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UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF MOLNUPIRAVIR IN PURE AND FORMULATION

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ABSTRACT

This study principally focuses on method development and validation of UV spectroscopy for assay of Molnupiravir in pure and pharmaceutical dosage forms. This method includes preparation of standard and working solutions of a Molnupiravir Drug and later aliquots of working solution of different concentrations were prepared and analysed. Then this established method had been validated for linearity, precision, accuracy, robustness, and ruggedness, limit of quantification and limit of detection. From the data obtained, it is clear that the method developed is sensitive and accurate within range of 2-12µg/ml. The correlation coefficient (R^2) was found to be 0.999. There is no interference observed with excipients in capsule formulation. The project method may be duly applied for the analysis of Molnupiravir in bulk for routine analysis.

Keywords: Ultraviolet Spectroscopy, validation, Molnupiravir, method development, assay

INTRODUCTION:

Antiviral Drugs are among the foremost prescribed agents worldwide to treat infections caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Molnupiravir **(Figure 1)** is an oral dosage antiviral drug showing its effect on nasopharyngeal SARS-COV-2, thus preventing progression of infection to severe

illness **[1, 2]**. It is a white crystalline powder with molecular weight 329.309 g/mol and melting point between 105-107 °C. It is chemically designated as [(2*R*,3*S*,4*R*,5*R*)-3,4-dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate. Molnupiravir is an antiviral drug candidate that is now being investigated in COVID-19 patients in phase III trials. Molnupiravir enhances the frequency of viral RNA alterations in animal models **[3-5]**.

By an extensive literature review we found that very few studies have been reported for the determination of molnupiravir using High-Performance Liquid Chromatography **[6]**. Another study on, LC-MS/MS studies for determination of Molnupiravir and its metabolite in human plasma **[7]**. UV spectroscopic methods for analysis of Molnupiravir in pure and pharmaceutical dosage forms have not been reported.

In this study, we tried to analyze Molnupiravir in pure and in pharmaceutical preparations. We selected the pharmaceutical preparation to be capsules and the solvent preferred as distilled water. All the optimization parameters were also considered, after development of UV method. The developed method was successfully validated and can be used to estimate the total drug content in the commercially available formulations of Molnupiravir.

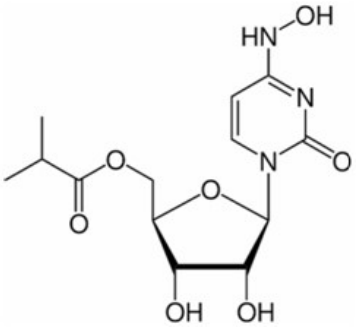

Figure 1: Chemical structure of Molnupiravir

MATERIALS AND METHODS:

Instruments and Reagents:

A gift sample of Molnupiravir with purity 99.98% was obtained from a Local manufacturing unit at Visakhapatnam. LAB INDIA (T_{60}) double beam UV/Visible spectrophotometer and ELITE analytical balance were the instruments used. The chemicals and reagents used are of analytical grade. Molnupiravir capsule formulation of 200 mg with a brand name of MOLFLU were purchased from the market.

Preparation of standard stock solution (1000µg/ml):

25mg of drug was added into a 25ml volumetric flask and dissolved using distilled water and finally made up to the mark with distilled water to get a concentration of 1000µg/ml, which is a standard stock solution of Molnupiravir.

Preparation of working standard solution (100µg/ml):

2.5ml of sample, from the above standard stock solution was transferred to a 25ml volumetric flask and made up to mark with distilled water to get a concentration of 100µg/ml.

Construction of calibration curve:

The working standard was then further diluted to get 8 µg/ml solution. It is scanned by a UV Spectrophotometer in the range of 200-400 using distilled water as blank. The maximum absorbance was found to be at wavelength 235 nm. Aliquots ranging from 2-12 µg/ml solutions were prepared by using distilled water as solvent. These samples were then analysed at λ_{max} 235nm to get respective absorbance. The values are then plotted to get a calibration curve.

Preparation of the Assay solution:

The proposed method was applied to analyze the commercially available "Molflu" capsule formulation.

RESULTS:

Method Validation:

Linearity:

Different aliquots of Molnupiravir were prepared in the range of 2-12µg/ml from the working standard solution (100µg/ml). These solutions were scanned on a Double beam UV-VIS spectrophotometer in the range of 200-400nm using distilled water as the blank. The spectrum was recorded at 235 nm **(Figure 2)**. The calibration plot was constructed as concentration versus absorbance and can be shown in **Figure 3** and **Table 1**. This shows a perfect linearity has been established.

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the intra-day variation study, six different solutions of the same concentration 8µg/ml were prepared and analyzed twice a day (Morning, and Evening) and the % RSD was calculated and reported **(Figure 4)**. In the inter-day variation study, the solutions of the same concentration 8µg/ml were prepared and analyzed six times, for two consecutive days and the absorbance was recorded **(Figure 5)**. The percentage relative standard deviations are found to be <2%.

Accuracy:

The accuracy of the method was determined by standard addition method the percent recovery of Molnupiravir was calculated. Known amounts of standard solutions were added at 80,100, 120% level to pre-quantified sample solution of Molnupiravir. The solutions were prepared in triplicate and the accuracy was indicated by % recovery

was calculated and reported in the **Table 2**. The percent recovery was found to be good.

Robustness:

The Robustness of the method was carried out by analyzing the sample of 8µg/ml using three different wavelengths (1of λ_{max}) and respective absorbance were recorded. The results shown in **Figure 6** indicate the method was robust.

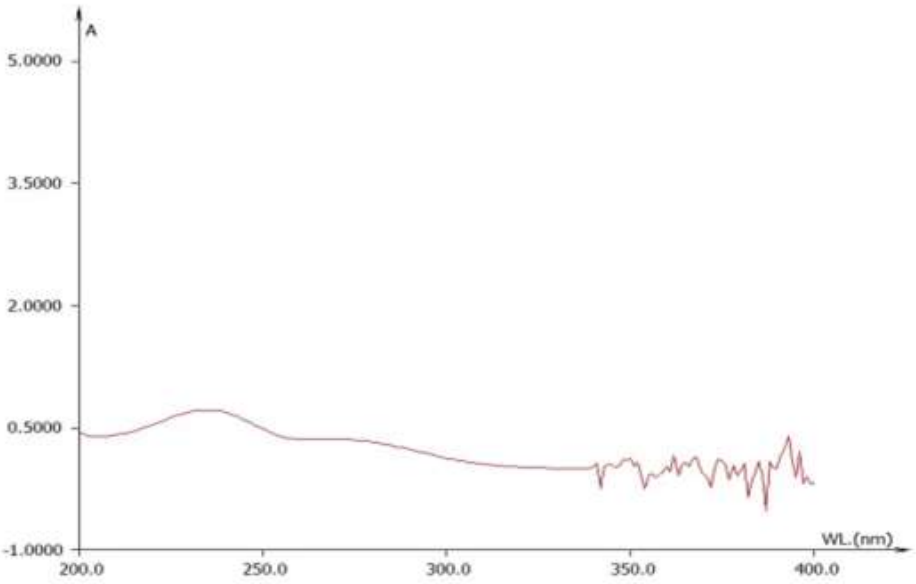

Figure 2: Spectrum obtained for pure drug

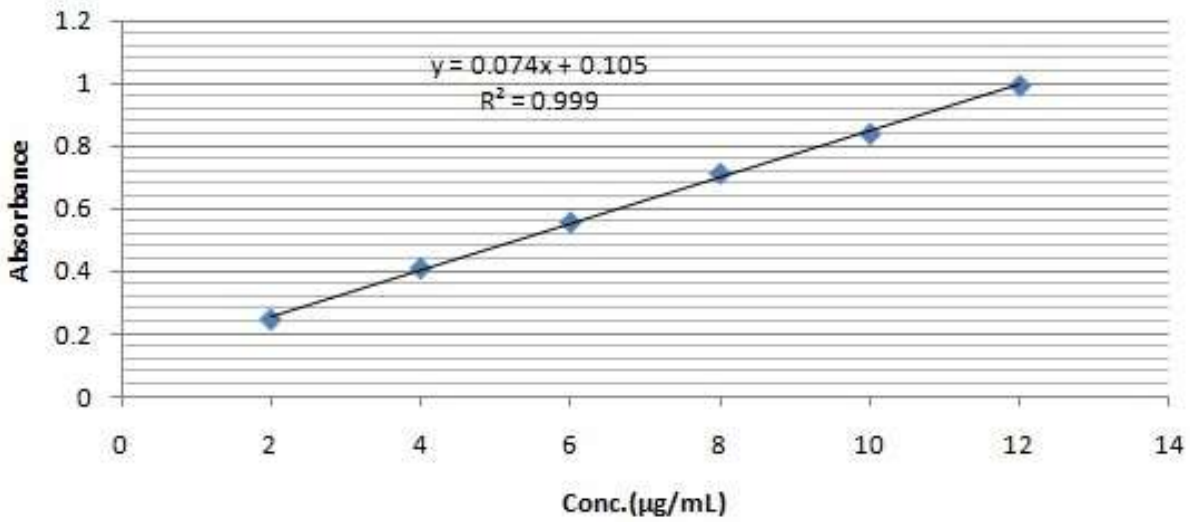

Figure 3: Calibration plot

Table 1: Linearity Data

Concentration (µg/ml)	Absorbance
2	0.2438
4	0.4075
6	0.5544
8	0.7102
10	0.8372
12	0.9910
Regression equation	Y = 0.074x+0.105
Correlation coefficient(R^2)	0.999

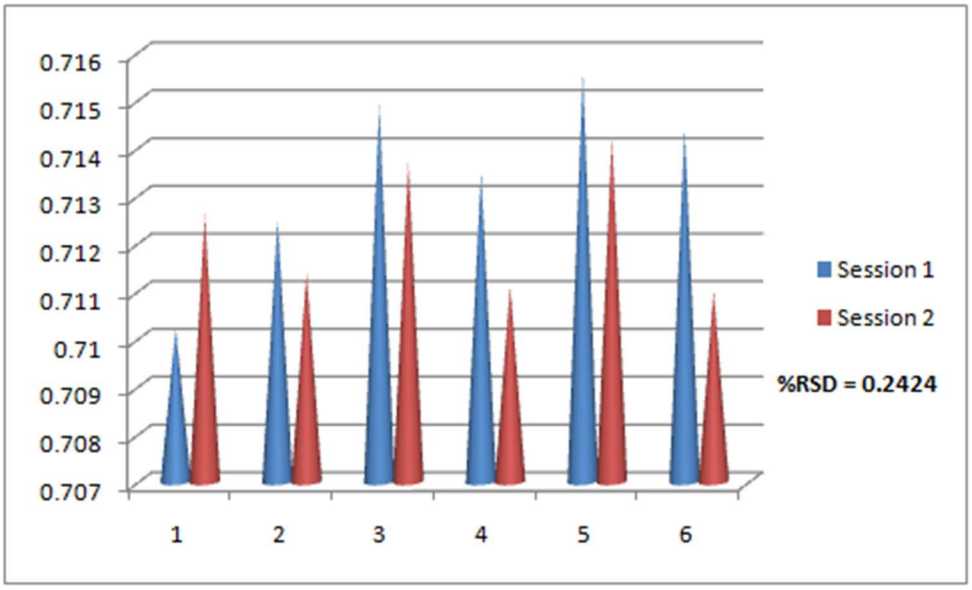

Figure 4: Intraday Precision

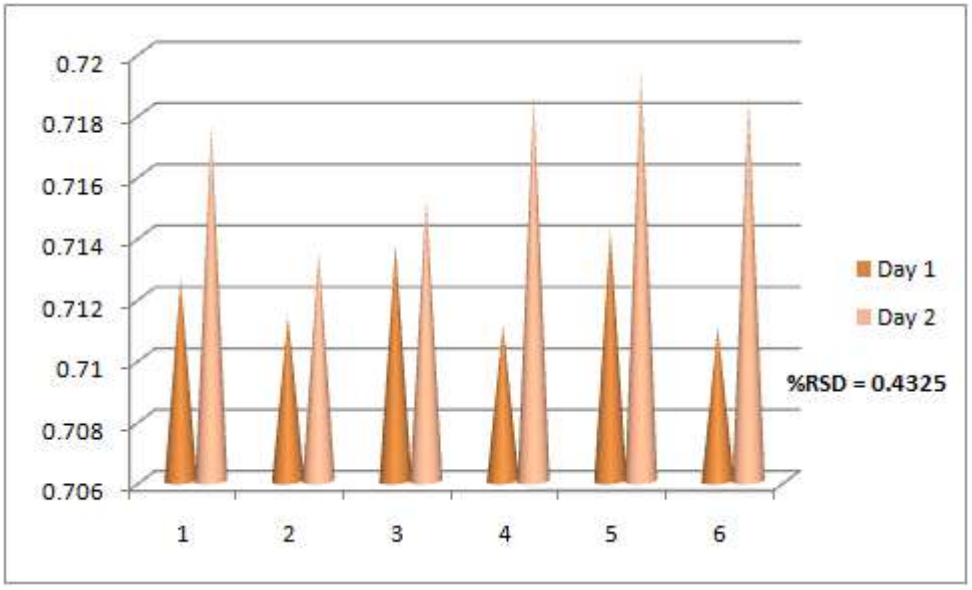

Figure 5: Interday Precision

Table 2: Accuracy of method

Level of addition	Formulation amount	Amount added	Theoretical amount	Experimental amount	% recovery	% mean recovery ±SD
80%	8	6.4	7.2	7.33	101.80	101.80±0.002
100%	8	8	8	7.93	99.12	99.12±0.002
120%	8	9.6	8.8	8.81	100.11	100.11±0.0009

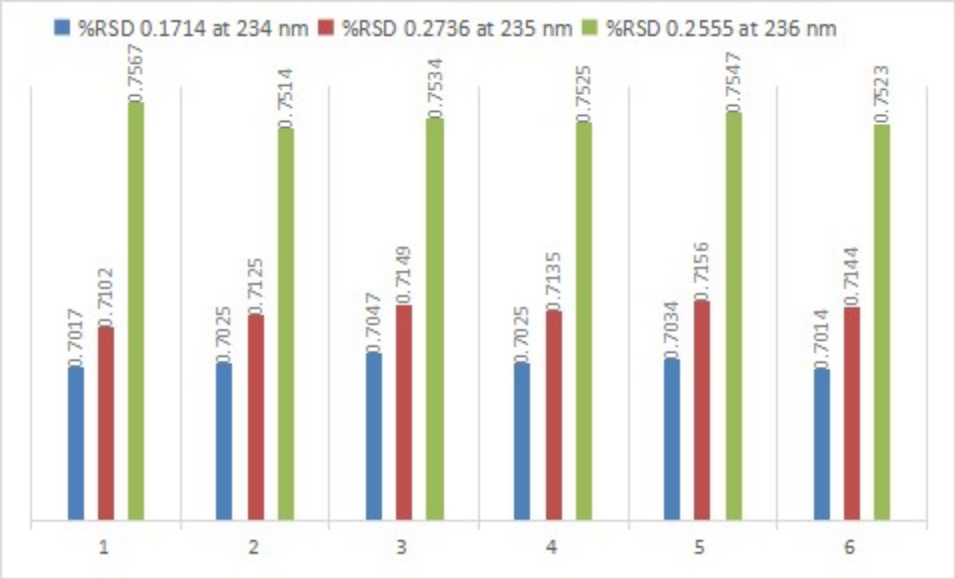

Figure 6: Robustness of method at wavelength 234nm, 235(λ_{max}), 236nm

Ruggedness:

The ruggedness of the method was carried out by analyzing the sample using two different analysts with same equipment and two different cuvettes by same analyst and respective absorbance were recorded. The results of first Analyst showed %RSD 0.1672 and second analyst showed %RSD 0.2184, which indicate that the methodology employed was rugged, since there is no significant difference between different operators.

Sensitivity:

Limit of detection (LOD) and limit of quantification (LOQ) of the drug was calculated from the calibration curve as 0.5017 µg/ml and 1.5068 µg/ml as respectively.

DISCUSSION

The method was developed and validated as per ICH guidelines. The method was validated in terms of linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. Beers law obeyed over the concentration range of 2-12 µg/ml, using multivariate analysis the linear equation Y=0.074x + 0.105 with a correlation coefficient if R^2= 0.999. The precision results show % RSD less than 2 at each level which indicates clearly that the method is precise

enough for the analysis of Molnupiravir. The accuracy of the method was checked by recovery studies. The high recovery with values indicates the accuracy of the developed methodology. The robustness and ruggedness studies reveal that the method is more sensitive. There was no interference ascertained from the excipients present in the formulation, indicated that the method is specific. Determination of Molnupiravir in capsule formulation showed that the drug was very close to the label amount. The percentage RSD values and all the characteristics parameters of the method are represented in the **(Table 3)**.

Assay of formulation*:*

The formulation procured was assayed by taking equivalent weight of 25 mg of molnupiravir in THE capsule formulation in a 25 ml volumetric flask using diluent as a distilled water and the final concentration was made using distilled water (8µg/ml). It was analyzed by screening in UV range of 400-200nm **(Figure 8)**.

Table 3: Summary of Results

Parameters		Results
Absorption maxima(nm)	**:**	**235nm**
Linearity range(µg/ml)	**:**	**2-12**
Regression equation	**:**	**Y = 0.074x + 0.105**
Correlation coefficient(R^2)	**:**	**0.999**
Accuracy	**:**	**99.12 -101.80%**
LOD(µg/ml)	**:**	**0.5017**
LOQ(µg/ml)	**:**	**1.5068**
Intraday precision(%RSD)	**:**	**0.0078**
Inter-day precision(%RSD)	**:**	**0.1449**

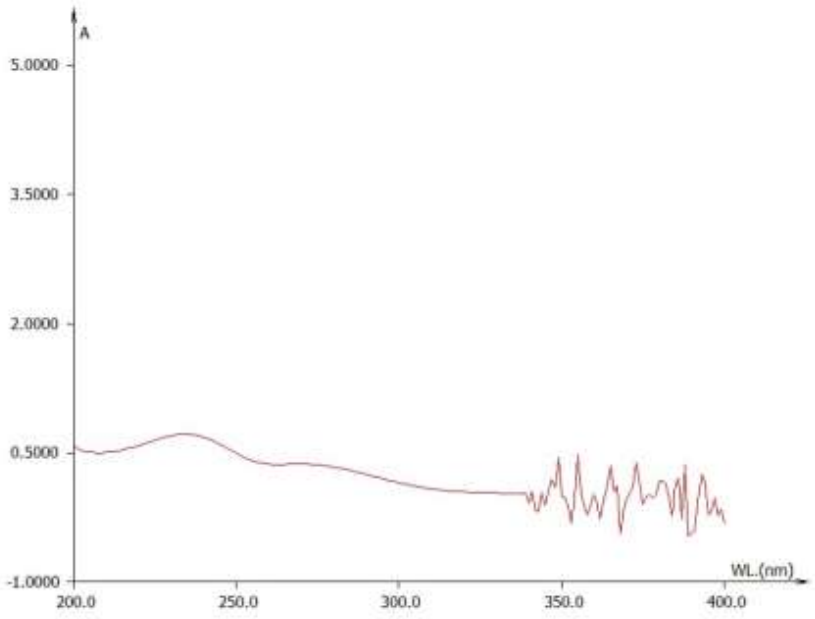

Figure 8: Spectrum obtained for formulation

CONCLUSION:

The proposed method was simple and found to be accurate, precise, robust and easy to determine. The proposed method gave a lower range of the calibration plot. The sample recoveries were in good agreement. The apparatus and reagents used seem to be accessible even for simple laboratories. Therefore, developed method can be recommended for routine and QC analysis of Molnupiravir. This methodology is also suitable for analysis of sample during accelerated stability studies, routine analysis of formulations and Active pharmaceutical ingredient.

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