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Simple Green Spectrophotometric & Chromatographic Assay of the Oral Antiviral Treatment of COVID-19: Molnupiravir -EIDD-2801

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Abstract

Although vaccination for "Coronavirus disease 2019" is currently available, effective antiviral therapy is of great importance. The presence of easily administrated oral antivirals was a goal since beginning of the pandemic. The end of 2021 witnessed the emergency use authorization to Merck oral antiviral drug Molnupiravir (MOL). This study presents three analytical procedures to assay MOL in its raw material and dosage form. Method I: direct spectrophotometric measuring at λ_{max} 233 nm using methanol as a solvent. Method II: HPTLC method, using methanol and glacial acetic acid as mobile phase followed by densitometric scanning of MOL bands at its λ_{max}. Method III: RP-HPLC-DAD procedure, where MOL is separated only in 5 minutes, using isocratic elution of acetonitrile and distilled water acidified with orthophosphoric acid (pH 3) with ratio 87:13 (flow rate 1 mL/min.). The DAD detection was done also at 233 nm. These methods were validated to be ready for MOL rapid quality control assay in its fast massive production with good linearity correlation coefficients in ranges of 2.5-20 µg/mL, 0.03-0.38 µg/band and 0.025-10 µg/mL, for methods I, II & III, respectively. Limits of detections of 0.53 µg/mL, 0.01 µg/band & 0.005 µg/mL of methods I, II & III, respectively show the proposed methods' sensitivity. In addition, the three methods were applied for assaying MOL in laboratory prepared capsules to prove the methods' selectivity. Finally, the greenness of the three proposed methods was assessed & compared to those of the previously reported methods for MOL single assay by AGREE metric for greenness assessment.

Keywords: Molnupiravir; Covid-19; Spectrophotometry; HPTLC; RP-HPLC; Validation; Green.

1. Introduction

Still the pandemic of "Coronavirus 2019" disease is the major concern of scientists and researchers worldwide. Several types of vaccines now exist that are highly effective.[1] However, rapid viral mutations, lack of full immunization and immunocompromised patients who may not be fully protected by vaccination, makes developing a treatment for this disease is essential.[2]

At the beginning of the crisis, the only available treatment was the symptomatic one together with trying already existing drugs such as antimalarial drug: hydroxychloroquine, antiparasitic drug: ivermectin and antibacterial: azithromycin and many more drugs. [2, 3] The huge hope was reaching an oral antiviral treatment specific for Covid-19 that can be readily prescribed to patients with moderate or mild infections without having to be administrated to hospital for treatment. [1]

Antiviral drugs such as Remdesivir and favipiravir work by inhibiting RNA polymerase enzyme (RPE) which proved to be the most successful and specific target against the virus causing Covid-19. However, current available form of Remdesivir is intravenous vials and favipiravir is an orally active antiviral but has poor pharmacokinetic profile and did not gain the EUA from the FDA.[2, 4]

A new RPE inhibitor developed by Merck is Molnupiravir (MOL, EIDD-28011/ MK-4482) ($C_{13}H_{19}N_3O_7$) ((2R,3S,4R,5R)-3,4-dihydroxy-5-(4-(hydroxyamino)-2-oxopyrimidin1(2H)-yl) tetrahydrofuran-2-yl) methyl isobutyrate, (Fig. 1).

MOL is an oral drug with good pharmacokinetics proved to inhibit "severe acute respiratory syndrom coronavirus-2" (SARS-CoV-2), the virus causing the "Covid-19 disease", and reduce its load. [2] In addition, MOL showed potential inhibitory effect on the new Omicron variant in some studies. [5]

Finally on December, 2021, the U.S. FDA "Food and Drug Administration" granted EUA for MOL to be

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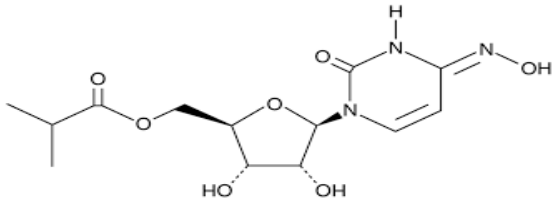

Figure 1: Chemical structure of Molnupiravir (MOL)

used in confirmed mild to moderate cases of Covid-19 (18+ years patients) who may be prone to severe complications and in case alternative authorized treatments are not an option. It should be administrated within five days from the beginning of symptoms by prescription only for five consecutive days.[6] Several companies started manufacturing the raw material and products of MOL, the oral Covid-19 antiviral medication, to supply to many low & middle-income countries.[7] Since there is a race to provide the Covid-19 antiviral pill in the market, it is of great urge to develop analytical methods for its assay in bulk powder and its marketed capsules. The methods must be simple and cheap yet sensitive to be used for its routine analysis in quality control labs especially in developing countries.

A literature survey for analytical methods for MOL single assay showed very limited results including a stability indicating HPLC method and a HPLC-MSMS method for assay of MOL in presence of its metabolite in biological matrix [8,9] No enough analytical methods are yet available and subjected to enough validation for this new drug single rapid assay. Thus, the aim of this work was to develop simple analytical techniques for MOL routine sample analysis.

2. Experimental

2.1. Instrumentation

2.1.1. UV/VIS Spectrophotometer for Method I

A UV-Vis spectrophotometer (Thermo-Spectronic) connected to Harvest computer system was used for the spectrophotometric measurements using 1-cm quartz cells.

2.1.2. HPTLC for Method II

For the second method, silica gel-60 HPTLC plates$_{(F254)}$, from E. Merck, Germany, (20x10 cm & 250 mm thickness) for MOL assay were used. A 100 µL Camag microsyringe & Linomat IV applicator (Switzerland) injected the sample (10 µL) as 5 mm bands on the plate. The bands were 4 mm apart and 15 mm away from the plate's bottom. Mobile phase of methanol & glacial acetic acid (10:0.05) developed the plates in Camag chamber (20x20 cm) followed by densitometric scanning using deuterium lamp and Camag scanner-III. CAMAG CATS software was also used for data analysis.

2.1.3. HPLC for Method III

HPLC-DAD system (Agilent 1200, USA) with an automated injector, quaternary pump, vacuum degasser and diode array detector was used. A reversed phase Interstil-ODS -3 (150 × 4.6 mm) 5µm column at 25°C was used for MOL assay.

2.2. Materials

HPLC-grade methanol and acetonitrile (Fischer Scientific, UK), orthophosphoric acid and glacial acetic acid (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) were used. Filtered double-distilled water was also used throughout the work. EIDD-2801 - Molnupiravir (MOL) (≥ 98 % purity) was obtained from Merck-SA, Dermstadt, Germany.

2.3. Standard solutions & calibration curves

A 250 µg/mL MOL stock standard solution was prepared in methanol (stored at 4°C for two weeks). Calibration standards, in the concentration range for each method, were prepared by further dilution using methanol. Calibration curves and regression equations for each method were constructed where the responses were plotted versus drug concentrations.

2.4. Analysis of laboratory-prepared pharmaceutical preparation

A portion of capsules powder (MOL active ingredient mixed well with small amount of common capsule excipient supplied by Pharco Pharmaceuticals Co., Alexandria, Egypt and composed of maize/ starch/ microcrystalline cellulose/ magnesium sterate/ hydroxyl propyl methyl cellulose/ silica) [10] equivalent to 25 mg MOL was weighed and accurately transferred into a 25-mL volumetric flask using 10 mL methanol. After sonication for 10 min, dilution to volume by methanol and filtration, appropriate (µL) portion of the prepared sample filterate was transferred into 10-mL volumetric flask and completed to volume with methanol to reach a concentration in the linearity range of each method. Each procedure was then performed as described earlier on the sample prepared.

2.5. Analysis of Spiked Human Plasma (Method III selectivity test)

Aliquots from a MOL diluted methanolic standard solution (75 µg/mL) prepared from its stock (250 µg/mL) were added to 250 µL plasma samples to prepare three samples with different MOL concentrations (3, 6, 10.5 µg/mL plasma). The solutions were vortexed for 3 min. after adding 500 µL acetonitrile for protein precipitation. Centrifugation was then done at 14000 rpm (-4 ºC, 15 min) and 20 µL of the supernatant was carefully injected into the HPLC system.

2.6. Procedure

2.6.1. Spectrophotometric method (Method I):

Direct measurement of absorbance readings "A" at λ_{max} 233 nm was done for determination of MOL. The

calibration standards were prepared in 10 mL volumetric flasks by taking appropriate volumes in the range of 0.1 – 0.8 mL from the MOL methanolic stock standard solution (250 µg/mL) and completed to volume. The prepared calibration standards in the concentration range stated in table 1 are measured against methanol as a blank in the spectrophotometer to determine their absorbance readings at λ_{max} 233 nm.

2.6.2. *HPTLC (Method II)*:

Using a micropipette, accurate volumes of 120 - 1520 µL of MOL standard stock solution (250 µg/mL) were added to 10-mL volumetric flasks then completed to volume using methanol to achieve calibration standards with concentration ranges of 3-38 µg/mL. Triplicate 10-µL portion of each solution was applied to the TLC plate to obtain final concentrations in table 1.

After saturating the chamber for 30-min with 20 mL of the previously mentioned mobile phase, the plate was developed for 12 min in ascending mode, and allowed to air-dry for 10 min. The plate was then scanned at λ_{max} of MOL (233 nm) under the following conditions: absorbance mode, deuterium lamp, 6 mm band width, 20 mm/sec. scanning speed and slit dimensions of 5 × 0.45 mm.

2.6.3. *HPLC (Method III):*

For the HPLC work, MOL stock solution was further diluted to a 50 µg/mL working solution. The calibration standards were then prepared by transferring accurate volumes of 5 – 2000 µL from MOL working solution (50 µg/mL) into 10-mL volumetric flasks and diluted to volume with methanol to reach the concentration ranges in table 1. Triplicate 20 µL injections were done for each solution and analyzed on the HPLC column at 1 mL/min flow rate using isocratic elution by a mobile phase of acetonitrile and distilled water acidified with orthophosphoric acid (pH 3) with ratio 87:13, v/v & DAD-detection also at 233 nm.

2.7. Validation parameters evaluation:

All validation parameters including: linearity and its range (by plotting calibration lines), limits of detection & quantitation, accuracy (by calculating % recoveries & % E_r), inter & intra-day precision (percentage relative standard deviation estimation), selectivity, robustness and solution stability have been assessed in details and measured to ensure methods' validation.

3. Results and Discussion

Three simple, green & cheap methods were developed for MOL assay in its drug substance and product with minimal analysis steps. Also, all validation parameters have been checked to ensure the three methods' validity.

The proposed methods have been compared to the two previously published methods for single assay of MOL in supplementary table 1. The proposed methods are considered greener and less time- consuming. The proposed methods also use simple instruments, unlike mass spectroscopic techniques that will not be widely available in developing countries' laboratories who need fast and simple quality control assay of MOL to face the pandemic with this oral antiviral therapy. Also it is well known that RP-HPLC methods are more applicable for pharmaceutical routine analysis compared to LC-MS/MS methods. The proposed HPLC method also provides higher sensitivity compared to the reported stability indicating HPLC method. Regarding the HPTLC method proposed; as previously documented in many researches, HPTLC is known to be better alternative than HPLC due to its high through put analysis where we can run several samples in one run thus reducing analysis time. In addition, besides its lower cost, HPTLC consumes much less amount of solvents and produce less waste compared to the other chromatographic methods. Thus for routine sample analysis, which is the target of this study, the reported HPTLC can be considered better option compared to the reported HPLC & HPLC-MS/MS methods. Although, direct spectrophotometry did not provide high sensitivity compared to the other reported chromatographic methods, it is a simple, cheap and rapid technique with low solvent consumption that still provides enough sensitivity for MOL routine analysis in its dosage forms which is the target of this study.

3.1. Method I: due to the novelty of the studied drug, no simple direct & valid spectrophotometric method was developed before for its quantitation. Common solvents as methanol, acetonitrile, water, aqueous sodium hydroxide (0.1 N) and aqueous hydrochloric acid (0.1 N) were tried to reach the highest Absorbance values of MOL. (Fig. 2) All solvents had comparable readings, except for sodium hydroxide (0.1 N) which caused decrease in absorbance value which may be attributed to the fact that MOL is an ester prodrug that may be hydrolyzed in alkaline medium. Thus, in order to decrease the number of sample preparations and unify the solvent used in the three methods, the solvent of choice was methanol.

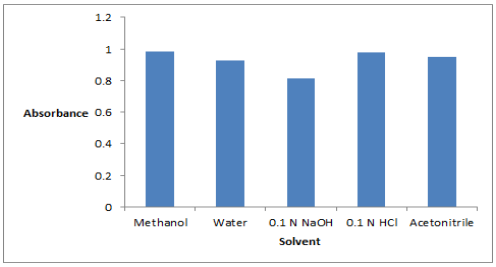

Figure 2: Effect of different diluting solvents on Absorbance values of MOL.

By simple spectrophotometric scanning of MOL, it showed two λ_{max} at 233 and 275 nm. (Fig. 3) Thus, simple assay of MOL was achieved using direct

measurements in methanol at 233 nm as it gave higher sensitivity compared to 275 nm.

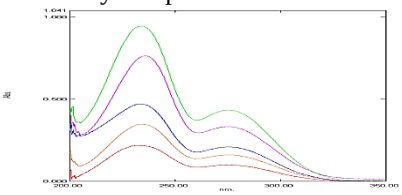

Figure 3: Absorption curve of increasing concentrations of MOL in methanol.

3.2. Method II: HPTLC is known to be a green technique that is used in separation of complex mixtures due to its minimal use of solvents, low waste, high sample through put and low cost per run. [10,11] MOL HPTLC assay was achieved at R_f (0.93 ± 0.02) using just methanol and glacial acetic acid (10:0.05, v/v respectively) with detection at 233 nm as shown in Fig. 4.

Different solvents were tried (methanol, water and ethanol). Most of these solvents and their combinations did separate MOL, but the cleanest assay with minimal solvent front was achieved using just methanol. Different volumes of ammonia and/or acetic acid were added to the mobile phase to adjust the system's pH and reduce peak tailing. Effect of pH was not significant. However, acidic medium was better as it reduced the solvent front, gave clearer chromatogram and yet achieved a symmetrical peak for MOL. The two λ_{max} of MOL were tried, and 233 nm was chosen for higher sensitivity.

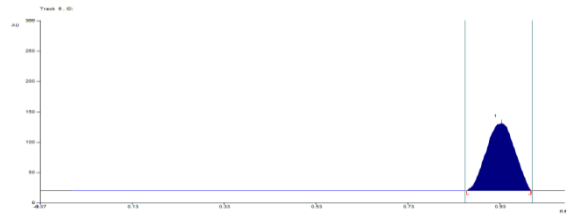

Figure 4: HPTLC (10 µL band volume) chromatogram of 0.38 µg/band MOL.

3.3. Method III: The proposed HPLC method was optimized to achieve a valid assay of the target drug.
For stationary phase selection and optimization: A simple ODS (150 × 4.6 mm) column was used and the results were comparable with trying a C_8 column of the same dimensions. However, the clearest separation with least noise was achieved using the ODS column.
For Mobile phase selection: Both acetonitrile and methanol were tried as being the usual organic modifiers but acetonitrile gave lower instrument pressure, more symmetrical & reproducible peaks with minimal tailing for MOL. Different aqueous phase buffers such as phosphate, acetate and formate at their different optimal pH values (pH 3.0–7.0) resulted in distortion of the baseline and huge solvent fronts which interfered in MOL peak. Meanwhile, just acidified water with orthophosphoric acid at pH (3) gave symmetrical MOL peaks at clear baseline. Increasing the pH beyond 3 caused significant peak tailing. **The chosen wavelength** for MOL detection using the HPLC method was 233 nm also as it caused significant increase in MOL sensitivity at R_t of 4.58 ± 0.08 (Fig. 5).

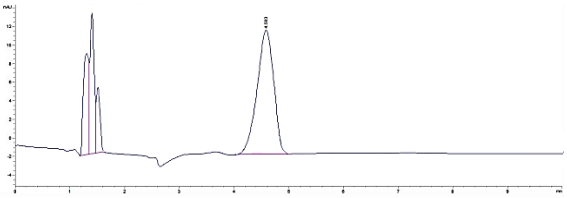

Figure 5: HPLC (10 µL injection volume) chromatogram of 10 µg/mL MOL.

System Suitability parameters were as follows: Capacity factor (k') = 2.53, Asymmetry factor (A_f) = 0.91 and Column efficiency (plates/m) = 2200. All within the acceptable FDA limits.[12]

3.4. Validation to the three methods

All validation parameters were verified according to the ICH guidelines. [13]

Linearity and Range: Calibration graphs were done by plotting either the "A" values of method I or peaks' areas of methods II & III versus MOL concentrations showed linearity with high r value (0.99 and higher). All regression parameters & the limits of detection and quantitation (LOD & LOQ) are summarized in Table 1. LOD and LOQ were calculated via ICH [13] [LOD=3.3 σ/S and LOQ=10 σ/S] (σ: intercept standard deviation & S: slope) for the spectroscopic method. For chromatographic Methods II & III with baseline noise: concentrations of "Signal/Noise" ratio of "3/1" and "10/1" were considered the LOD and LOQ, respectively. (Supplementary Fig. 1 & 2)

Table 1: Regression parameters of the proposed methods for determination of MOL.

Parameter	Method I	Method II	Method III
Linearity range	2.5-20 µg/mL	0.03-0.38 µg/band	0.025-10 µg/mL
LOQ	1.60 µg/mL	0.03 µg/band	0.02 µg/mL
LOD	0.53 µg/mL	0.01 µg/band	0.005 µg/mL
Intercept, (a)	9.44×10^{-2}	993.52	31.15
Slope, (b)	4.29×10^{-2}	2549.66	26.43
Correlation coefficient, (r)	0.9997	0.9982	0.9995
Standard deviation of intercept, S_a	6.84×10^{-3}	18.14	2.42
Standard deviation of slope, S_b	5.73×10^{-4}	86.18	0.48
Standard deviation of residuals, $S_{y/x}$	8.35×10^{-3}	25.41	4.15
F	5595.83	875.25	3013.36

Significance F	5.26×10^{-6}	8.48×10^{-5}	1.33×10^{-5}

Accuracy & Precision: Replicates (n=5) at 3 concentration levels were analyzed on same day for "accuracy & intra-day precision" and on different days for "accuracy & inter-day precision" evaluations. The results in Table 2 indicate high accuracy & precision of the three methods. All % recoveries, % E_r and % RSD are within acceptable limits ± 2 %.

Table 2: Accuracy and Precision validation.

Method	Conc. (µg/mL in methods I & III – method II in µg/band)	Mean % Recovery ± RSD%[a]	E_r %[b]
(a) Accuracy and intra-day precision (repeatability)			
Method I	2.5	101.75 ± 0.90	1.75
	10	100.94 ± 0.51	0.94
	20	99.92 ± 0.65	-0.08
Method II	0.03	98.30 ± 0.88	-1.70
	0.10	101.22 ±1.21	1.22
	0.30	100.55 ± 0.97	0.55
Method III	0.025	101.68 ± 1.20	1.68
	1	101.85 ±1.05	1.85
	10	100.22 ± 0.70	0.22
(b) Accuracy and inter-day precision			
Method I	2.5	101.55 ± 1.50	1.55
	10	100.20 ± 0.99	0.20
	20	101.36 ± 1.20	1.36
Method II	0.03	99.50 ± 1.01	-0.50
	0.10	100.65 ±1.33	0.65
	0.30	101.50 ±1.25	1.50
Method III	0.025	101.22 ± 1.90	1.22
	1	98.76 ±1.77	-1.24
	10	100.99 ± 0.97	0.99

a. Percentage relative standard deviation
b. Percentage relative error.

Selectivity: Selectivity was demonstrated by applying the methods for MOL assay in presence of its capsules' probable excipient without any interference (Table 3). In addition, MOL HPLC & HPTLC peaks were evaluated for their purity. The HPLC purity angle of all samples was within the acceptable threshold limit, showing no interference. (Fig.6a) Overlapped spectra recorded at different time intervals across the HPLC peaks also indicate peak purity (Fig.6b).

Table 2: Application in laboratory prepared pharmaceutical preparations.

Lab-prepared labs	% Found ± RSD % *(n=5)*			
	Method I	**Method II**	**Method III**	**Reported method [9]**
	100.98 ± 0.87	101.65 ± 1.99	101.50 ± 1.45	101.98 ± 0.55
Students' *t- test* (*t*)*	0.29	0.19	0.66	--------
Variance ratio F-test (F)*	0.41	0.69	0.69	--------

*Theoretical values of *t* and *F*: 2.31 & 6.39, respectively, at 95% confidence limit.

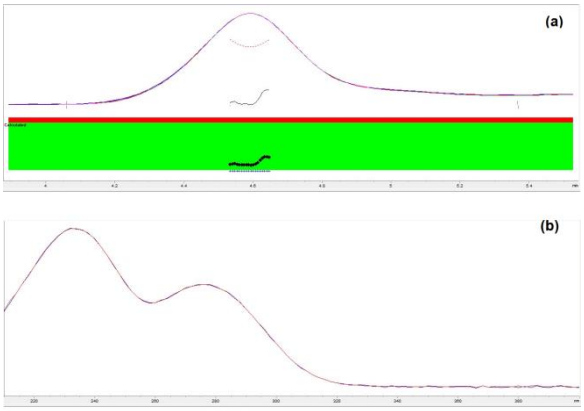

Figure 6: (a) Purity plot of MOL peak & (b) Absorption spectra illustrating peak purity of MOL obtained from its laboratory prepared capsule solutions for the HPLC method.

To demonstrate the HPLC method's selectivity, assay of MOL was done in spiked plasma samples. The purpose of MOL assay in plasma by the proposed HPLC method was only to ensure the method's selectivity that even in complex matrix, it was able to determine MOL concentration accurately. High % recoveries from 95 to 97% and low % RSD values ≤ 2% for the three different spiked MOL concentrations when compared to equivalent standard concentrations proved the high selectivity of the developed HPLC method in this study (Supplementary table 2 & supplementary fig.3).

For HPTLC method: The purity of MOL sample spots was shown by comparing their R_f and spectra with those of the standards. Peak purity was tested by comparing spectra at different points: S, M & E which are peak start, peak apex & peak end, respectively. The calculated r (S, M) and r (M,E) values were not less than 0.999, which indicates the peaks' homogeneity.

Robustness: Intended significant changes in the conditions resulted in no variations neither on peaks' areas nor MOL retention. Table 4 shows that RSD % values of all peak areas were less than 2 and standard deviations of R_t or R_f were also very low.

MOL Solution Stability: After preparation of MOL standard and sample solutions, they were left at room temperature for 1 to 6 h and then analyzed by the three methods at different time intervals. No change in the results of all methods indicating the stability of drug solutions for at least 6 h, which is sufficient time to complete the drug analysis.

3.5. Analysis of Pharmaceutical Preparation

Since MOL capsules are still not available in our markets, the applicability of the methods was tested by determination of MOL in presence of common capsules ingredients. The recovery results were acceptable and also RSD% values less than 2 (Table

3) indicate the methods are selective for MOL even in presence of other excipients and can be used for MOL assay in its capsules. The three methods were also compared to a reported method [9] using *t- test* & F-test and the results were comparable with no significant difference. The reported method was an HPLC method with isocratic elution using acetonitrile: water (20:80 v/v) & detection at 240 nm.

Table 4: Robustness determination (n= 3) for MOL assay using the proposed chromatographic methods.

HPTLC Parameters tested	HPTLC method		HPLC Para-meters tested	HPLC–DAD method	
	RSD % peak areas	R_f ± SD		RSD % peak areas	R_t ± SD
1) Mobile phase composition [methanol: gl. Acetic acid (10: 0.1, 10: 0.2 and 10: 0.05 (v/v))]	1.09	0.93 ± 1.15 × 10^{-2}	**1) Mobile phase ratio** [±1 % aqueous phase]	0.62	4.587 ±5.69 × 10^{-3}
2) Mobile phase volume [15, 20 and 25 mL]	0.55	093 ± 5.77 × 10^{-3}	**2) Flow rate** [1 ± 0.1 mL/min]	0.59	4.591 ± 6.66 × 10^{-3}
3) Duration of saturation [30, 40 and 50 min]	0.50	0.94 ± 5.77 × 10^{-3}	**3) Colum n temp.** [25° C ± 2° C]	0.99	4.598 ± 5.57 × 10^{-3}
4) Time from chromatog raphy to scan [10, 20, 30 and 60 min]	0.87	0.93± 1.15 × 10^{-2}	**4) pH of the aqueou s phase** [3 ± 0.2]	1.01	4.599 ± 5.86 × 10^{-3}
5) λ (± 2 nm)	1.10	0.94 ± 1.00 × 10^{-2}	**5) λ (± 2 nm)**	0.94	4.591 ± 1.00 × 10^{-3}

3.6. Assessment of Method Greenness

Green and eco -friendly practices have been recently adopted in different analytical procedures such as using green sample pretreatment, using environmentally friendly solvents and reagents, consuming less energy and shortening analysis times.[14,15] Greenness assessment was done using "Analytical GREEnness" Metric Approach [16] (AGREE), a new method introduced in 2020. AGREE assessment is based on the twelve principles of "Green Analytical Chemistry" (GAC) and is represented in a clock-like graph composed of 12 sections representing the 12 GAC principles. Each section is assessed and represented in a color of green, yellow & red. The overall greenness performance of the 12 sections is colored, written in the middle and scored within 0 to 1. As shown in table 5, the clock-like graph shows an overall AGREE score of 0.76, 0.69 and 0.61 for our spectrophotometric, HPTLC and HPLC methods, respectively. All were with green color indicating low impact on the environment. Only one red was found in the three methods representing the off-line sampling that occurs in the analytical quality control laboratories of pharmaceuticals. The spectrophotometric method was the greenest method due to lack of any reagents used and high number of samples that can be analyzed in an hour. The HPTLC method greenness followed the spectrophotometric method and greener than the HPLC method and this is attributed to the less waste, less solvents and no buffer and more number of samples that can be analyzed in an hour. In comparison with the reported methods [8, 9], the proposed spectrophotometric and HPTLC methods showed greener assessment than both reported methods. Our HPLC method showed similar greenness to that the reported HPLC [9] method but with an advantage of higher sensitivity. The reported HPLC-MS/MS method [8] showed the least green assessment due to the analysis in biological fluids and many tedious extraction procedures and due to high waste.

Table 5: Assessment of the greenness of the 3 proposed and 2 reported methods using AGREE [16] tool.

Spectrophotometric method	HPTLC method	HPLC method
		

Reported HPLC method [9]	Reported HPLC-MS/MS method [8]
	

4. Conclusion

Since MOL is a newly marketed drug that has been launched as the first oral antiviral against SARS-Cov-

2, no valid analytical method is currently available for its routine assay. Three simple yet sensitive methods were developed to analyze MOL in its capsules. The proposed methods have the advantages of being simple and of low cost without the use of complicated instruments which make them suitable for developing countries who need valid MOL assay for fast launching of the drug in the hope to face this pandemic with an oral treatment.

Conflicts of interest

There are no conflicts to declare.

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References

1. Jayk Bernal A., Gomes da Silva M.M. and Musungaie D.B., et al, Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. *N. Engl. J. Med.*, **386**, 509-520 (2021). doi: 10.1056/NEJMoa2116044

2. Imran M., Kumar Arora M. and Asdaq S.M.B., et al, Discovery, development, and patent trends on Molnupiravir: A prospective oral treatment for COVID-19. *Molecules*, **26**, 5795 (2021). doi: 10.3390/molecules26195795

3. Lam S., Lombardi A. and Ouanounou A., COVID-19: A review of the proposed pharmacological treatments. *Eur. J. Pharmacol.*, **886**, 173451 (2020). doi: 10.1016/j.ejphar.2020.173451

4. Lee C-C., Hsieh C-C. and Ko W-C., Molnupiravir—A Novel Oral Anti-SARS-CoV-2 Agent. *Antibiotics*, **10**, 1294 (2021). doi:10.3390/antibiotics10111294

5. Li P., Wang Y., Lavrijsen M. and Lamers M.M., et al, SARS-CoV-2 Omicron variant is highly sensitive to molnupiravir, nirmatrelvir, and the combination. *Cell Res.*, **32**, 322–324 (2022). doi:10.1038/s41422-022-00618-w

6. U.S. Food and Drug Administration, FDA, Coronavirus (COVID-19) Update: FDA Authorizes Additional Oral Antiviral for Treatment of COVID-19 in Certain Adults, December 23, 2021 (accessed 21 July 2022) https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-oral-antiviral-treatment-covid-19-certain

7. The Medicines Patent Pool (MPP) https://medicinespatentpool.org/news-publications-post/27-generic-manufacturers-sign-agreements-with-mpp-to-produce-molnupiravir (accessed 9 March 2022).

8. Amara A., Penchala S.D. and Else L., 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.*, **206**, 114356 (2021). doi: 10.1016/j.jpba.2021.114356

9. Reçber T., Timur S.S., Kablan S.E., 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.*, **214**, 114693 (2022). doi.org/10.1016/j.jpba.2022.114693

10. El-Kimary E.I., Khamis E.F., Belal S.F., Abdel Moneim M.M., Robust Chromatographic Methods for the Analysis of Two Quaternary Mixtures Containing Paracetamol, Codeine, Guaifenesin and Pseudoephedrine or Phenylephrine in their Dosage Forms. *J. chromatogr. Sci.*, **57**, 828-837 (2019). https://doi.org/10.1093/chromsci/bmz057

11. Youssef R., Khamis E., El-Sayed M., Abdel Moneim M., Development and validation of a high-performance thin-layer chromatographic method for the assay of ternary mixtures containing cetirizine dihydrochloride in pharmaceutical dosage forms. *JPC-J. Planar Chromat.*, **27**, 58-65, 2014. doi:10.1556/JPC.27.2014.1.11

12. US Food and Drug Administration, Guidance for industry, bioanalytical methods validation (2001).

13. International Conference on Harmonization (ICH), Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Current Step 4 Version, Parent Guidelines on Methodology, 1996, incorporated in November 2005.

14. Thanoon, E., Saeed A., Development of Green Spectrophotometric Method for Determination of Metoclopramide Hydrochloride. *Egypt. J. Chem.*, **64**, 3451 - 3456 (2021). doi:10.21608/EJCHEM.2021.43857.2923

15. Gamal H., Attia, N., Green Synthesis Approach for Renewable Textile Coating and Their Mechanical and Thermal Properties. *Egypt. J. Chem*., **63**, 3109-3115 (2020). doi:10.21608/EJCHEM.2020.20618.2234

16. Pena-Pereira, F., Wojnowski, W., Tobiszewski, M., AGREE—Analytical GREEnness Metric Approach and Software. *Anal chem.*, **92**, 10076-10082 (2020). https://doi.org/10.1021/acs.analchem.0c01887