ratio (1:1, v/v) as a blank.

Construction of the calibration curve

A calibration graph was constructed representing the relation between the absorption value at 230 nm of different standard solutions in concentration range from 2.5-20 µg/mL against corresponding concentrations and the regression equation was computed to be used later in calculation of unknown concentrations of MOL.

III. Results and discussion

Direct Spectrophotometric technique offers a significant approach for drug analysis either in bulk, different dosage forms whether the drug is single or in combination. Spectrophotometric technique is simple, cost effective and doesn't require any expensive instruments or sophisticated programs. In addition, it could be considered a green and environmentally friendly technique. It is mainly used for the routine analysis and tracking any change in the concentration of drugs under study during manufacture or storage to be able to select the best additives and manufacturing steps for the aim of delivering a safe product to the patient with the right concentration.

In the presented work the main aim was to develop a simple, fast, cheap and ecofriendly spectrophotometric method for MOL routine analysis either in raw powder or in pharmaceutical dosage form. For the sake of attaining good absorbance without any significant interference from the excipients in the dosage form, initial trials were performed to select adequate and optimum conditions for measurements. Parameters, such as an ideal solvent & final diluent for MOL, detection wavelength, and concentration of the targeted standard solutions were carefully studied.

Optimization of experimental parameters

Solvent & final diluent selection

MOL was found to be soluble in water (5.8 mg/mL) **(24)**, phosphate buffer pH 7.2 (1.0 mg/mL), DMF (30.0 mg/mL), DMSO (30.0 mg/mL) **(25)**, methanol (35.0 mg/mL), ethanol (28.0 mg/mL), and acetonitrile (25.0 mg/mL) **(23)**. Thus after different trials, the stock standard solution was prepared in ethanol to assure complete dissolution of the drug where ethanol is a green solvent and more preferred than methanol. Where methanol is considered one of the most toxic solvents and the analysts may be to its hazards through skin contact, inhalation of its fumes or by ingestion. Thus, replacing methanol with a greener alternative as ethanol was intended.

During the preparation of working solution and the calibration set, it was found that using a diluent of ethanol and water in ratio (1:1, v/v), after trying other different ratios, was sufficient for drug dissolution. The addition of water offered some merits to the method where it minimized the cost of the method and, reduced the use of organic solvent and decreased the generated waste.

Wavelength selection

The zero order spectrum of MOL was scanned in the wavelength range of 200.0-400.0 nm as illustrated in Figure 2. Two absorbance peaks were observed at 230.0 and 270.0 nm **(25)**. From the recorded spectrum, a wavelength of 230.0 nm was selected as the maximum wavelength where MOL exhibited maximum absorbance.

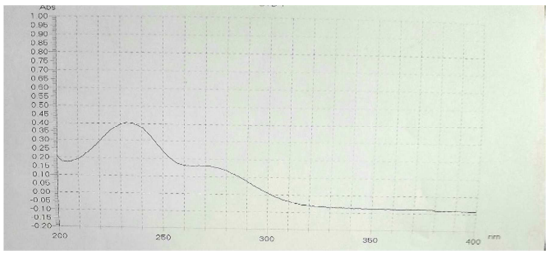

Figure 2: Zero-order absorption spectrum of MOL (10 µg/mL) in a mixture of ethanol: water in ratio (1:1, v/v) as a diluent

IV. Method validation

The analytical methods were suitably settled and validated, regarding the accuracy, precision, linearity, specificity, the limit of detection (LOD) and the limit of quantification (LOQ), in agreement with the ICH guidelines **(26)**.

Specificity

Was appraised by calculating the response of the blank, placebo, analyte samples and any expected or known species (for example excipients). It would typically be anticipated that no response would be found that intrudes with the response of the analyte. The obtained results approved that there is no significant absorbance for diluent or placebo. The results demonstrate that the proposed method is specific for quantification of MOL in bulk powder and pharmaceutical formulation.

Linearity

Standard solutions over a concentration range of 2.5-20.0 µg/mL for MOL, each in three replicates, were measured via the UV system at 230.0 nm using a mixture of ethanol and water in ratio (1:1, v/v) as a diluent. The calibration graphs obtained for the drugs were linear over the investigated range with RSD% of less than 1% based on three successive readings. The correlation coefficient of 0.9997 suggested that the adopted UV method exhibits an excellent linearity over the concentration ranges of 2.5-20.0 µg/mL for MOL.

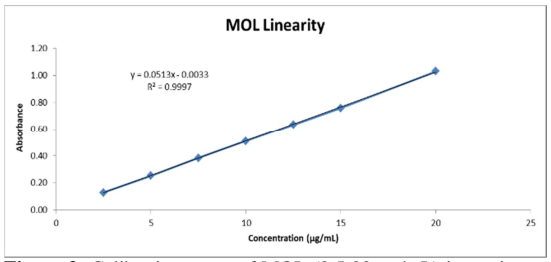

Figure 3: Calibration curve of MOL (2.5-20 µg/mL) in a mixture of ethanol: water in ratio (1:1, v/v) as a diluent at 230 nm

The validation parameters for MOL were expressed in Table 2. The Detection and quantification limits (LOD and LOQ) were also estimated as per ICH guidelines (26-47) and the results were tabulated in Table 2.

Table 2

Validation parameters for determination of Molnupiravir using the proposed direct spectrophotometric method

Parameters	MOL
Linear range (µg/mL)	2.5-20.0
Equation of calibration curve	y = 0.0513x - 0.0033
SD for slope of the calibration curve	0.006
Correlation coefficient (R^2)	0.9997
LOD (µg/mL)	0.37
LOQ (µg/mL)	1.11

Accuracy

Accuracy could be defined as the closeness of conformism between the actual quantity and test result. The accuracy of the proposed spectrophotometric method was evaluated by carrying out recovery studies at distinctive spiked levels (5, 10 & 15 µg/mL). Three determinations were performed at each level and the results were recorded. The amounts recovered and the percent recovery values were calculated, results are displayed in Figure 4. The percentage recovery was found to be within the limit of 98.79 -101.26% with RSD% was 1.28.

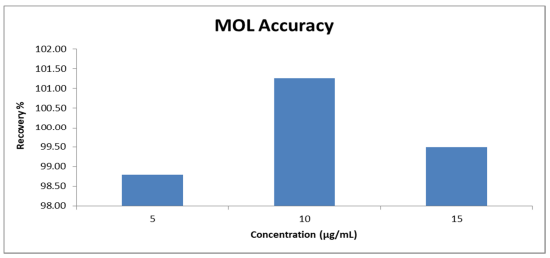

Figure 4: Accuracy of MOL via UV at 230.0 nm

Precision

Precision was done on three different levels, repeatability (six determination of standard solution 10.0 µg/mL), intraday (analyst I & analyst II) and interday (day I & day II), where each analyst prepares 6 different tests. Absorbance was determined and compared. Percentage relative standard deviation RSD% was used as indicator for precision; where results must be less than 2.0%. The results revealed that the % RSD was in the satisfactory range for the different standard solutions, RSD% was 0.266 for the first day and 0.375 for the second day precision. Intraday and Interday precisions were performed as assay% for six tests against standard solutions. The average assay at intraday precision was 100.59% with RSD% of 1.26 and at interday precision was 99.85% with RSD% of 1.16. Therefore, the method precision was demonstrated to be adequate; results are illustrated in Figures 5 & 6.

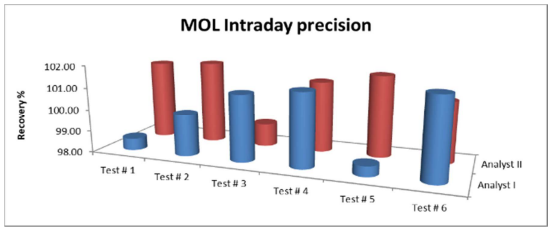

Figure 5: Intraday (Analyst I & Analyst II) precision of MOL

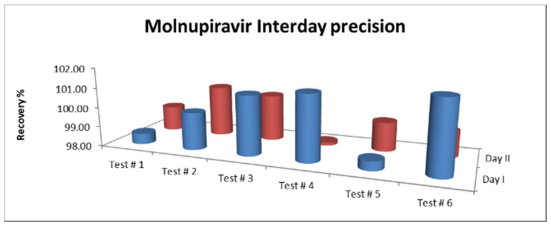

Figure 6: Interday (Day I & Day II) precision of MOL

Assay determination for MOL by the proposed UV method

Potency results are shown in Table 3, average of three determinations for the MOL 200 mg capsules, assays was 98.97% and RSD % was 0.71.

Table 3

Potency results for capsules (MOL 200 mg per capsule, R&D prepared samples)

EXP.#	MOL assay %
I	98.16
II	99.46
III	99.3
Average	98.97
RSD %	0.71

V. Statistical Analysis

All the results obtained by the proposed UV method were compared with those of the reported method of MOL (22), where no significant difference between both methods was observed, as shown in Table 4.

Table 4

Statistical comparison between the results of the proposed UV method and the reference method

Item	Proposed method	Reference method (22)	
Mean	99.32	100.15	
SD	0.58	1.04	
n		5	5
t-test		1.22 (2.31) *	-
F-value		5.89 (6.39) *	-

*The values in parenthesis are corresponding to the theoretical values of t and F at the 95% confidence level

Conclusions

In this study, economic, easy, fast and ecofriendly direct spectrophotometric method was optimized and validated in accordance to the ICH guidelines for the quantitative assay of MOL in bulk and pharmaceutical dosage form. The adopted method demonstrated its applicability and validity with respect to linearity, sensitivity, recovery, precision, and specificity. The proposed method could be applied routinely for MOL determination efficiently in quality control labs with no need for any separation step or sophisticated instruments or programs. The method could be applied in any step of the drug production starting from the purchase of the raw powder passing through packaging, transportation, storage and marketing in order to assure the effectiveness of the treatment.

1. Conflicts of interest

"There are no conflicts to declare"

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