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RESEARCH ARTICLE

New UV Spectrophotometric Method for the Estimation of Molnupiravir used in the treatment of COVID-19

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Abstract:

Background:

Antiviral drugs gained more importance due to SARS-COV-2 infection and many drugs are under investigation to end the pandemic. Molnupiravir is an investigational medicinal product being developed by Merck Sharp and Dohme in collaboration with Ridgeback for the treatment of COVID-19.

Objective:

A new, simple, and economical UV-spectrometric method was developed and validated for the estimation of Molnupiravir in a bulk and pharmaceutical dosage form.

Methods:

The maximum wavelength was found to be 236 nm. The developed method was validated according to ICH guidelines and found to be linear within the range of 10-50µg/ml with a correlation coefficient (R^2) 0.9989.

Results:

The %RSD for precision, accuracy, LOD, LOQ, Ruggedness, and Robustness were within the range of acceptable limits as per ICH Q2 (R1). The accuracy of the method was determined at three concentration levels and found to be 99.87%, 99.53%, and 99.84%.

Conclusion:

All parameters obtained are within acceptable limits as per ICH guidelines. The molnupiravir was exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions and its stability data was determined which will be useful for further formulation development.

Keywords: Molnupiravir, COVID-19, Spectrophotmetric, Stability, ICH, [Q_2 (R_1)].

Article History	Received: July 15, 2022	Revised: January 06, 2023	Accepted: January 19, 2023

1. INTRODUCTION

The Molnupiravir, N-Hydroxy-5[1]-0-isobutyryl-3, 4-dihydrocytidine (C2R, 3S, 4R, 5R)-3, 4-dihydroxy-5-((4Z)-4-(hydroxyimino)-2-oxo-3, 4-dihydropyrimidin-1(2H)-yl) oxolan-2-yl) methyl 2-methyl propanoate, was approved by UKS medicines and health product regulatory agency on 04 November 2021 and on 23 December 2021 was granted emergency use of authorization by FDA developed by Merck Sharp and Dohme in collaboration with Ridge back for treatment of COVID-19 [1].

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An oral bioavailable isopropyl ester prodrug of the ribonucleoside analog -d-N 4-Hydroxycytidine (NHC, also known as EIDD-1931), molnupiravir (MPV), also known as EIDD-2801/MK-4482, has antiviral activity against several RNA viruses. With its active metabolite NHC showing broad-spectrum antiviral efficacy against SARS-CoV-2, MERS-CoV, SARS-CoV, and related zoonotic group 2b or 2c bat-CoVs, pharmacokinetic (PK) profiling revealed that MPV is orally bioavailable in ferrets and nonhuman primates. Additionally, it demonstrated improved effectiveness against CoV with mutations conferring resistance to the nucleoside analog inhibitor, Remdesivir. In mice models of SARS-CoV or MERS-CoV infection, prophylactic and therapeutic MPV treatment markedly improved pulmonary function, decreased

viral titer, and prevented body weight loss [2]. An immunocompromised mouse model's response to molnupiravir treatment revealed suppression of SARS-CoV-2 replication in vivo, supporting the drug's potential for the treatment of COVID-19. Data from a phase 2a clinical trial presented at the 2021 Conference on Retroviruses and Opportunistic Infections (CROI) demonstrated that, in comparison to the placebo, treatment with molnupiravir significantly decreased the time to the negativity of infectious virus from nasopharyngeal swabs in COVID-19 participants [3].

Molnupriavir was recently approved for the treatment of COVID-19, there are no spectrophotometric methods available for this drug [2 - 14]. Therefore the new spectrophotometric method with forced degradation was developed and validated in bulk as well as in formulation for Molnupiravir.

2. MATERIALS AND METHODS

The Molnupiravir standard was provided by Swapnroop Research Pvt. Ltd., India. The Molnupiravir tablet containing 200 mg of Molnupiravir and the inactive ingredient used in the drug matrix was obtained from the market under the brand name “Molflu 200”. The analytical grade methanol was obtained from Merck life Science Pvt. Ltd. (Mumbai). The UV visible double beam spectrometer with matched quartz cells (1 cm) of Shimadzu with model no. 1800 was used for analysis.

2.1. Preparation of Standard Stock Solution

The accurately weighed 10mg Molnupiravir was transferred in a volumetric flask containing 10ml methanol to prepare 1000 µg/ml, and from that 100 µg/ml was prepared as a working standard.

2.2. Selection of Wavelength

The standard solution of Molnupiravir was scanned on a UV spectrophotometer between 200 nm to 400 nm on spectrum mode using diluents as blank. The Molnupiravir shows λmax at 236 nm (Fig. **1**).

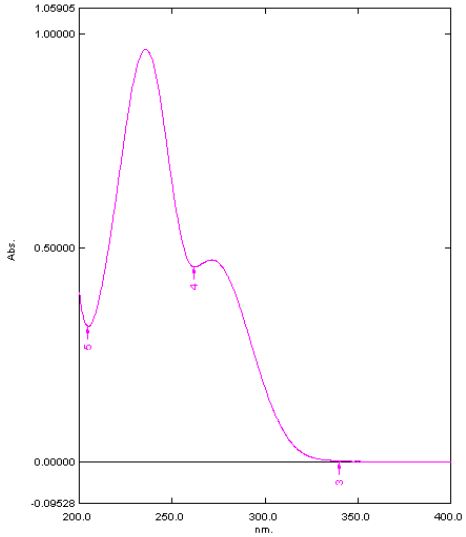

Fig. (1). UV Spectra of 20 µg/ml Molnupiravir.

2.3. Method Validation

The new method was validated by following “Validation of Analytical procedures: Text and methodology Q2 (R1)”, guidelines given by the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use. The objective of the analytical procedure will state different validation characteristics like linearity, precision, repeatability, intermediate precision, specificity, detection limit, quantitation limit, linearity, and range [15].

2.4. Linearity

Five points calibration curve was obtained in a concentration range from 10– 50 µg/ml for Molnupiravir. The response of the drug was found to be linear in concentration range and the linear regression equation was y = 0.042357x+0.123828 with a correlation coefficient of 0.9989. The results are shown in Table **1** and Fig. (**2**).

Table 1. Results of linearity range and absorbance.

Concentration (ppm)	Absorbance
10	0.55148
20	0.96286
30	1.38023
40	1.85464
50	2.22342
y-intercept	0.12382
Slope	0.042357

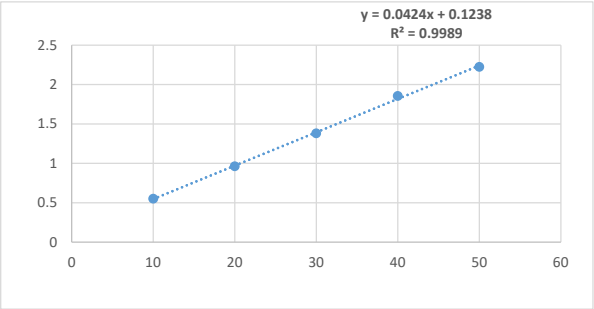

Fig. (2). Calibration curve in the concentration range 10–50 µg/ml.

2.5. Precision

The inter-day and intra-day precision of the method was determined at three concentration levels 10, 20, and 30 µg/ml. Three replicates of each concentration were determined, and the % RSD of found concentration was determined. The results are given in Tables (**2** - **5**).

2.6. Repeatability

The method's repeatability was determined by analyzing six replicates within short time intervals. The results are given in Table **6**.

2.7. Accuracy

The accuracy of the method was determined by the standard addition method at 80%, 100%, and 120%

concentration levels. The samples were analyzed on a UV spectrophotometer and the concentration found was compared with the found concentration standards of the same concentration. The percent recovery was obtained in the range of 99.87 – 99.84%. The results are shown in Table **7**.

2.8. Robustness

The robustness of the method was determined by small but deliberate changes in wavelength and the drug was analyzed. The results of %RSD are given in Table **8**.

Table 2. Results of intraday precision–morning.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.55035	10.0	100	-
10	0.53998	9.815	98.15	98.796
-	0.54035	9.824	98.24	-
-	0.95947	19.709	98.545	-
20	0.94995	19.484	97.423	97.646
-	0.94613	19.394	96.972	-
-	1.36481	29.269	97.563	-
30	1.36951	29.379	97.93	98.001
-	1.37686	29.553	98.51	-
-	-	-	Mean	98.147
-	-	-	SD	0.588862
-	-	-	%RSD	0.599975

Table 3. Results of intraday precision –evening.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.54293	9.885	98.85	-
10	0.53841	9.778	97.78	97.556
-	0.53101	9.604	96.04	-
-	0.94196	19.297	96.481	-
20	0.94996	19.484	97.424	96.911
-	0.94412	19.366	96.83	-
-	1.350	28.919	96.399	-
30	1.358	29.108	97.028	97.159
-	1.371	29.415	98.050	-
-	-	-	Mean	97.208
-	-	-	SD	0.325356
-	-	-	%RSD	0.3347

Table 4. Results of interday precision – day – I.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.52774	9.5268	95.268	-
10	0.53680	9.7405	97.405	97.017
-	0.54093	9.8379	98.379	-
-	0.92998	19.0136	95.068	-
20	0.94948	19.4735	97.367	96.233
-	0.94012	19.2528	96.264	-
-	1.351	28.943	96.477	-
30	1.347	28.849	96.163	96.110
-	1.341	28.707	95.691	-
-	-	-	Mean	96.453
-	-	-	SD	0.492008
-	-	-	%RSD	0.5101

2.9. Ruggedness

The ruggedness of the method was analyzed by two different analysts at three concentration levels. The results are given in Tables **9** and **10**.

Table 5. Results of interday precision day – II.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.54003	9.8107	98.167	-
10	0.53235	9.6356	96.356	95.218
-	0.52292	9.4132	94.132	-
-	0.94837	19.4474	97.237	-
20	0.94008	19.2518	96.259	96.176
-	0.92963	19.0066	95.033	-
-	1.349	28.896	96.320	-
30	1.341	28.849	96.163	96.032
-	1.340	28.683	95.613	-
-	-	-	Mean	95.8086
-	-	-	SD	0.09755
-	-	-	%RSD	0.1018

Table 6. Results of repeatability.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration
30	1.38147	29.6914	98.971
30	1.37120	29.4490	98.163
30	1.36880	29.3923	97.974
30	1.36754	29.3626	97.875
30	1.35810	29.1397	97.132
30	1.35609	29.0922	96.974
-	-	Mean	97.843
-	-	SD	0.72849
-	-	%RSD	0.7445

Table 7. Results of accuracy.

% Recovery level	Stock Solution of dosage form	API added	Concentration in ppm	Absorbance	Found Concentration	Conc. found in Std. API	% Recovery	Mean
80%	1 ml	0.8 ml	18 ppm	0.881	17.85	17.86	99.97%	-
-	1 ml	0.8 ml	18 ppm	0.870	17.60	17.65	99.73%	99.87%
-	1 ml	0.8 ml	18 ppm	0.850	17.13	17.14	99.91%	-
100%	1 ml	1 ml	20 ppm	0.943	19.33	19.34	99.92%	-
-	1 ml	1 ml	20 ppm	0.947	19.43	19.49	99.68%	99.53%
-	1 ml	1 ml	20 ppm	0.940	19.25	19.45	98.99%	-
120%	1 ml	1.2 ml	22 ppm	1.041	21.65	21.74	99.57%	-
-	1 ml	1.2 ml	22 ppm	1.037	21.55	21.78	98.93%	99.84%
-	1 ml	1.2 ml	22 ppm	1.048	21.80	21.58	101.03%	-

Table 8. Results of robustness (change in wavelength)

Conc. µg/ml	Absorbance			Found Concentration			% Found Concentration			Mean
	R1	R2	R3	R1	R2	R3	R1	R2	R3	
30 ppm (λ=236)	1.361	1.351	1.359	29.208	28.972	29.160	97.36	96.57	97.20	97.04
30 ppm (λ=237)	1.357	1.348	1.350	29.113	28.901	28.948	97.04	96.33	96.49	96.62

(Table 8) contd.....

Conc. µg/ml	Absorbance			Found Concentration			% Found Concentration			Mean
	R1	R2	R3	R1	R2	R3	R1	R2	R3	
30 ppm (λ=238)	1.350	1.345	1.349	28.948	28.830	28.924	96.49	96.47	96.47	96.35
-	-	-	-	-	-	-	Mean			96.671
-	-	-	-	-	-	-	SD			0.347707
-	-	-	-	-	-	-	%RSD			0.359680

Table 9. Results of ruggedness analyst–I.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.55035	10.0	100	-
10	0.53998	9.815	98.15	98.796
-	0.54035	9.824	98.24	-
-	0.95947	19.709	98.545	-
20	0.94995	19.484	97.423	97.646
-	0.94613	19.394	96.972	-
-	1.36481	29.269	97.563	-
30	1.36951	29.379	97.93	98.001
-	1.37686	29.553	98.51	-
-	-	-	Mean	98.147
-	-	-	SD	0.588862
-	-	-	%RSD	0.599975

Table 10. Results of ruggedness analyst–II.

Concentration (ppm)	Absorbance	Found Concentration	% Found Concentration	Mean % Found Concentration
-	0.54011	9.827	98.27	-
10	0.55003	10.0	100	98.68
-	0.53797	9.777	97.77	-
-	0.95747	19.681	98.405	-
20	0.94981	19.500	97.500	97.61
-	0.94514	19.390	96.950	-
-	1.3671	29.352	97.84	-
30	1.3602	29.189	97.29	97.77
-	1.37155	29.457	98.19	-
-	-	-	Mean	98.02
-	-	-	SD	0.577148
-	-	-	%RSD	0.588806

2.10. Limit of Detection and Limit of Quantitation

2.10.1. LOD:

It is the lowest amount of analyte in the sample that can be detected. LOD can be calculated from the following formula.

2.10.2. LOQ:

It is the lowest amount of analyte in a sample that can be quantitatively determined. LOQ can be calculated from the following formula:

The LOD and LOQ were found to be 7.59 µg/ml and 23.01 µg/ml.

2.11. Force Degradation Study

In the view of determining stability indicating data of the drug, the forced degradation was carried out by thermal, oxidative, acid, alkali, and photolytic degradation. The details of the procedure of forced degradation are given in the below sections [16 - 20].

2.11.1. Thermal Degradation

The 0.5 ml of MLP standard stock solution was diluted to 4.5 ml of water, and then the solution was kept at 40°C for 2 hours. The solution was cooled to room temperature, and then it was diluted with a suitable solvent to 25 µg/ml and then absorbance was taken on UV spectrophotometry (Fig. **3**).

2.11.2. Oxidative Degradation

The 0.5 ml of MLP standard stock solution was diluted to 4.5 ml of 3% H_2O_2. Then the solution was kept at 40°C for 15 minutes. The solution was boiled at 100°C for 15 minutes and then cooled to room temperature. The solution was diluted with diluent to 25 µg/ml and then absorbance was taken on UV spectrophotometry (Fig. **4**).

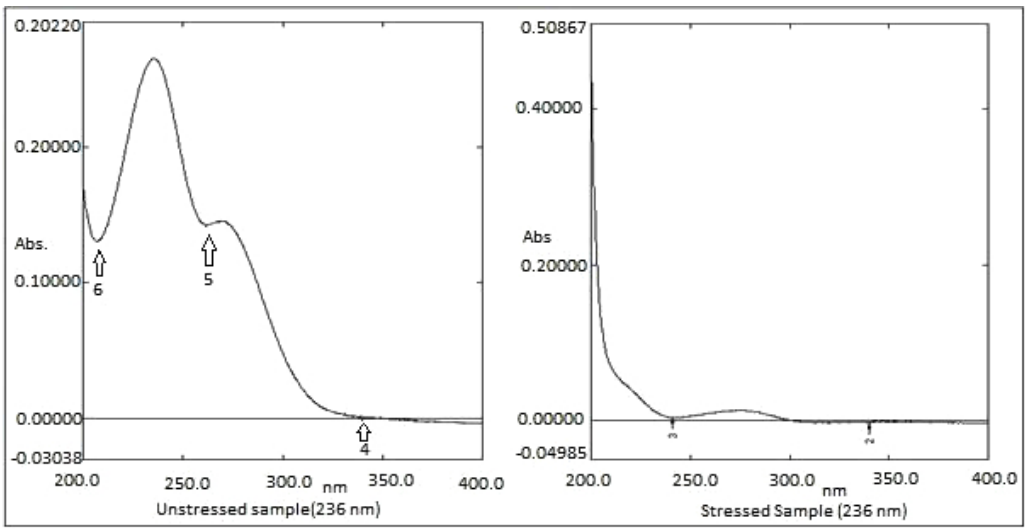

Fig. (3). Comparative results of stressed to unstressed samples for thermal degradation.

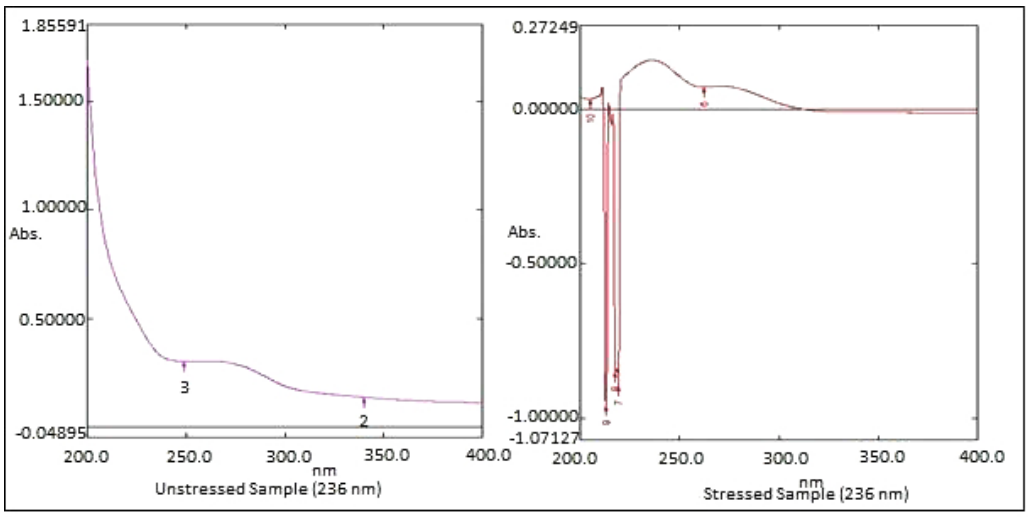

Fig. (4). Comparative results of stressed to unstressed samples for Oxidative degradation.

2.11.3. Acid and Alkali Hydrolysis

The 0.5 ml of MLP standard stock solution was diluted to 4.5 ml of 0.1M HCI or 0.1M NaOH. The solution was kept at 40°C for 2 hours. The solution was cooled at room temperature after they were neutralized with a suitable amount of HCI or NaOH. Then the solution was diluted with a suitable diluent to 25 µg/ml and then absorbance was taken on UV spectrophotometry (Figs. **5** and **6**).

2.11.4. Photolytic Degradation

The 0.5 ml of MLP standard stock solution was diluted to 4.5 ml with water. The solution was kept under UV light combined with a tungsten lamp for 24 hours at room temperature diluent to 25µg/ml and then absorbance was taken on UV spectrophotometry (Fig. **7** and Table **11**).

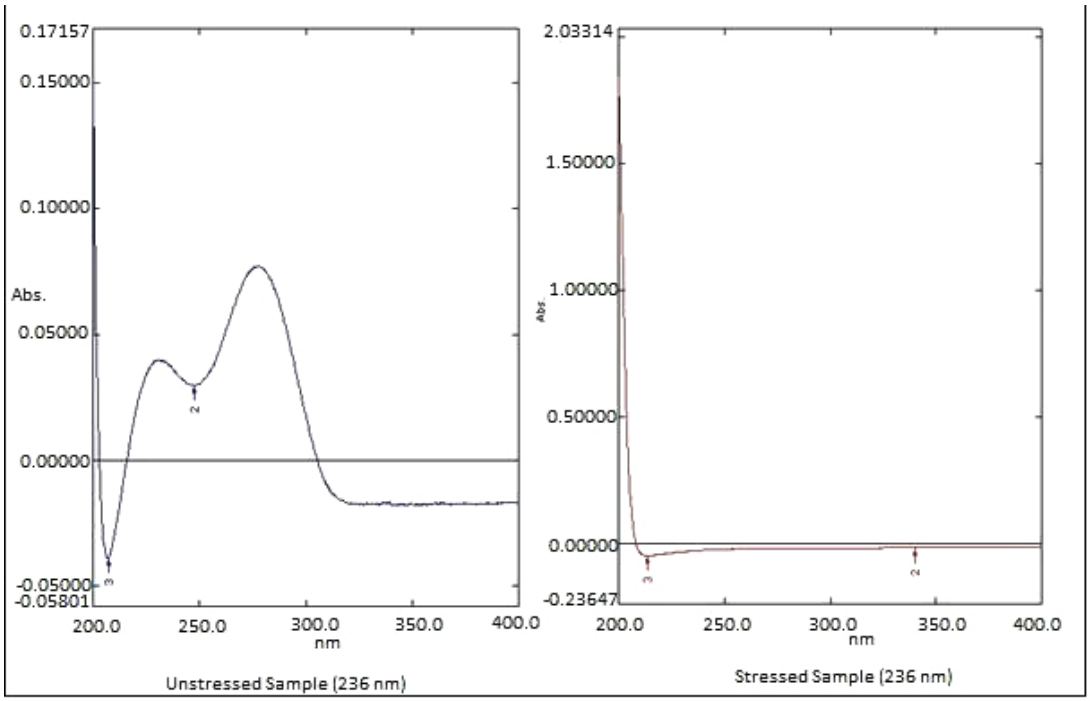

Fig. (5). Comparative results of stressed to unstressed samples for Acid degradation.

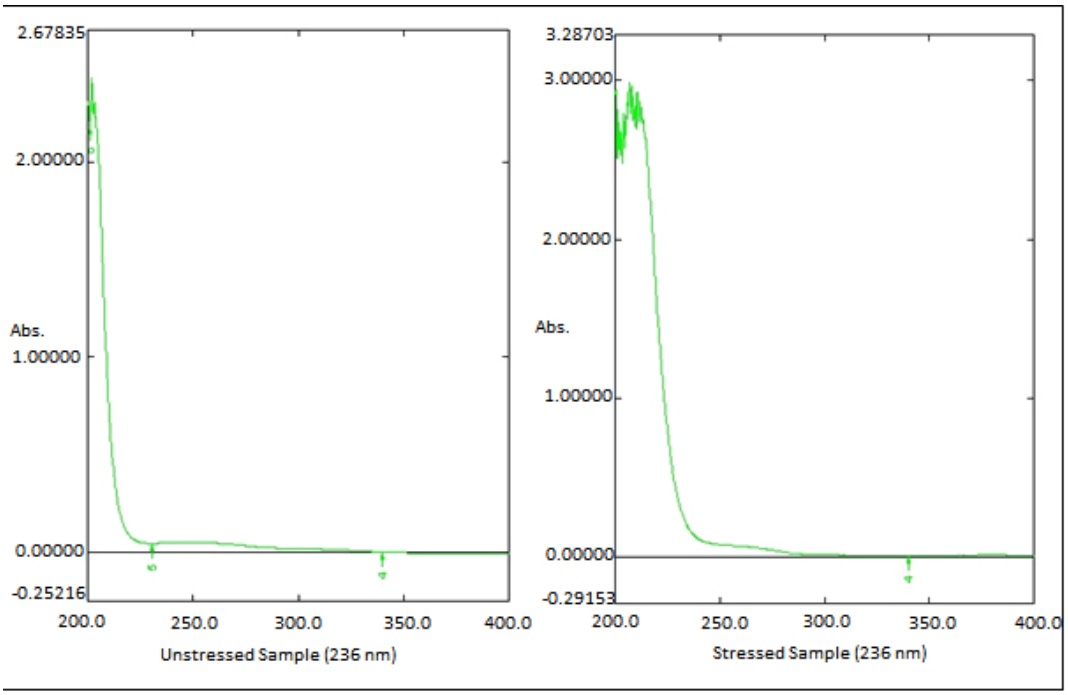

Fig. (6). Comparative results of stressed to unstressed samples for Base degradation.

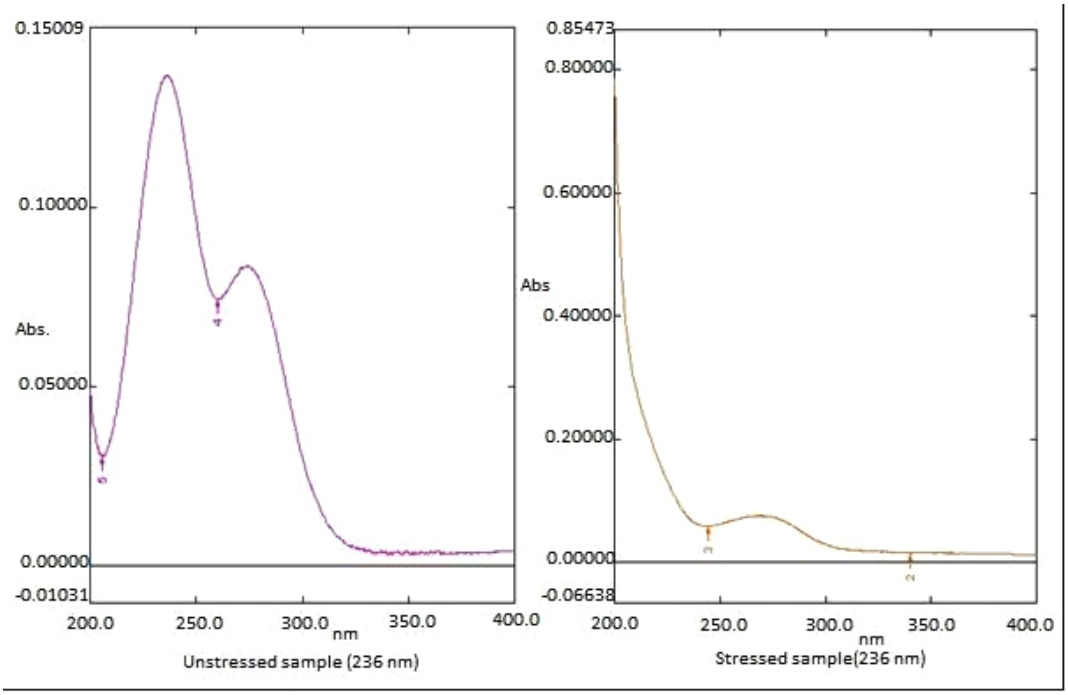

Fig. (7). Comparative results of stressed to unstressed samples for photolytic degradation.

Table 11. Results of forced degradation.

Degradation Parameter	Absorbance of Unstressed Sample (236nm)	Absorbance of Stressed Sample (236nm)	% Degradation
Thermal Degradation	0.26532	0.00679	2.559%
Oxidative Degradation	0.33719	0.16051	47.602%
Acidic Degradation	0.03770	0.02690	71.352%
Basic Degradation	0.16367	0.04633	28.306%
Photolytic Degradation	0.13666	0.6917	50.614%

Note: The % degradation was obtained by using the following formu Table (la (Table **11**), % Degradation=Stressed sample/Unstressed sample×100.

3. RESULTS AND DISCUSSION

The correlation coefficient of linearity shows that the method was linear over the range of 10-50 µg/ml. The %RSD for inter-day and intra-day precision and repeatability was found to be within the acceptable limit as per ICH Q_2 (R_1) guidelines. The accuracy of the method was determined at 80,100,120% levels and the results obtained were within the range of acceptable limit as per ICH Q_2 (R_1) guidelines. The %RSD for ruggedness and robustness were within the acceptable range as per ICH Q_2 (R_1) guidelines. The sensitivity of the method was determined by obtaining LOD and LOQ and their values obtained were within the acceptable limit as per ICH Q_2 (R_1) guidelines. The forced degradation behaviour of Molnupiravir was tried to determine and percent degradation in thermal, oxidative, acidic, basic, and photolytic degradation were reported, which are helpful for determining the stability indicating the method by using hyphenated techniques.

CONCLUSION

Since there is no method available for the determination of Molnupiravir by UV spectrophotometry, therefore in the present study, a new, simple, economical UV spectrophotometric method for determination of Molnupiravir in bulk and dosage form was developed and validated for the first time. The developed method was linear, accurate, precise, robust, rugged and having stability indicating characteristics that is capable of determining Molnupiravir in the presence of degradation products. The present analytical method was validated as per ICH Q_2 (R_1) guidelines and it meets specific acceptance criteria.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No humans/ animals were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors are very much thankful to the Management and Principal, Dr.M.J.Chavan, of Amrutvahini College of Pharmacy, Sangamner for providing necessary facilities to carryout this work.

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