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Stability Indicating Eco-Friendly HPLC Method for Molnupiravir

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Abstract: ***The growing interest of Green Analytical Chemistry (GAC) principles through replacement of polluting analytical procedures with greener ones, has encouraged us to develop an eco-friendly stability-indicating HPLC with photo diode array method (HPLC-PDA) for determination of Molnupiravir. Effective separation was accomplished using SunQ C18 column (4.6×250 mm, 5 µm particle size) with isocratic elution of the mobile phase composed of Methanol: Water (60: 40 v/v). Quantification of Molnupiravir was based on measuring the peak area at 236 nm. Molnupiravirpeak eluted at retention time 3.78 ±0.02 min. The developed HPLC procedure was carefully validated in terms of system suitability, linearity and range, precision, accuracy, specificity, robustness, detection and quantification limits. Forced degradation conditions of neutral, acidic and alkaline hydrolysis, oxidation, photo degradation and thermal degradation were studied. Peak purity was established using Borwin software and the results obtained were satisfactory.***

Keywords: Molnupiravir; HPLC-PDA; Eco-friendly; Forced degradation; Stability-indicating

1. Introduction

Molnupiravir (MVR) is an antiviral medication that inhibits replication of certain RNA viruses and is used in the treatment of Covid-19 in those infected by SARS-CoV-2. It inhibits viral reproduction by promoting widespread mutations in the replication of viral RNA by RNA-directed RNA polymerase [1]. It is metabolized into a ribonucleoside analog that resembles cytidine, β-D-N^4-Hydroxycytidine 5′-triphosphate (also called EIDD-1931 5′-triphosphate or NHC-TP). During replication, the virus's enzyme incorporates NHC-TP into newly made RNA instead of using real cytidine.

2. Literature Survey

Few reports described that there is LC-MS/MS method for simultaneous quantification of molnupiravir and its metabolite in human plasma [2] and saliva and another method is development and validation of assays for the quantification of its metabolite in human plasma and in peripheral blood mononuclear cell lysates [3] but there is no method in literature on stability indicating RP-HPLC.

This work describes the development, validation, and application of an eco-friendly reliable, simple and highly specific HPLC-PDA method for the quantitative analysis of MVR in its API form.

3. Materials and Methods

3.1 Chemicals and Reagents:

Working standard MVR was received as gift sample from Natco Pharma, Hyderabad. Methanol HPLC grade was purchased from Merck laboratories and HPLC grade water used generated using Lab Ultima system.

3.2 Instruments used

Analysis was carried out by Jasco MD-2010 plus HPLC with PDA detector using Borwin software, column used was SunQC18 (4.6mm*250mm, 5µm particle size), Digital weighing balance (shimadzu AY 120), Jet Vac water Jet Vacuum Pump (Biomedica), NewtronicsPhotostability chamber.

3.3 Method development

3.3.1 Selection of solvent and wavelength

The drug is freely soluble in water hence UV spectrum of MVR was recorded in distilled water. The drug showed good absorbance at wavelength 236 nm hence it was selected as detection wavelength.

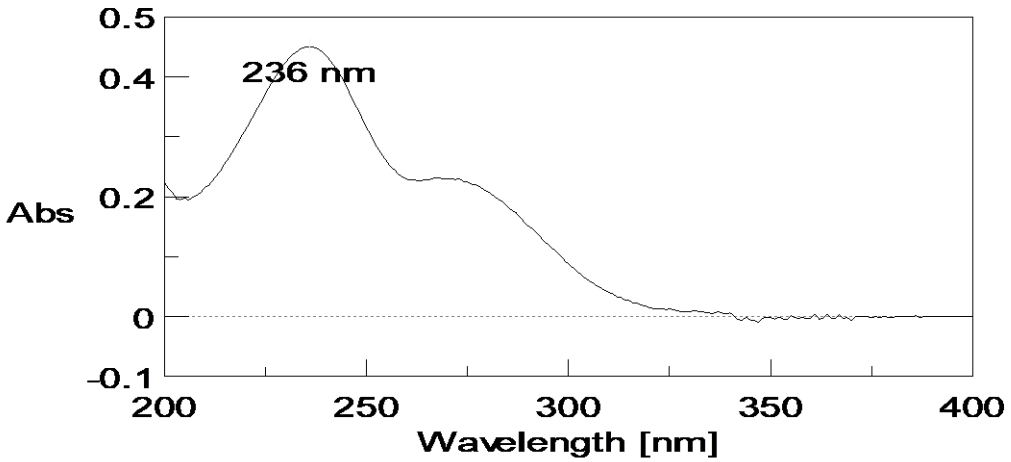

Figure 1: UV spectrum of MVR in distilled water

3.3.2 Preparation of standard stock solution
Standard stock solution of MVR was prepared by taking 10 ml volumetric flask and taking 10mg of drug and dissolved in distilled water to get a concentration of 1000µg/ml.

3.3.3 Preparation of working standard solutions
The working standard solution of MVR was prepared by diluting 1 ml of the standard stock solution to 10 ml with distilled water in a 10 ml volumetric flask to get the concentration of 100µg/ml.

3.3.4 Preparation of standard dilutions for linearity study
Appropriate dilutions were made from working standard solution to obtain solutions in the range of 20 to 100 ppm.

3.4 Forced degradation studies

Forced degradation studies were performed on MVR. The drug was subjected to stress conditions which include acid hydrolysis (0.1N HCl), base hydrolysis (0.1 N NaOH), thermal (60 ºC), oxidation (6% H_2O_2) and photolytic (exposure to UV light and cool white fluorescent light). The conditions for stress degradation were optimized to obtain recovery of Molnupiravir in the range of 70 to 90 %. The monitoring period for acid, base and oxidation was at 4 hrs and for thermal (60ºC in hot air oven) and photolytic as per ICH guidelines [4] (in photostability chamber by Newtronics)

Procedure for preparation of sample solutions of degradation
Working standard solution of 100 ug/ml was prepared, to4 ml of it, 1ml ofstress reagent (0.1 N HCl/NaOH/ 6 % H_2O_2) was added, shaken for some time and kept aside for 4 hrs. Then, after completion of 4 hrs, volume was made up to 10 ml. This solution was injected into HPLC. For hydrolysis under different pH, the solution was neutralized before injection.

3.4.6.1 Acid Hydrolysis
The % degradation after acid hydrolysis was found to be 24.23 %

3.4.6.2 Base Hydrolysis
The % degradation after base hydrolysis was found to be 13.46 %

3.4.6.3 Oxidative Hydrolysis
The % degradation after oxidative hydrolysis was found to be 25.15 %

3.4.6.4 Thermal degradation
About 100mg of MVR was transferred into a clean, dry petri dish. Petridish was placed in the oven at a temperature of 60° C for 4hrs. After completion of 4 hrs the sample was removed. Appropriately weighed and diluted to get 40 ug/ml, this solution was injected into HPLC. The % degradation was found to be 14.93 %

3.4.6.5 Photolytic degradation
Accurately weighed 100mg MVR and transferred into a clean, dry petri dish. Petridish was placed. After completion of required illumination the sample was removed. Appropriately weighed and diluted to get 40 ug/ml, this solution was injected into HPLC. The % degradation on exposure to UV light and fluorescence light was found to be 18.41 % and 26.48 respectively

Summary of degradation

Stress conditions	% recovered	Peak purity (peak front)	Peak purity (peak tail)
Acid, 0.1 N HCl 4 hrs at RT	75.77	993.71	992.56
Base, 0.1 N NaOH 4 hrs at RT	86.54	998.62	997.97
Thermal, 60ºC for 4 hrs	85.07	997.00	995.89
Oxidative 6% H_2O_2 for 2 hrs	74.85	995.23	994.80
UV 200 watt hrs/sq. m	81.59	991.84	991.31
Fluorescence 1.2 million lux hours	73.52	999.25	998.72

3.5 Validation of the method

The analytical method was validated with respect to parameters such as linearity and range, precision, accuracy, robustness, specificity, LOD and LOQ

3.5.1 Linearity

The linearity of the method was determined in the concentration range of 20-100µg/ml for MVR. Each solution was injected and calibration curve was plotted using peak area versus concentration, data of MVR was shown in Fig.3. The correlation coefficient was found to be 0.9862and the regression equation was y = 52785x-686202 for MVR.

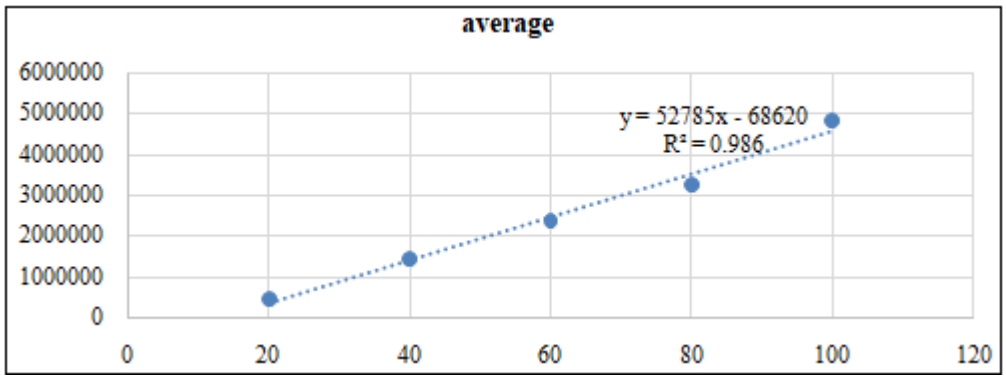

Figure 3: Linearity

3.5.2 Precision

Precision studies were carried out by studying six replicate injections of the standard drug on one day. This process is called intraday precision. The results were calculated in terms of %RSD.

Precision studies were also carried out by injecting six replicate injections of the standard drug on three different days. This process is called interday precision. The results were calculated in terms of %RSD.

The % RSD for Intraday precision and Interday precision was found to be 1.34 & 1.38 respectively.

3.5.3 Accuracy

Accuracy (% Recovery) was evaluated at three different concentrations equivalent to 80, 100 and 120% of the target concentration of active ingredients, by adding a known amount of standard and sample solutions in a same volumetric flask and calculates the % recovery for each concentration. The results are shown in Table 1.

Table 1: Recovery studies

S. No	Amount of marketed formulation added (ug/ml)	Amount of API added	Total amount of the drug (ug/ml)	Amount recovered	% recovery
1	20	16	36	36.46	101.28
2	20	20	40	40.29	100.75
3	20	24	44	44.81	101.85

nm= nano meter, ug= micro gram, ml= millilitre

3.5.4 Specificity

The specificity studies were done retention time and peak purity for both sample and standard found to be same as shown in table 2.

Table 2: Specificity Studies

Name	Retention time (min)	Peak purity (peak front)	Peak purity (Peak tail)
Standard	3.80	999.98	999.20
Sample	3.80	999.60	999.42

Min= minutes

3.5.5 Robustness

To evaluate the robustness of the method the chromatographic conditions were deliberately varied and degree of reproducibility was evaluated. Robustness was carried out on standard drug solution. Robustness of the proposed method was assessed with respect to change in flow rate of mobile phase (1ml/min ± 0.05ml/min). The results are shown in table 3

Table 3: Robustness

Sr. No	Flow rate	Peak areas	Tailing factor
1	0.95ml/min	1474117	1.35
2	1.05 ml/min	1450013	1.37

ml=millilitre, min= minutes

4. Results and Discussion

A simple, accurate reverse phase high performance liquid chromatographic method for determination of MVR has been developed. In RP-HPLC various columns are available, but here SunQC18 (4.6mm*250mm, 5µm particle size), i. e. column was preferred because using this column peak shape, resolution were good and a mobile phase comprising of methanol and water as amixture in the ratio of (60: 40 v/v) in an isocratic elution mode with maintaining the column temperature at ambient were preferred (Table 4).

Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents (methanol, acetonitrile, water). The drug was found to be soluble in Water.

Table 4: Chromatographic conditions

HPLC system	Jasco MD-2010 plus
column	SunQC18 (4.6mm*250mm, 5µm)
Mobile phase	Methanol: water (60: 40 v/v)
Flow rate	1.0 ml/min
Injection volume	20 ul/min
Total run time	6 min
Mode of	Isocratic

operation	
Detector	Photo Diode Array

ml = MilliLitre; min = Minutes; µm = Micro meter;
µl = Micro Litre; mm = Milli meter

The retention time of MVR was 3.78 ±0.02 min, the linearity was found to be in the range of 20-100µg/ml. The correlation coefficient was 0.9862, The proposed method was validated according to the ICH guidelines (Table 5)

Table 5: System Suitability Test Parameters.

S. no	Parameters	MVR
1	Range	20-100µg/ml
2	Detection wavelength	236 nm
3	Theoretical plates	2450
4	Tailing factor	1.45
5	Retention time	3.78 ±0.02 min

Forced Degradation Studies were carried out at different stress conditions like acidic, alkaline, thermal, photostability andoxidative condition for MVR and to study the percentage degradation of MVR.

- Linearity was found to be in the range of 20-100 µg/ml.
- The slope, intercept and correlation coefficient values were found to be y=52785x-68620& 0.986 respectively.
- The Limit of Detection was 1.59 µg/ml.
- The Limit of Quantification was4.81 µg/ml.
- Precision of developed method was studies as:

a) Intraday precision % RSD-1.34
b) Interday precision % RSD-1.38

Low %RSD values indicate that the method is precise.

- The recovery studies were carried out in three levels i. e.80%, 100% and 120% by adding known amount of standard drug. The recovery values were found to be within the limits indicating that the method is accurate.
- In Acidic, alkaline, thermal, photolytic and oxidation conditions the drug was stable and degradation shown was as per ICH guidelines.

The validated liquid chromatographic method was applied for determination of MVR. MVRwas stable under acid, base, photolytic, thermal and oxidative conditions.

5. Conclusion

A simple, accurate stability indicating RP-HPLC analytical method has been developed and validated. The results of degradation studies reveal that the drug does not show peak for degradation product under any degradation conditions.

6. Future Scope

As the drug shows degradation in every stress conditions hence to reduce degradation various changes can be made like changes in PH, temperature conditions, mobile phase and further the drug can be used for formulation studies.

7. Abbreviations

HPLC	High Perfromance Liquid Chromatography
UV	Ultra Violet
LC-MS	Liquid Chromatography-Mass Spectroscopy
um	micro meter
mm	milli meter
ug	micro gram
nm	nano meter
cm	centimeter
ul	Micro litre
ml	Micro litre
min	minutes
%	percentage
°C	Degree celsius
RSD	Relative Standard Deviation

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