


 Cite this: *RSC Adv.*, 2022, 12, 34512

Development and validation of molnupiravir assessment in bulk powder and pharmaceutical formulation by the RP-HPLC-UV method

 Abdelaziz M. Annadi,^a Noha M. El Zahar,^{bc} Nour El-Din A. Abdel-Sattar,^a Ekram H. Mohamed,^d Safwat A. Mahmoud^{*e} and Mohamed S. Attia^{id *a}

An accurate, sensitive and selective RP-HPLC-UV method has been established for the estimation of Molnupiravir (MOL) in pure bulk powder and pharmaceutical formulation. Separation was achieved on an Inertsil C₁₈ column (150.0 mm × 4.6 mm, 5.0 μm), using a mobile phase of 20 mM phosphate buffer pH 2.5: acetonitrile (80 : 20, v/v%) in isocratic mode with a flow rate of 1.0 mL min⁻¹. The λ_{max} of MOL prepared in the chosen diluent (ethanol : water in equal proportions) was found to be 230.0 nm. The constructed calibration curve was found to be linear in the concentration range of 0.2–80.0 μg mL⁻¹. The recovery% of MOL using the proposed method was 100.29%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.04 μg mL⁻¹ and 0.12 μg mL⁻¹, respectively. No significant interference was detected in the presence of the common pharmaceutical formulation excipients. The method was validated following the ICH recommendations. All the obtained results were statistically compared with those using reported methods and there were no significant differences. The method developed in this work was successfully employed for the assessment of MOL in bulk powder and pharmaceutical formulation.

 Received 12th August 2022
 Accepted 12th November 2022

 DOI: 10.1039/d2ra05066h
rsc.li/rsc-advances

Introduction

The rise of SARS-CoV-2 variants of concern (VoCs) has aggravated the COVID-19 pandemic situation. The existing monoclonal antibodies and vaccines have reduced efficacy against these VoCs to some extent. Antivirals play an important role in affecting the conserved proteins of SARS-CoV-2. They are not usually affected by VoC mutations and thus should be efficient against the arising variants. The orally available antiviral MOL (molnupiravir, MK-4482, EIDD-2801) effectively inhibits certain RNA virus replication and so has been ascertained to be active against infections caused by different variants of SARS-CoV-2.^{1–4} MOL shows strong potential resistance against the emerging VoCs where the initial efficacy data in COVID-19 patients have been recently revealed.^{5–10} Consequently, MOL represents a promising cost-effective cure for the treatment of COVID-19 while demonstrating high safety and efficacy in ongoing

clinical trials.^{11–15} MOL is considered to be the first oral, direct-acting antiviral proved to be extremely efficient at reducing nasopharyngeal SARS-CoV-2 infective virus and viral RNA.^{16–19} The chemical structure of MOL is shown in Fig. 1.

To the best of our knowledge, no simple RP-HPLC-UV methods for the determination of MOL in bulk and pharmaceutical formulations have been previously reported. A literature review reveals limited methods for analysis, with no clear, well-defined, validated methods (Table 1). MOL levels have been quantified using LC-MS/MS in human plasma¹⁷ and animal models,²⁰ while the validation parameters have not been completely reported. One validated method has been described for quantifying MOL and its metabolite by LC-MS/MS in human plasma and saliva.²¹ Herein, a simple RP-HPLC-UV method was established and validated for the assessment of MOL in bulk powder and pharmaceutical formulation as per ICH guidelines.

Experimental

Materials and reagents

MOL, purity 98%, was obtained from Optimus Drugs Pvt LTD, India. 200 mg per capsule (R&D prepared samples) was used as the pharmaceutical formulation dosage form. American Society for Testing and Materials (ASTM) grade I water was obtained daily from the central laboratory. Potassium dihydrogen phosphate, sodium dihydrogen phosphate, dipotassium hydrogen phosphate, *o*-phosphoric acid, acetic acid, and anhydrous

^aChemistry Department, Faculty of Science, Ain Shams University, Abbassia, Cairo, 11566, Egypt. E-mail: mohd_mostafa@sci.asu.edu.eg

^bPharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Organization of African Unity Street, Abbassia, Cairo 11566, Egypt

^cMedicinal Chemistry Department, Faculty of Pharmacy, King Salman International University, Ras-Sedr, South Sinai, Egypt

^dPharmaceutical Chemistry Department, Faculty of Pharmacy, The British University in Egypt, ElSherouk City, Cairo, Egypt

^ePhysics Department, Faculty of Science, Northern Border University, Arar, Saudi Arabia. E-mail: samahmoud2002@yahoo.com



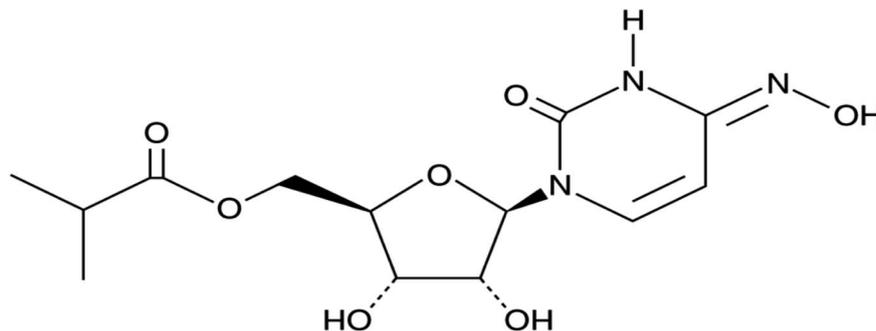


Fig. 1 Chemical structure of MOL.

sodium acetate were supplied by Scharlau, Barcelona, Spain. The solvents used, such as acetonitrile, ethanol and methanol, were also supplied by Scharlau, Barcelona, Spain.

The chosen diluent for standard and test sample preparation was a mixture of ethanol and water in equal proportions.

Apparatus

An electronic balance, pH meter (Mettler Toledo, 235, MA, USA), sonicator and UV-visible spectrophotometer (Shimadzu UV JAPAN 1801) were used for development of the method. Chromatographic separation and analysis were conducted using high-performance liquid chromatography with a PDA-detector Agilent 1260 HPLC system (Deutschland GmbH, Waldbronn, Germany).

MOL solubility measurements in different solvents

For each solvent, about 500 mg of MOL was transferred into 10 mL of solvent, sonicated for 1 h at 37 °C, and then cooled to room temperature to yield the stock solution, which was then diluted to be in the targeted concentration range of the MOL assay method. The recovery amount (mg mL^{-1}) was reported for each solvent.

Preparation of stock and standard solutions

20.0 mg of MOL was dissolved in 100 mL of diluent to yield the stock solution, which was then diluted to prepare different

standard solutions in the concentration range $0.2\text{--}80.0 \mu\text{g mL}^{-1}$ for the MOL assay method calibration curve.

Chromatographic conditions

MOL was analyzed using an Inertsil C_{18} column (150.0×4.6 mm, $5 \mu\text{m}$) and a mobile phase of 20 mM phosphate buffer pH 2.5 : acetonitrile (80 : 20, v/v%) in isocratic mode, with a flow rate of 1.0 mL min^{-1} . The buffer was filtered using a $0.45 \mu\text{m}$ nylon membrane filter, and the used mobile phase was stable for more than 5 days without any physical changes or any fluctuation in the system's suitability parameters. The wavelength of detection was adjusted to be 230.0 nm. The temperature was set to 30 °C for the column, and 25 °C for the sample tray. The injection volume was $50.0 \mu\text{L}$.

Method validation

The analytical method was suitably developed and validated, regarding the accuracy, precision, linearity, specificity, limit of detection (LOD) and the quantification limit (LOQ), in agreement with the requirements of the ICH guidelines.^{24–38}

Linearity

Standard solutions in the concentration range $0.2\text{--}80.0 \mu\text{g mL}^{-1}$ for MOL were injected into the RP-HPLC-UV system. Each solution was measured three times. The response signal was measured at 230.0 nm. The calibration curve was constructed

Table 1 A comparison between literature methods and the method developed in this work

Method	Mobile phase	Stationary phase	Linear range $\mu\text{g mL}^{-1}$	Ref.
LC-MS-MS	1 mM ammonium acetate in water (pH 4.3) and 1 mM ammonium acetate in acetonitrile	Polar atlantis C18 column	$2.5\text{--}5000 \text{ ng mL}^{-1}$	21
LC	Ammonium formate and ACN	Waters Xselect HSS T3 (75×4.6 mm, $2.5 \mu\text{m}$)	—	22
RP-HPLC-PDA	ACN : water (20 : 80 v/v)	Discovery® HS C18 column (75×4.6 mm, $3 \mu\text{m}$)	0.1–60.0	23
RP-HPLC-UV	20 mM phosphate buffer pH 2.5 : acetonitrile; 80 : 20, v/v%	Inertsil C_{18} column (150×4.6 mm, $5 \mu\text{m}$)	0.2–80.0	This work



Solubility of MOL in different solvents (mg/mL)

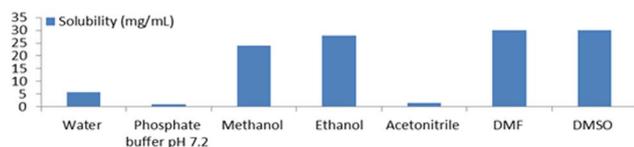


Fig. 2 Solubility of MOL in different solvents.

using the corresponding concentrations for each response signal, and the regression equation was computed.

Accuracy

The accuracy of the method is the proximity between the true quantity and the test result. The values of the method recovery reflect the accuracy of the procedure, and this was done by spiking the active drug to the placebo at three different concentrations (5, 10 and 20 $\mu\text{g mL}^{-1}$).

Precision

The precision of the method was checked by measuring a 10.0 $\mu\text{g mL}^{-1}$ standard solution of MOL six times. The precision was evaluated on three different levels: repeatability (six determinations of the standard solution), intraday (analyst I and analyst II) and interday (day I and day II). Each analyst prepared six different tests. The peak areas as the recovery% were determined and compared. The percentage relative standard deviation RSD% was used as an indicator for precision, and should be less than 2.0%.

Selectivity

The selectivity was evaluated by measuring the response of the blank, placebo, and analyte samples and any potential or identified species such as the formulation excipients. No response that interferes with the analyte's response should be obtained.

Detection and quantification limits

The LOD is the lowest level of analyte that gives a qualitative response, while the LOQ is the lowest concentration that can be quantified consistently with a defined level of recovery and precision. The LOD and LOQ were calculated as per ICH guidelines.^{24–38}

Robustness

The robustness of the optimized method was investigated by assessing small deliberate changes in the values in the method development parameters such as the column temperature and buffer pH value. The quantitative effect of the variables was determined by considering the value of the recovery%, and the acceptable limits should be $\pm 2\%$ for the peak response and retention times of the analyte.

Solution stability

The stability of the sample solutions was evaluated by analyzing a known MOL concentration (10.0 $\mu\text{g mL}^{-1}$). Replicates ($n = 3$) were exposed to different temperature conditions, including lab temperature (15–25 $^{\circ}\text{C}$) for 8, 12 and 24 h, cooling temperature (5 ± 3 $^{\circ}\text{C}$) for 5 days, and freezer temperature (-20 $^{\circ}\text{C}$) for 10 days. The results were investigated by comparing them with those from the assays of freshly prepared solutions of reference standards.²³

Applications to pharmaceutical formulation

Standard solution preparation

20 mg of MOL was dissolved in 100 mL of diluent to yield solution A. Then, 5 mL from solution A was diluted into 100 mL of diluent, and filtered using a 0.45 μm nylon syringe filter. The first 5 mL was discarded, and the nominal concentration was 0.01 mg mL^{-1} .

Test solution preparation

The content of ten capsules (MOL 200 mg per capsule, R & D prepared samples) was mixed well and ground to a fine powder.

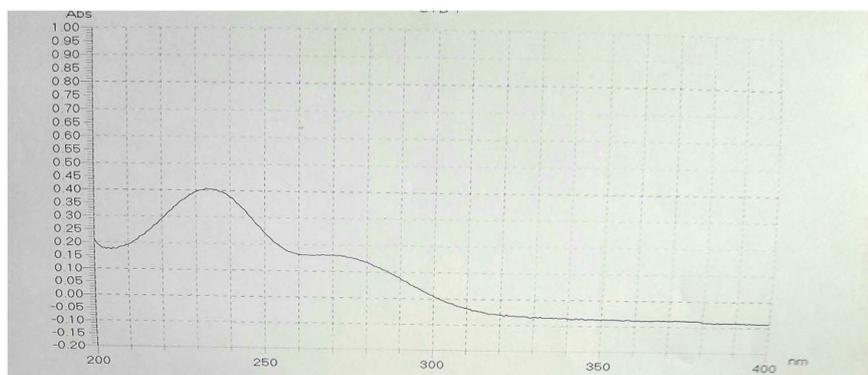


Fig. 3 UV spectrum of MOL (10 $\mu\text{g mL}^{-1}$) in ethanol:water (1 : 1, v/v) showing λ_{max} at 230 nm.



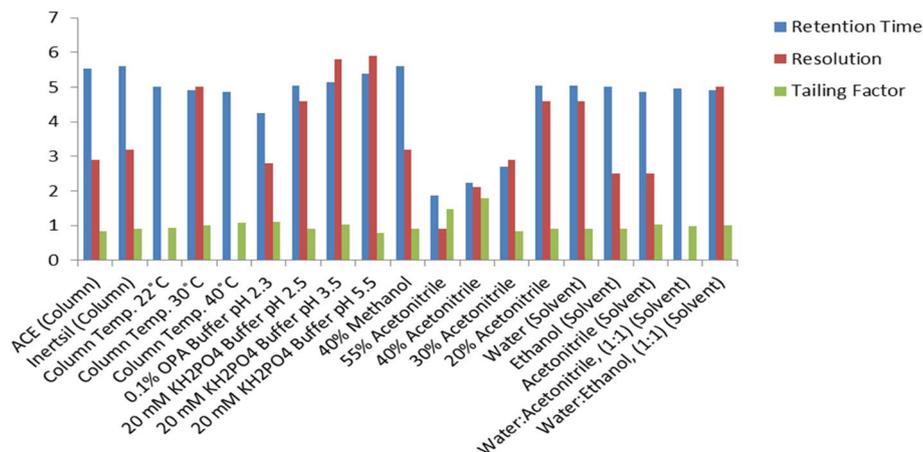


Fig. 4 Effect of change of different chromatographic parameters on the retention time, resolution between the MOL peak at 4.9 min and the peak at 2.5 min and the tailing factor of the MOL peak using the Inertsil column.

The weight equivalent to 20 mg of MOL was dissolved in a 100 mL volumetric flask containing 80 mL of diluent, then sonicated for 15 min at 25 °C and diluted to the required volume with diluent. 5.0 mL of this ethanolic solution was diluted in a 100 mL volumetric flask using the same diluent, and filtered using a 0.45 µm nylon syringe filter. The first 5 mL was discarded, and the nominal concentration was 0.01 mg mL⁻¹.

Results and discussion

Optimization of experimental parameters

In the RP-HPLC-UV method, initial trials were performed with the aim to select the most suitable chromatographic condition parameters. These parameters, such as the solvent, mobile phase components and their percentages, detection wavelength, pH value, type of column and concentration range for the target standard solutions, were carefully studied.

Solvent and diluent selection

MOL is soluble in water (5.8 mg mL⁻¹),³⁹ phosphate buffer pH 7.2 (1 mg mL⁻¹), ethanol (28 mg mL⁻¹), DMF (30 mg mL⁻¹), DMSO (30 mg mL⁻¹),⁴⁰ methanol (24 mg mL⁻¹) and acetonitrile (1.4 mg mL⁻¹) (Fig. 2). Thus, the stock and working standard solutions were prepared in ethanol:water (1:1, v/v) as the diluent.

Wavelength selection

The UV spectrum of MOL was recorded by scanning the wavelength range of 200.0–400.0 nm (Fig. 3). Two absorbance peaks were observed at 230.0 and 270.0 nm.⁴⁰ From the UV spectrum, a wavelength of 230.0 nm was selected as the maximum wavelength.

Organic modifier selection

Different ratios of methanol (40–60%, v/v) and acetonitrile (10–40%, v/v) as organic modifiers were evaluated (Fig. 4 and 5). The

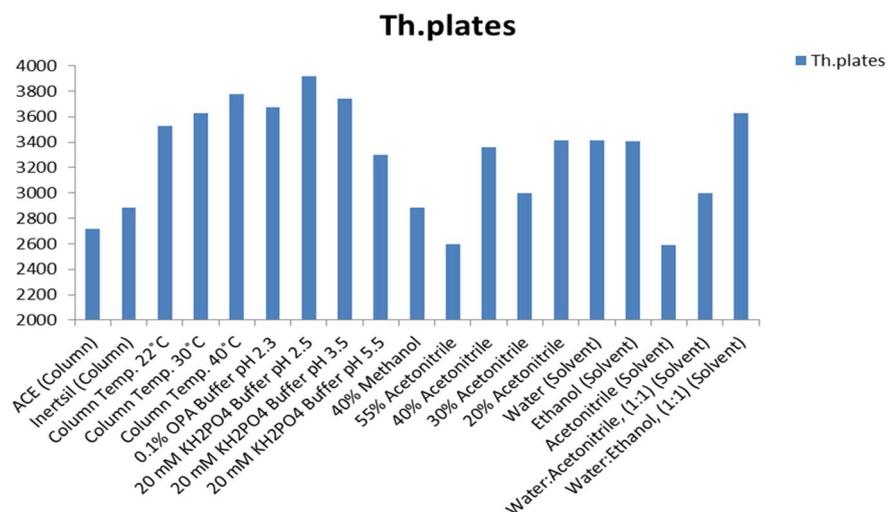


Fig. 5 Effect of the change of the chromatographic parameters on the theoretical (Th.) plates of the MOL peak using the Inertsil column.



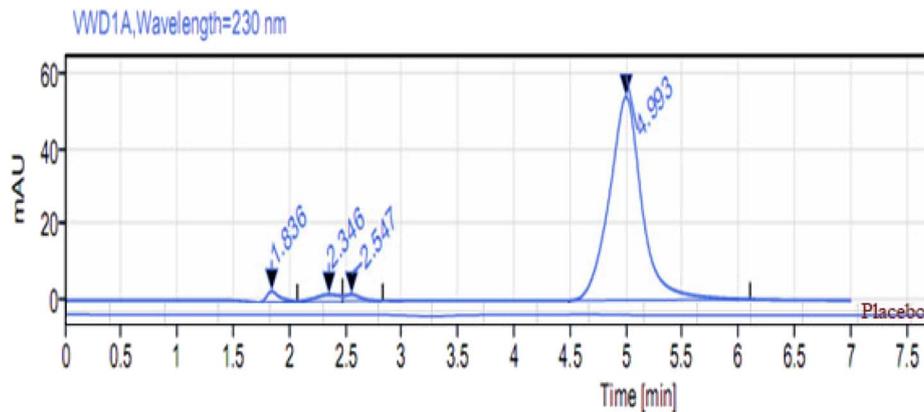


Fig. 6 HPLC chromatogram of the placebo and MOL ($10 \mu\text{g mL}^{-1}$) at 4.9 min in ethanol : water (1 : 1, v/v) at $\lambda_{\text{max}} = 230 \text{ nm}$, using the Inertsil column.

Table 2 Validation parameters

Parameter	MOL
Linear range ($\mu\text{g mL}^{-1}$)	0.2–80
SD of the calibration curve	1.339
Correlation coefficient (r^2)	1.0
Equation of calibration curve	$y = 110.43x + 1.1332$
Accuracy levels	5, 10 and $20 \mu\text{g mL}^{-1}$
Recovery%	100.67, 99.96 and 100.24%
RSD%	0.36, 0.45 and 0.30%
Precision	
Intraday precision RSD%	0.51%
Interday precision RSD%	0.57%
LOD ($\mu\text{g mL}^{-1}$)	0.04
LOQ ($\mu\text{g mL}^{-1}$)	0.12

results show that methanol gives a longer run time than acetonitrile and causes peak broadening. Using a mixture of acetonitrile and buffer gives better separation and resolution for the investigated drug.

Moreover, as the ratio of methanol in the mobile phase decreases, the retention of MOL increases. Therefore, acetonitrile was chosen as the organic modifier of choice as it permitted the separation of MOL within a short analytical run time, a favorable resolution factor and exceptional sensitivity.

Buffer selection

Several buffers in different proportions were tested including phosphate buffer with varying strength (5.0–30.0 mM). The results revealed that 20.0 mM phosphate buffer resulted in a stable baseline and sharp peak. Similarly, 0.1% *o*-phosphoric acid solution as a buffer was tested and gives a stable baseline and a sharp peak shape but with low resolution. The 20.0 mM phosphate buffer was used as the buffer of choice throughout this study (Fig. 4 and 5).

Buffer pH selection

The effect of varying the mobile phase pH on the selectivity and retention times of MOL was studied using mobile phases adjusted to different pH values ranging from 2.5 to 5.5. The pK_a of MOL is 8.21;²⁵ hence variation of the pH value of the mobile phase did not meaningfully affect the retention time. A pH value of 2.5 was chosen as the most suitable pH value, generating well-defined symmetrical peaks for MOL with high sensitivity through a short analytical run time (Fig. 4 and 5).

Column selection

Different brands of C_{18} ($150.0 \times 4.6 \text{ mm}$, $5.0 \mu\text{m}$) column were tested. Inertsil had a longer run time than ACE, which exhibited

MOL Intraday precision

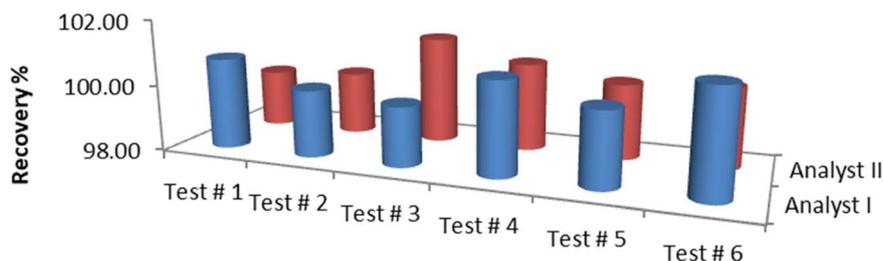


Fig. 7 Intraday (analyst I and analyst II) precision of MOL.



MOL Interday precision

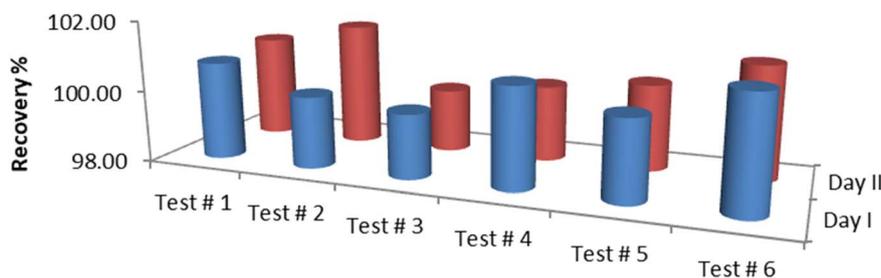


Fig. 8 Interday (day I and day II) precision of MOL.

Table 3 Assay results of MOL capsules

Exp. #	MOL assay%
I	100.14
II	100.42
III	101.19
Average	100.58
RSD%	0.54

Table 4 Statistical comparison between the results of the proposed HPLC method and the reference method

Parameter	Proposed method	Reference method ²³
Mean	99.37	100.15
SD	0.87	1.04
<i>n</i>	5	5
<i>t</i> -test	1.06 ^a (2.31)	—
<i>F</i> -value	2.60 ^a (6.39)	—

^a The values in parentheses correspond to the theoretical values of *t* and *F* at the 95% confidence level.

low resolution (Fig. 4 and 5). Hence, the Inertsil column was used in this method.

System suitability

The main reason for carrying out system suitability tests was to verify the equipment performance; the RSD% for the average area for six replicate injections of the working standard was calculated.

In the RP-HPLC method, the RSD% for the peak area was found to be less than 1.5%. The tailing parameter for the analyte peak was found to be not more than 1.2 and the theoretical plates were not less than 3000.

Selectivity

No significant responses for the diluent or placebo (formulation excipients) were detected at the given retention time of MOL (Fig. 6). These results demonstrate that the proposed method is selective for MOL quantification in bulk powder and

pharmaceutical formulation. The peak purity index was more than 0.9992 for all preparations.

Linearity and range

The MOL standard calibration curve was linear with RSD% less than 1% based on three successive readings, and the correlation coefficient r^2 was 1.0, showing that the adopted HPLC method had outstanding linearity over the concentration range 0.2–80.0 $\mu\text{g mL}^{-1}$ for MOL.

Accuracy and recovery

The accuracy of the method was assessed by using recovery studies with different levels of spiking. At each level, three determinations were achieved and the results were recorded. The percent recovery values were calculated and were found to be within the limit (100.67, 99.96 and 100.24%) with RSD% of 0.36, 0.45 and 0.30, as shown in Table 2.

Precision

The results of precision tests performed on the standard solutions of MOL showed that the RSD% was in the acceptable range. The average assay for intraday (analyst I and analyst II) precision was 100.42% with RSD 0.51% while the interday (day I and day II) precision was 100.56% with RSD 0.57%. RSD% was 0.34 for the repeatability of six determinations of the standard solution. Therefore, the method precision was confirmed to be satisfactory, and the results are summarized in Fig. 7 and 8.

Robustness

The recovery% and retention time were not affected by increasing the column temperature from 25 to 35 °C or by increasing the pH value from 2.5 to 5.5, while the theoretical plates are increased by an increase in column temperature and a decrease in pH value (Fig. 4 and 5). The findings indicated that minor modifications in the values provided for the chromatographic conditions do not influence the outcomes for the recovery and retention time of MOL. According to ICH, the evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. Therefore, we used the results from the



optimization of the experimental parameters as a guide. Fig. 4 and 5 indicate that even when the changes in the chromatographic parameters were large, the method was still robust.

Stability of the solution

The stability of the MOL solutions was investigated. The results confirmed that the solutions were stable for 12 h at room temperature (15–25 °C) as the assay% decreased from 100 to 94% and small peaks appeared at 2.3 and 2.5 min, indicating that the MOL was hydrolyzed. Additionally, MOL was stable for 5 days at a cool temperature (5 ± 3 °C) and 10 days in a freezer (–20 °C), stored in amber colored flasks protected from the light. We don't recommend storing the aqueous solution for more than one day.⁴⁰

Applications of the proposed method

The assay results are shown in Table 3. The average of three determinations for the MOL 200 mg capsule (R & D sample) assay was 100.58% and the RSD% was 0.54.

Statistical analysis

All the results obtained by the proposed HPLC method were compared with those obtained by the reported method for MOL,²³ and no significant difference between the methods was observed, as shown in Table 4.

Conclusions

In this work, a simple, fast and reliable RP-HPLC-UV method was optimized and validated according to the ICH guidelines for the determination of MOL in bulk and pharmaceutical formulation. The optimized method demonstrated excellent linearity, sensitivity, recovery, precision and selectivity. The established method was effectively implemented to analyze the studied drug, and it is suggested that this can be applied successfully to the routine work of quality control.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

Authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number "IF_2020_NBU_242".

References

- 1 R. Abdelnabi, C. S. Foo, S. De Jonghe, P. Maes, B. Weynand and J. Neyts, Molnupiravir Inhibits Replication of the Emerging SARS-CoV-2 Variants of Concern in a Hamster Infection Model, *J. Infect. Dis.*, 2021, **224**(5), 749–753.

- 2 C. J. Gordon, E. P. Tchesnokov, R. F. Schinazi and M. Gotte, Molnupiravir promotes SARS-CoV-2 mutagenesis *via* the RNA template, *J. Biol. Chem.*, 2021, **297**(1), 100770.
- 3 F. Graham, Daily briefing: Inside Merck's COVID drug, molnupiravir, *Nature*, 2021, DOI: [10.1038/d41586-021-02792-0](https://doi.org/10.1038/d41586-021-02792-0).
- 4 W. Holman, W. Holman, S. McIntosh, W. Painter, G. Painter, J. Bush, *et al.*, Accelerated first-in-human clinical trial of EIDD-2801/MK-4482 (molnupiravir), a ribonucleoside analog with potent antiviral activity against SARS-CoV-2, *Trials*, 2021, **22**(1), 561.
- 5 R. Abdelnabi, C. S. Foo, S. J. F. Kaptein, X. Zhang, T. N. D. Do, L. Langendries, *et al.*, The combined treatment of Molnupiravir and Favipiravir results in a potentiation of antiviral efficacy in a SARS-CoV-2 hamster infection model, *EBioMedicine*, 2021, **72**, 103595.
- 6 M. Imran, M. Kumar Arora, S. M. B. Asdaq, S. A. Khan, S. I. Alaqel, M. K. Alshammari, *et al.*, Discovery, Development, and Patent Trends on Molnupiravir: A Prospective Oral Treatment for COVID-19, *Molecules*, 2021, **26**(19), 5795.
- 7 F. Kabinger, C. Stiller, J. Schmitzova, C. Dienemann, G. Kokic, H. S. Hillen, *et al.*, Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis, *Nat. Struct. Mol. Biol.*, 2021, **28**(9), 740–746.
- 8 S. H. Khoo, R. Fitzgerald, T. Fletcher, S. Ewings, T. Jaki, R. Lyon, *et al.*, Optimal dose and safety of molnupiravir in patients with early SARS-CoV-2: a Phase I, open-label, dose-escalating, randomized controlled study, *J. Antimicrob. Chemother.*, 2021, **76**(12), 3286–3295.
- 9 E. Mahase, Covid-19: Molnupiravir reduces risk of hospital admission or death by 50% in patients at risk, MSD reports, *Bmj*, 2021, **375**, n2422.
- 10 B. Malone and E. A. Campbell, Publisher Correction: Molnupiravir: coding for catastrophe, *Nat. Struct. Mol. Biol.*, 2021, **28**(11), 955.
- 11 G. P. Ahlqvist, C. P. McGeough, C. Senanayake, J. D. Armstrong, A. Yadaw, S. Roy, *et al.*, Progress Toward a Large-Scale Synthesis of Molnupiravir (MK-4482, EIDD-2801) from Cytidine, *ACS Omega*, 2021, **6**(15), 10396–10402.
- 12 B. Malone and E. A. Campbell, Molnupiravir: coding for catastrophe, *Nat. Struct. Mol. Biol.*, 2021, **28**(9), 706–708.
- 13 L. Menendez-Arias, Decoding molnupiravir-induced mutagenesis in SARS-CoV-2, *J. Biol. Chem.*, 2021, **297**(1), 100867.
- 14 G. R. Painter, M. G. Natchus, O. Cohen, W. Holman and W. P. Painter, Developing a direct acting, orally available antiviral agent in a pandemic: the evolution of molnupiravir as a potential treatment for COVID-19, *Curr. Opin. Virol.*, 2021, **50**, 17–22.
- 15 J. Reina, Plitidepsin, an inhibitor of the cell elongation factor eEF1a, and molnupiravir an analogue of the ribonucleoside cytidine, two new chemical compounds with intense activity against SARS-CoV-2, *Revista espanola de quimioterapia*, 2021, **34**(5), 402–407.
- 16 W. Fischer, J. J. Eron, W. Holman, M. S. Cohen, L. Fang and L. J. Szewczyk, *et al.* Molnupiravir, an Oral Antiviral Treatment



- for COVID-19. *medRxiv : the preprint server for health sciences*, 2021.
- 17 W. P. Painter, W. Holman, J. A. Bush, F. Almazedi, H. Malik and N. Eraut, *et al. Human Safety, Tolerability, and Pharmacokinetics of Molnupiravir, a Novel Broad-Spectrum Oral Antiviral Agent with Activity Against SARS-CoV-2. Antimicrobial agents and chemotherapy*, 2021.
 - 18 Y. Wang, P. Li, K. Solanki, Y. Li, Z. Ma, M. P. Peppelenbosch, *et al.*, Viral polymerase binding and broad-spectrum antiviral activity of molnupiravir against human seasonal coronaviruses, *Virology*, 2021, **564**, 33–38.
 - 19 C. Willyard, How antiviral pill molnupiravir shot ahead in the COVID drug hunt, *Nature*, 2021, DOI: [10.1038/d41586-021-02783-1](https://doi.org/10.1038/d41586-021-02783-1).
 - 20 K. Rosenke, F. Hansen, B. Schwarz, F. Feldmann, E. Haddock, R. Rosenke, *et al.*, Orally delivered MK-4482 inhibits SARS-CoV-2 replication in the Syrian hamster model, *Nat. Commun.*, 2021, **12**(1), 2295.
 - 21 A. Amara, S. D. Panchala, L. Else, C. Hale, R. FitzGerald, L. Walker, *et al.*, The development and validation of a novel LC-MS/MS method for the simultaneous quantification of Molnupiravir and its metabolite ss-d-N4-hydroxycytidine in human plasma and saliva, *J. Pharm. Biomed. Anal.*, 2021, **206**, 114356.
 - 22 S. Jain, S. Giri, N. Sharma and R. P. Shah, LC and LC-HRMS studies on stability behavior of molnupiravir an anti-COVID 19 drug, *J. Liq. Chromatogr. Relat. Technol.*, 2022, 1–10.
 - 23 T. Recber, S. S. Timur, S. Erdogan Kablan, F. Yalcin, T. C. Karabulut, R. Neslihan Gursoy, *et al.*, A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies, *J. Pharm. Biomed. Anal.*, 2022, **214**, 114693.
 - 24 M. S. Attia, M. H. Khalil, M. S. A. Abdel-Mottaleb, M. B. Lukyanova, Yu. A. Alekseenko and B. Lukyanov, *Int. J. Photoenergy*, 2006, **2006**, 1–9.
 - 25 M. S. Attia, A. O. Youssef, Z. A. Khan and M. N. Abou-Omar, *Talanta*, 2018, **186**, 36–43.
 - 26 M. S. Attia, A. O. Youssef and A. A. Essawy, *Anal. Methods*, 2012, **4**, 2323–2328.
 - 27 M. S. Attia, K. Ali, M. El-Kemary and W. M. Darwish, *Talanta*, 2019, **201**, 185–193.
 - 28 M. S. Attia, W. H. Mahmoud, A. O. Youssef and M. S. Mostafa, *J. Fluoresc.*, 2011, **21**, 2229–2235.
 - 29 M. S. Attia, M. N. Ramsis, L. H. Khalil and S. G. Hashem, *J. Fluoresc.*, 2012, **22**, 779–788.
 - 30 M. S. Attia, W. H. Mahmoud, M. N. Ramsis, L. H. Khalil, A. M. Othman, S. G. Hashem and M. S. Mostafa, *J. Fluoresc.*, 2011, **21**, 1739–1748.
 - 31 M. S. Attia, A. M. Othman, E. Elraghi and H. Y. Aboul-Enein, *J. Fluoresc.*, 2011, **21**, 739–745.
 - 32 A. A. Elabd and M. S. Attia, *J. Lumin.*, 2016, **169**, 313–318.
 - 33 M. S. Attia, S. A. Elsaadany, K. A. Ahmed, M. M. El-Molla and M. S. A. Abdel-Mottaleb, *J. Fluoresc.*, 2015, **25**, 119–125.
 - 34 S. G. Hashem, M. M. Elsaady, H. G. Afify, M. El-Kemary and M. S. Attia, *Talanta*, 2019, **199**, 89–96.
 - 35 M. S. A. Abdel-Mottaleb, M. Saif, M. S. Attia, M. M. Abo-Aly and S. N. Mobarez, *Photochem. Photobiol. Sci.*, 2018, **17**, 221–230.
 - 36 W. E. Omer, M. A. El-Kemary, M. M. Elsaady, A. A. Gouda and M. S. Attia, *ACS Omega*, 2020, **5**, 5629–5637.
 - 37 L. M. Abdullah, M. S. Attia and M. S. A. Abdel-Mottaleb, *Egypt. J. Chem.*, 2019, **62**, 247–255.
 - 38 M. S. Attia, A. O. Youssef, A.-S. S. H. Elgazwy, S. M. Agami and S. I. Elewa, *J. Fluoresc.*, 2014, **24**, 759–765.
 - 39 D. Bank. <https://go.drugbank.com/drugs/DB15661>.
 - 40 cayman chem, *M. Physicochemical properties*, <https://www.caymanchem.com/product/29586/eidd-2801>.

