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Green adherent spectrophotometric determination of molnupiravir based on computational calculations; application to a recently FDA-approved pharmaceutical dosage form

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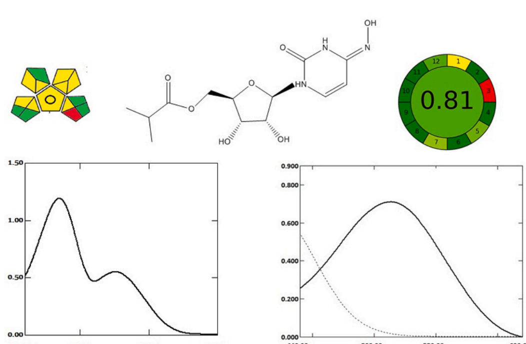
HIGHLIGHTS

- Molnupiravir is an oral antiviral drug.
- Green adherence and minimal environmental impact.
- Diazo coupling of molnupiravir with 8-hydroxyquinoline.

GRAPHICAL ABSTRACT



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ABSTRACT

Molnupiravir is an oral antiviral drug developed to provide significant benefit in reducing hospitalizations or deaths in mild COVID-19. Integrated green computational spectrophotometric method was developed for the determination of molnupiravir. Theoretical calculations were performed to predict the best coupling agent for efficient diazo coupling of molnupiravir. The binding energy between molnupiravir and various phenolic coupling agents, α-naphthol, β-naphthol, 8-hydroxyquinoline, resorcinol, and phloroglucinol, was measured using Gaussian 03 software based on the density functional theory method and the basis set B3LYP/6-31G(d). The results showed that the interaction between molnupiravir and 8-hydroxyquinoline was higher than that of other phenolic coupling agents. The method described was based on the formation of a red colored chromogen by the diazo coupling of molnupiravir with sodium nitrite in acidic medium to form a diazonium ion coupled with 8-hydroxyquinoline. The absorption spectra showed maximum sharp peaks at 515 nm. The reaction conditions were optimized. Beer's law was followed over the concentration range of 1–12 μg/ml molnupiravir. Job's continuous variation method was developed and the stoichiometric ratio of molnupiravir to 8-hydroxyquinoline

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was determined to be 1:1. The described method was successfully applied to the determination of molnupiravir in pure form and in pharmaceutical dosage form. The results showed that the proposed method has minimal environmental impact compared to previous HPLC method.

1. Introduction

Trends toward the development of environmentally friendly analytical chemistry play an essential role in protecting the environment by monitoring pollutants in air, water, or soil. Many solvents and reagents are used in analytical procedures, resulting in toxic residues. Hazardous solvents should be replaced with more environmentally friendly ones to shorten analysis time, reduce sample volume, and consume less energy. Green analytical chemistry was introduced to eliminate the adverse effects of analytical procedures on the environment. The green chemistry approach has attracted considerable interest from chemists, especially those involved in environmentally friendly laboratory practices in analytical chemistry [1–3]. Computational chemistry represents an integral approach, in HPLC [4,5], spectrophotometry [6,7] and electrochemistry [8], to the development of greener methods based on theoretical testing of compound interactions and reducing the use of toxic solvents [9]. Many analytical methods have been developed to demonstrate the quality of the drug materials including HPLC [10], TLC [11], specroflorimetry [12–17] as well as spectrophotometry [18–28].

Color-based spectrophotometric methods are a simple, cost-effective continental tool widely used in quantitative pharmaceutical analysis [29,30]. Among the color-based spectrophotometric methods, diazotization coupling is generally used for the determination of compounds containing free aromatic amino groups. The reaction is based on the coupling of the diazonium salts with various coupling agents [31–34]. It is believed that the selection of the best coupling agent can improve the spectrophotometric determination of the compound of interest. In addition, computer-aided calculations can lead to shorter analysis time, smaller sample volume, and thus a more environmentally friendly analytical procedure.

Molnupiravir [MLP], Fig. 1, is a new oral bioavailable ribonucleotide antiviral drug that shows significant benefit in reducing hospitalizations or deaths in mild COVID-19 and may be an important weapon in the fight against SARSCoV-2. MLP is activated by metabolism in the body. Once it enters the cell, it becomes an RNA-like component. In the first step, RNA polymerase incorporates these components into the RNA genome of the virus. In the next step, the RNA-like components are paired with components of the viral genome. The viral RNA contains multiple mutations as it replicates to produce new viruses, preventing the pathogen from replicating. This viral drug causes mutations in other RNA viruses and prevents their spread [35–38].

Although LC-MS /MS [39,40] and HPLC [41] have been reported for the determination of MLP in the presence of its active metabolite and in the presence of various antiviral drugs. Unfortunately, the use of organic solvents in LC-MS /MS and HPLC can lead to environmental contamination and potential health risks to analysts if improperly disposed of. One study states that an average chromatographic process can generate up to 1 L of organic waste per day, which adds up to millions of liters of hazardous waste per year. Considering these hazardous impacts, greener analytical methods should be developed to replace them with more environmentally friendly methods [42].

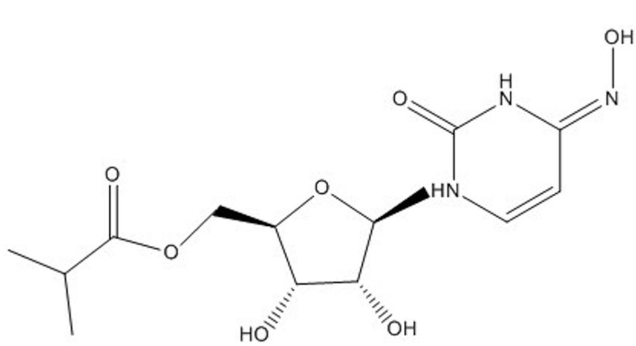


Fig. 1. Chemical structure of MLP.

In this work, integral calculations were performed to select the best coupling agent for diazo coupling of MLP and to provide a more environmentally friendly analytical method. The best coupling agent, 8-hydroxyquinoline, was used for the spectrophotometric determination of MLP in pure form and in pharmaceutical preparation. The method was based on the formation of a red colored azo dye upon the reaction of MLP with sodium nitrite in acidic medium to form a diazonium ion coupled with 8-hydroxyquinoline. In addition, the environmental friendliness of the described method was evaluated on a scientific basis using the national environmental method index [43], the analytical eco-scale [44], the green analytical procedure index [45], and the AGREE evaluation method [46].

2. Experimental

2.1. Materials

Pure analytical standard of MLP [98.99%] was kindly supplied by EPICO Pharmaceutical Company, Tenth of Ramadan city, Egypt. Molnupiravir EVA Pharma® capsules [200 mg MLP per capsule] was purchased from local pharmacy store. Sodium nitrite (Win lab, UK), solution was prepared as 4% aqueous solution (w/v). 8-hydroxyquinoline (Koch-Light Laboratories Ltd, England) was prepared in 1% (w/v) sodium hydroxide solution in 50% (v/v) aqueous ethanol. Hydrochloric acid (El-Nasr Company, Egypt), was prepared as 0.5 M aqueous solution. Sodium hydroxide (El-Nasr Company, Egypt), was prepared as 2 M aqueous solution.

2.2. Apparatus

Shimadzu UV–Visible 1650 Spectrophotometer, Tokyo, Japan.

2.3. Standard solutions

A standard stock solution of MLP [100 μg/ml] was prepared by dissolving 10 mg analytical standard pure MLP in 50 mL ethanol, and the volume was made up to 100 mL with ethanol.

2.4. Procedures

2.4.1. Computational calculations for the study of the interaction energy between MLP and different coupling agents

The structural formulas of MLP, the coupling agents [α-naphthol, β-naphthol, 8-hydroxyquinoline, resorcinol, phloroglucinol] and the corresponding complexes were drawn and optimized in Gauss-view software using the density functional theory method at B3LYP/6-31G (d) basis set level [47]. The energy of the optimized structures was measured. In addition, the binding energy of the diazotized MLP - coupling agent products, ΔE, was calculated as follows:

$$\Delta E = E_{A\text{–}B} - E_{A} - n\,E_{B}.(1)$$

where A is the energy of an optimized MLP, B is an optimized energy of the coupling agents.

Table 1
Computationally calculated binding energy between various phenolic coupling agents and MLP.

Compounds under the study	Binding energy	
	ΔE (Hartree)*	ΔE (KJ/mol)
MLP-8-hydroxyquinoline	−0.0205	−53.81
MLP- α naphthol	−0.0160	−42.00
MLP- β naphthol	−0.0120	−31.51
MLP-phloroglucinol	−0.0112	−29.41
MLP- resorcinol	−0.0110	−28.88

*1Hartree = 2625.5 KJ/mol.

2.4.2. *General procedure*

Aliquots of MLP standard solution [100 μg/mL] corresponding to [10–120 μg] were transferred to a series of 10-ml volumetric flasks, followed by adding 0.5 mL of hydrochloric acid [0.5 M] and 0.5 mL sodium nitrite [4%]. The prepared solutions were shaken for 5 min to provide enough time for efficient diazotization of MLP. 1 mL of 8-hydroxyquinoline [0.35%] was added. Then 0.5 mL sodium hydroxide [2 M] was added and the prepared solutions were allowed to stand for 5 min. Finally, the volume was completed to the mark with ethanol. The absorbance of the red colored azo dye complexes was measured at 515 nm against a blank treated similarly.

2.4.3. *Application of the proposed method for the determination of the pharmaceutical dosage form*

Ten Molnupiravir EVA Pharma® capsules were accurately weighed, and average weights were calculated. The capsule contents were mixed thoroughly and definite weight corresponding to one capsule was placed in a 100 mL volumetric flask and the volume was made up to 50 mL with ethanol. The solution was shaken and completed the mark with ethanol. The obtained solution was serially diluted to obtain different concentrations. Then, the general procedure for the described method was followed.

3. Results and discussion

MLP is the first orally administered antiviral drug to show significant benefit in reducing hospitalizations or deaths in mild COVID-19 and could be an important weapon in the fight against SARS-CoV-2 [35–38]. Since MLP has recently entered the market, the development of validated analytical methods is recommended to enable accurate determination and quality control approach of MPL in different analytical laboratories. Previous work has proposed the use of LC-MS /MS [39,40] and HPLC [41] for the determination of MLP, recommending the disposal of organic wastes that have a negative impact on the environment. Spectrophotometry represents a simple continental tool for quantitative analysis of drugs. The diazotization- based spectrophotometric method is commonly used for the determination of compounds with a free amino group [29,30]. MLP is an amino group-containing compound suitable for diazotization with nitrous acid and coupling of the resulting complex with phenolic coupling agents.

3.1. *Computational calculations for selecting the best phenolic coupling agent provided an efficient diazo coupling of MLP*

Computational calculations provide integral approach to more environmentally friendly analytical methods. Density functional theory [DFT] is a computational theory used to predict the electronic structure of compounds. DFT derives the actual properties of the compound based on a measurement of electron density. It is assumed that relevant data can be predicted from the measurement of the electron density of a compound [47]. In order to evaluate the binding efficiency between MLP and various phenolic coupling agents [α-naphthol, β-naphthol, 8-hydroxyquinoline, resorcinol, phloroglucinol], DFT was performed at B3LYP/6-31G (d) basis set level. The studied compounds were geometrically optimized and the minimum energy of MLP, coupling agents and MLP-coupling agent complexes was determined. The binding energy between MLP and coupling agents was calculated to evaluate the strength of the interaction, Table 1. The coupling agent with higher binding energy could be the best for diazotization of MLP compared to the other coupling agents. From the data given in Table 1, 8-hydroxyquinoline could be the suitable reagent for diazotization of MLP.

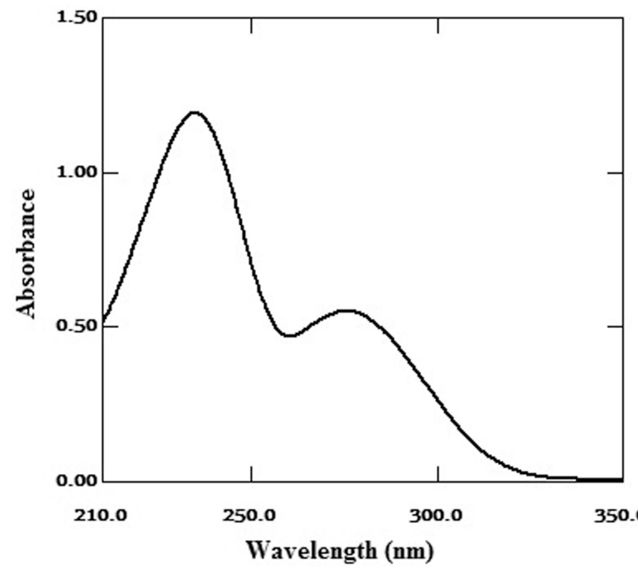


Fig. 2. UV absorbance spectrum of MLP [10 μg/mL].

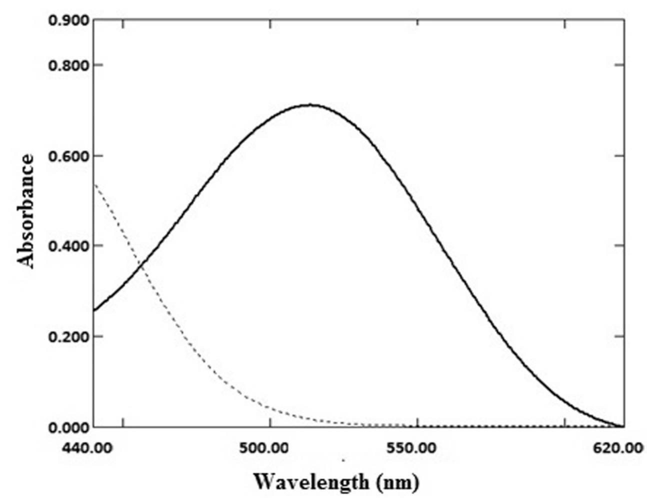


Fig. 3. Absorption spectra of the reaction product between MLP (8ug/mL) and 8-hydroxyquinoline [ ] and reagents only [− − − −] at 515 nm.

3.2. *Spectral characteristics*

The UV absorption spectra of MLP showed an absorption maximum at 272 nm, Fig. 2. Since MLP is an amine-containing compound, an attempt was made to develop an analytical method for its determination. The diazotization reaction with an optimally selected phenolic coupling agent, 8-hydroxyquinoline, yields an intensely red colored azo dye product that can be measured spectrophotometrically. The method described was based on diazotization of MLP with sodium nitrite in acidic medium. Subsequently, the diazonium salt formed was coupled with 8-hydroxyquinoline. The medium was made alkaline with sodium

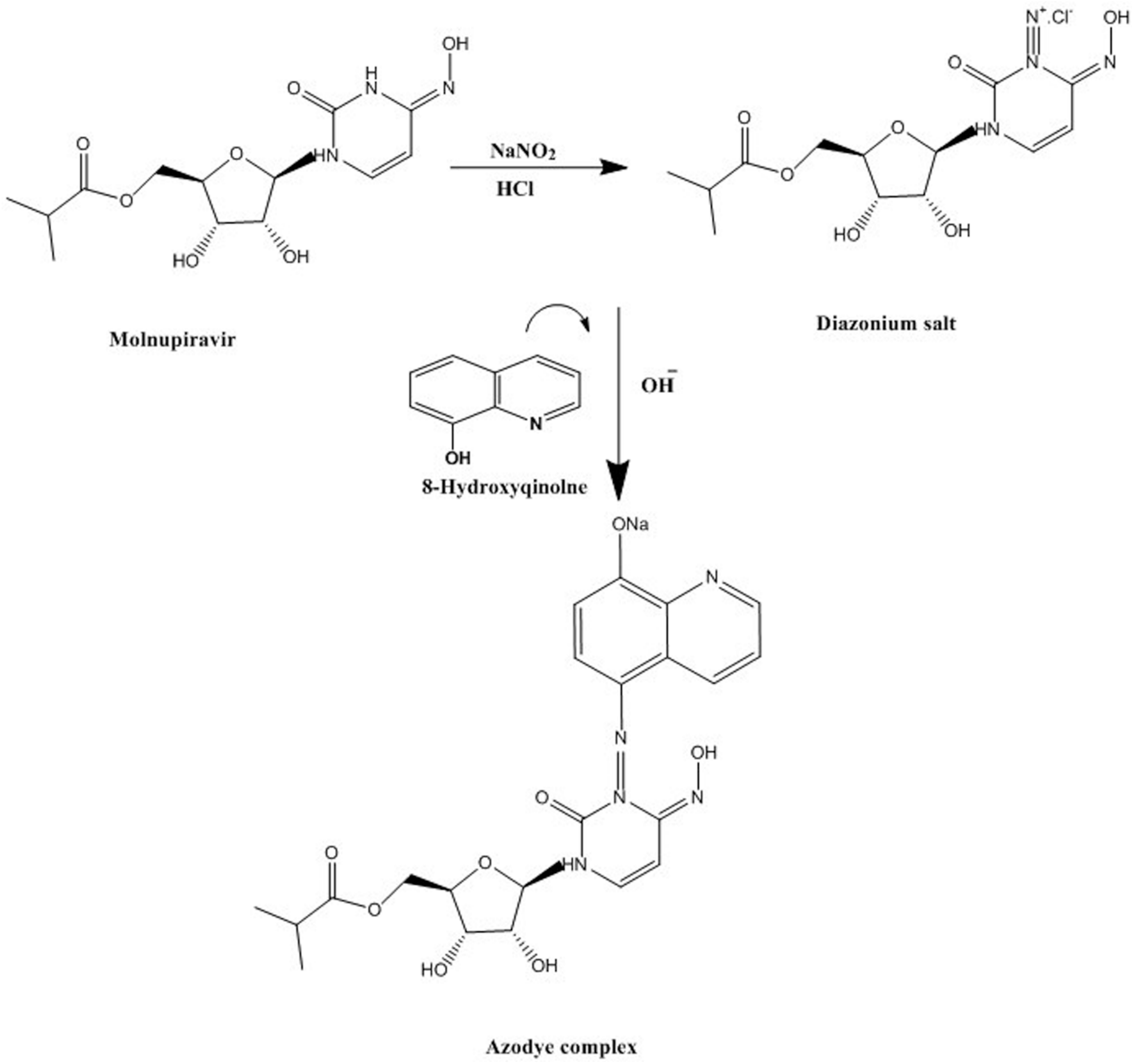


Fig. 4. Scheme for the diazotization reaction pathway of MLP followed by diazo coupling with 8-hydroxyquinoline.

hydroxide, and the developed color was measured in comparison with the reagent blank at 515 nm, as shown in Fig. 3. The proposed diazotization reaction pathway of MLP and diazo coupling with 8-hydroxyquinoline was shown in Fig. 4.

3.3. Optimization of the reaction conditions

The diazotization reaction is mainly based on many parameters. These parameters include the amounts of hydrochloric acid, sodium nitrite, 8-hydroxyquinoline, and sodium hydroxide. A high acidity condition is recommended for the diazo coupling reaction to obtain a strong nitrosating agent. It was found that 0.5 mL of 0.5 M hydrochloric acid was the best condition, as shown in Fig. 5a. Sodium nitrite plays an essential role in the conversion of amines to diazo compounds. It was found that 0.5 mL of a 4% sodium nitrite solution provided efficient absorption intensity, as shown in Fig. 5b. In addition, the results showed that 1 mL of 0.35% 8-hydroxyquinoline was efficient for the determination of MLP, as shown in Fig. 5c. Sodium hydroxide restores the stability of the benzene ring and provides an effective medium for the diazotization of MLP. The results show that 0.5 mL of 2 M sodium hydroxide provides the best conditions, as shown in Fig. 5d.

3.4. Molar ratio assessment of the reaction

Job's method of continuous variation [48] have been suggested to measure the stoichiometry of the chemical reactions. In the current study, Job's method of continuous variation was applied regarding to the simplicity. Graphical representation of the absorbance values at 515 nm versus MLP mole fraction was created as shown in Fig. 6. The stoichiometry of the complex formed between MLP and 8-hydroxyquinoline was found to be [1:1].

3.5. Validation of the method

The described method was validated according to the guidelines of ICH. The linearity of the method was evaluated by generating different calibration curves on different days. Calibration curves were generated within concentration ranges selected based on sensitivity parameters. Each concentration was repeated three times. The concentration ranges, regression equations, and other statistical parameters are listed in Table 2. The general procedures for drug determination were valid for triplicate determination of [3, 6, 9 μg/mL] MLP. Accuracy was expressed as % recovery, as shown in Table 2. Precision, expressed as percent relative standard deviation, was determined by the determination of [3, 6, 9 μg/mL] MLP. For repeatability, it was performed within one day and for mean precision, it was performed on three consecutive days. The low

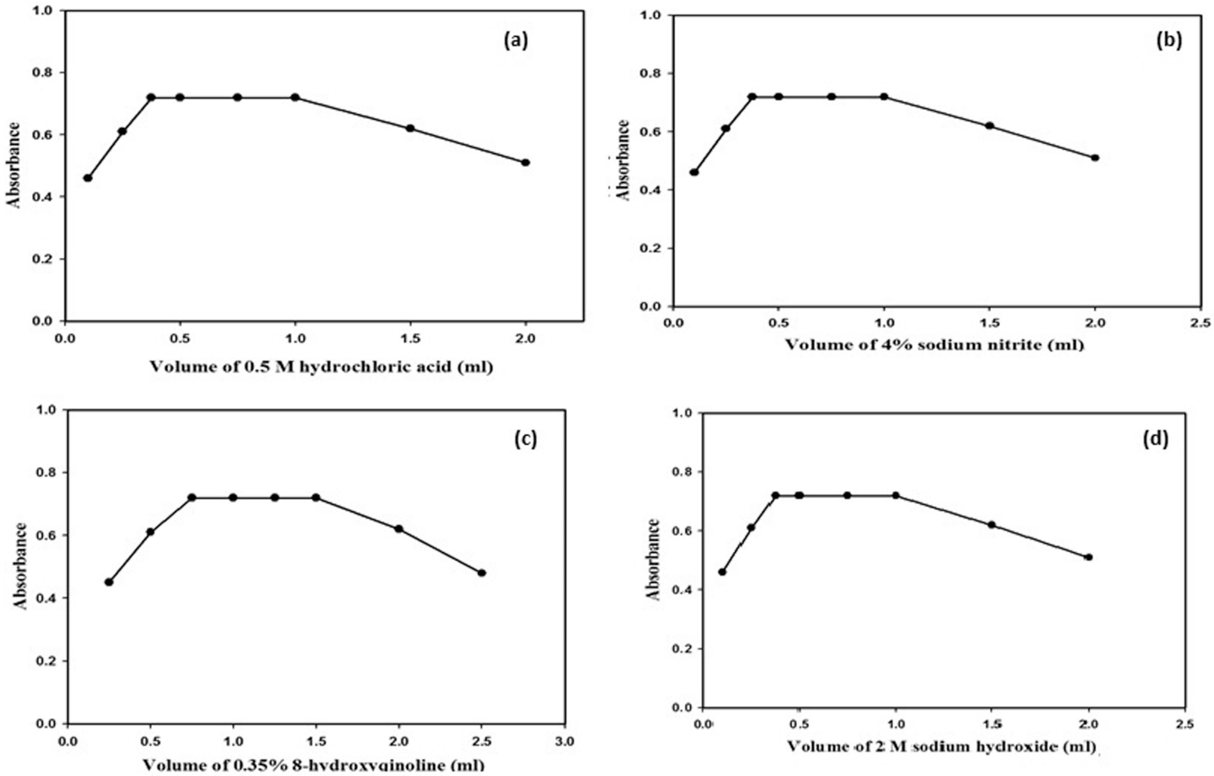


Fig. 5. Optimization for different factors affecting the diazotization of MLP with 8-hydroxyquinoline.

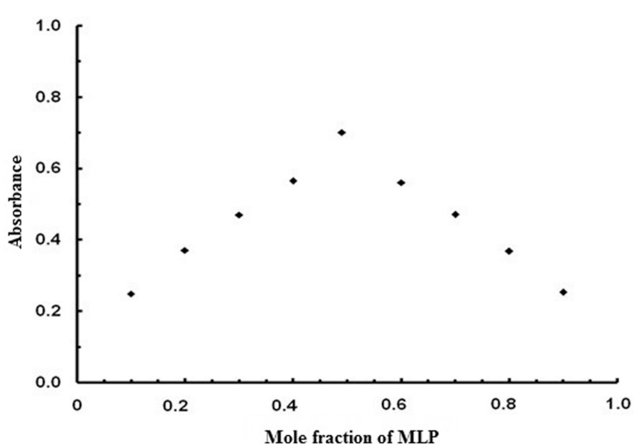


Fig. 6. Graphical representation of the absorbance values at 515 nm versus MLP mole fraction considering the reaction of [1.37 × 10^{-3} M] FV with [1.37 × 10^{-3} M] 8-hydroxyquinoline using job's method.

values of %RSD indicate high precision of the proposed method, as shown in Table 2.

3.6. *Application of the proposed method for determination of the pharmaceutical capsule*

The described method succeeded to determine MLP in the pharmaceutical capsules. The results were statistically compared with the results of the previously reported HPLC method [41]. By applying *t*-test and *F*-test, no significant differences were found at 95% confidence level, indicating the acceptability of the described method for the spectrophotometric determination of MLP in the pharmaceutical dosage form, Table 3.

Table 2
Regression and validation parameters for the described spectrophotometric determination of MLP.

Parameters		Obtained data
Wavelength (nm)		515
Linearity range (μg/ml)		1.00–12.0
– Regression values		0.0420
– Slope		0.0011
– Intercept		
Coefficient of determination (r^2)		0.9996
Accuracy (%R)[a]		99.820
Precision (%RSD)[b]	Repeatability	0.623
	Intermediate precision	0.782

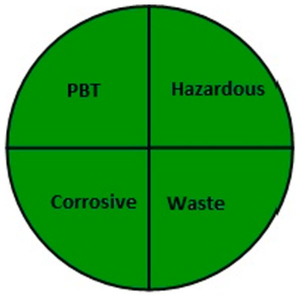
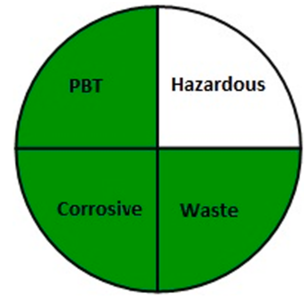
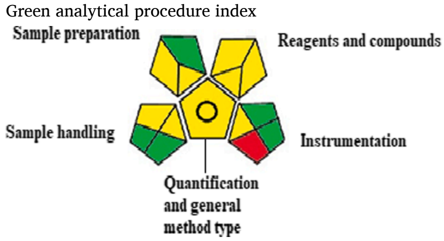
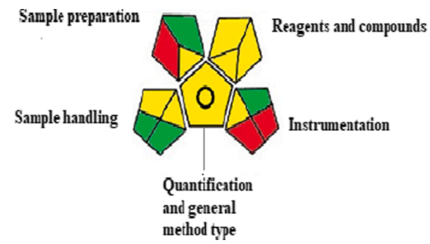
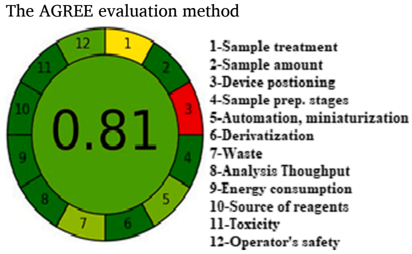
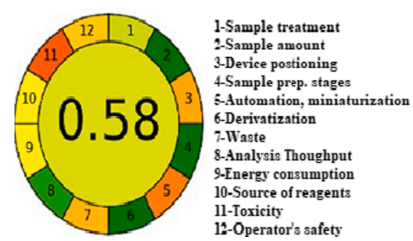
[a] Value corresponding to 9 determinations (3 concentrations repeated 3 times).
[b] Values corresponding to 9 determinations (3 concentrations repeated 3 times).

Table 3
Application of the proposed method for spectrophotometric determination of MLP in the pharmaceutical preparation with statistical assessment of the obtained results with results of the reported HPLC method.

Parameter	Proposed method	Reported HPLC method [41]
Mean[a]	99.68	100.29
S.D[a]	1.060	1.396
Variance[a]	1.1233	1.947
t-test	0.252 (2.306)[b]	
F-value	1.688 (6.388)[b]	

[a] Mean recovery, % of five measurements.
[b] The values in the parenthesis are the corresponding theoretical values of t and F at (p = 0.05).

Table 4
Greenness evaluation and comparison of the developed method and reported one using described metrics.

Proposed method	Reported HPLC method [41]
National environmental method index	
	
Green analytical procedure index	
	
The AGREE evaluation method	
	

3.7. Greenness assessment and comparison of the proposed method with the reported HPLC method

Although there are many principles presented for green practices evaluation of analytical procedures, the use of national environmental index analytical eco-scale, green analytical procedure index and AGREE together were recommended for full assessment of an analytical procedure providing synergistic results. The developed spectrophotometric method was evaluated and compared to the reported HPLC method using the mentioned tools as provided in Table 4. In general, national environmental index result of the spectrophotometric method reveled the greener adherence in comparison to the reported HPLC method. Regarding to this tool did not take in consideration the amount of solvent used nor other aspects of the procedure, the analytical eco-scale score was additionally calculated, to complement national environmental index, regarded the quantities of solvents consumed and provided more information about the environmental impact of the methods. As the obtained score of the applied method was 79, this revealed an excellent green analysis method with minimal negative effect on the environment and human health. The green analytical procedure index presented a detailed overview for different steps of the analysis procedure such as sample preparation, sample handling (collection, preservation, transport & storage), chemicals used, and instrumentation. Every factor of the analytical procedures was colored green through yellow to red identifying low, medium to high negative environmental impact, respectively. The proposed method possessed the highest number of green zones and lowest number of red zones (5 green zones and one red zone). While the reported HPLC method seemed to be lower in greenness (4 green and 3 red zones). The details obtained from green analytical procedure index and the easy detection of non-eco-friendly practices makes it superior to previously mentioned methods. However, the construction of the chart is time consuming and complex. Finally, AGREE tool was applied, providing the greenness profile as a numerical value, (0.81) for spectrofluorometric method and (0.58) for the reported HPLC method, confirming the greenness superiority of the applied method. AGREE method merges the advantages and addresses the cons of the aforementioned tools. It considers the quantities of reagents, simple to construct and highlights the weaknesses of a studied method. In summary, the results obtained from all the assessment tools provided a detailed greenness profile, complemented each other, and confirmed compliance with green practices for the most part [48,49].

4. Conclusion

In this work, integral computational calculations were done to choose the best coupling agent for the diazo-coupling of molnupiravir and provide greener analytical method. 8-hydroxyquinoline, provided the best coupling agent for diazotization of molnupiravir. The method was developed, optimized and applied for spectrophotometric determination of molnupiravir in the pure and in pharmaceutical preparation. Additionally, the greenness of the described method was evaluated on a scientific basis and the results revealed that the proposed method had minimum impact on the environment in comparison to previously reported HPLC method.

CRediT authorship contribution statement

Ahmed H. Abdelazim: Conceptualization, Project administration, Writing – original draft. **Mohammed A.S. Abourehab:** Methodology. **Lobna M. Abd Elhalim:** Software, Visualization. **Ahmed A. Almrasy:** Validation. **Sherif Ramzy:** Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J. Namieśnik, Trends in environmental analytics and monitoring, Crit. Rev. Anal. Chem. 30 (2000) 221–269.
[2] I. Pacheco-Fernández, V. Pino, Green solvents in analytical chemistry, Curr. Opin. Green Sust. Chem. 18 (2019) 42–50.
[3] M. Bustamante-Torres, D. Romero-Fierro, S. Hidalgo-Bonilla, E. Bucio, in: Green Sustainable Process for Chemical and Environmental Engineering and Science, Elsevier, 2021, pp. 219–237.
[4] K.A. Attia, N.M. El-Abasawi, A. El-Olemy, A.H. Abdelazim, M. El-Dosoky, Simultaneous determination of elbasvir and grazoprevir in their pharmaceutical preparation using high-performance liquid chromatographic method, J. Chromatogr. Sci. 56 (2018) 731–737.
[5] K.A. Attia, N.M. El-Abasawi, A. El-Olemy, A.H. Abdelazim, Application of an HPLC method for selective determination of phenazopyridine hydrochloride: theoretical and practical investigations, J. AOAC Int. 100 (2017) 1400–1406.
[6] A.H. Abdelazim, S. Ramzy, Spectrophotometric quantitative analysis of remdesivir using acid dye reagent selected by computational calculations, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 276 (2022) 121188.
[7] A.H. Abdelazim, S. Ramzy, Spectrophotometric quantitative analysis of lesinurad using extractive acid dye reaction based on greener selective computational approach, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 277 (2022) 121292.
[8] K.A. Attia, N.M. El-Abasawi, A.H. Abdel-Azim, Experimental design of membrane sensor for selective determination of phenazopyridine hydrochloride based on computational calculations, Mater. Sci. Eng., C 61 (2016) 773–781.
[9] J. Stevens, Virtually going green: The role of quantum computational chemistry in reducing pollution and toxicity in chemistry, Phys. Sci. Rev. 2 (2017).
[10] K.A. Attia, N.M. El-Abasawi, A. El-Olemy, A.H. Abdelazim, Validated stability indicating high performance liquid chromatographic determination of lesinurad, J. Chromatogr. Sci. 56 (2018) 358–366.
[11] A.H. Abdelazim, S. Ramzy, Application of different quantitative analytical techniques for estimation of aspirin and omeprazole in pharmaceutical preparation, BMC Chem. 16 (2022) 1–8.
[12] S. Ramzy, A.H. Abdelazim, M.A. Hasan, Application of green first derivative synchronous spectrofluorometric method for quantitative analysis of fexofenadine hydrochloride and pseudoephedrine hydrochloride in pharmaceutical preparation and spiked human plasma, BMC Chem. 16 (2022) 1–11.
[13] S. Ramzy, A.H. Abdelazim, A.OE. Osman, M.A. Hasan, Spectrofluorimetric quantitative analysis of favipiravir, remdesivir and hydroxychloroquine in spiked human plasma, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 281 (2022) 121625.
[14] A. El-Olemy, A.H. Abdelazim, S. Ramzy, M.A. Hasan, A.W. Madkour, A.A. Almrasy, M. Shahin, Application of different spectrofluorimetric approaches for quantitative determination of acetylsalicylic acid and omeprazole in recently approved pharmaceutical preparation and human plasma, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 262 (2021) 120116.
[15] K.A. Attia, A. El-Olemy, S. Ramzy, A.H. Abdelazim, M.A. Hasan, T.F. Mohamed, Z. A. Nasr, G.F. Mohamed, M. Shahin, Development and validation of a highly sensitive second derivative synchronous fluorescence spectroscopic method for the simultaneous determination of elbasvir and grazoprevir in pharmaceutical preparation and human plasma, New J. Chem. 44 (2020) 18679–18685.
[16] K.A. Attia, A. El-Olemy, S. Ramzy, A.H. Abdelazim, M.A. Hasan, R.F. Abdel-Kareem, Simultaneous determination of elbasvir and grazoprevir in their pharmaceutical formulation by synchronous fluorescence spectroscopy coupled to dual wavelength method, Spectrochim, Acta Part A: Mole. Biomole. Spectrosc. 248 (2021) 119157.
[17] K.A. Attia, A. El-Olemy, S. Ramzy, A.H. Abdelazim, M.A. Hasan, M.K. Omar, M. Shahin, Application of different spectrofluorimetric methods for determination of lesinurad and allopurinol in pharmaceutical preparation and human plasma, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 244 (2021) 118871.
[18] A.H. Abdelazim, S. Ramzy, Simultaneous spectrophotometric determination of finasteride and tadalafil in recently FDA approved Entadfi™ capsules, BMC Chem. 16 (2022) 1–8.
[19] A.H. Abdelazim, M.A. Abourehab, L.M. Abd Elhalim, A.A. Almrasy, S. Ramzy, Different spectrophotometric methods for simultaneous determination of lesinurad and allopurinol in the new FDA approved pharmaceutical preparation; additional greenness evaluation, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. (2022) 121868.
[20] S. Ramzy, A.H. Abdelazim, Application of different spectrophotometric methods for quantitative analysis of direct acting antiviral drugs simeprevir and sofosbuvir, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 272 (2022) 121012.
[21] A.H. Abdelazim, S. Ramzy, A.H. Abdel-Monem, A.A. Almrasy, A. Abdel-Fattah, M. Shahin, Quantitative spectrophotometric analysis of celecoxib and tramadol in their multimodal analgesia combination tablets, J. AOAC Int. (2022).
[22] (!!! INVALID CITATION !!! {.}).
[23] A.H. Abdelazim, M. Shahin, A.S. Abu-Khadra, Application of different chemometric assisted models for spectrophotometric quantitative analysis of velpatasvir and sofosbuvir, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 252 (2021) 119540.
[24] A.H. Abdelazim, M. Shahin, Different chemometric assisted approaches for spectrophotometric quantitative analysis of lesinurad and allopurinol, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 251 (2021) 119421.
[25] A.A. Mohamed, A. El-Olemy, S. Ramzy, A.H. Abdelazim, M.K. Omar, M. Shahin, Spectrophotometric determination of lesinurad and allopurinol in recently approved FDA pharmaceutical preparation, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 247 (2021) 119106.
[26] A.M. Zeid, A.H. Abdelazim, M. Shahin, Simultaneous spectrophotometric quantitative analysis of elbasvir and grazoprevir using assisted chemometric models, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc 252 (2021) 119505.
[27] K.A. Attia, N.M. El-Abasawi, A. El-Olemy, A.H. Abdelazim, A.I. Goda, M. Shahin, A. M. Zeid, Simultaneous spectrophotometric quantitative analysis of velpatasvir and sofosbuvir in recently approved FDA pharmaceutical preparation using artificial neural networks and genetic algorithm artificial neural networks, Spectrochim. Acta Part A: Mole. Biomole. Spectrosc. 251 (2021) 119465.
[28] K.A.M. Attia, N.M. El-Abasawi, A.H. Abdelazim, Colorimetric estimation of alfuzosin hydrochloride in pharmaceutical preparation based on computational studies, Anal. Methods 8 (8) (2016) 1798–1805.
[29] T.D. Nguyen, H.B. Le, T.O. Dong, T.D. Pham, Determination of fluoroquinolones in pharmaceutical formulations by extractive spectrophotometric methods using ion-pair complex formation with bromothymol blue, J. Anal. Meth. Chem. 2018 (2018) 1–11.
[30] C.S.R. Lakshmi, M.N. Reddy, Spectrophotometric determination of azathioprine in pharmaceutical formulations, Talanta 47 (5) (1998) 1279–1286.
[31] N.V. Sreekumar, B. Narayana, P. Hegde, B.R. Manjunatha, B.K. Sarojini, Determination of nitrite by simple diazotization method, Microchem. J. 74 (1) (2003) 27–32.
[32] J. Shah, M.R. Jan, M.A. Khan, Determination of furosemide by simple diazotization method in pharmaceutical preparations, J. Chin. Chem. Soc. 52 (2) (2005) 347–352.
[33] K. Satyanary, P. Nagesara, Simple and selective s spectrophotometric methods for the determination of mosapride citrate by diazo coupling reaction in pharmaceutical formulations, Int. J. Pharm. Pharmaceut. Sci. 4 (2012) 363–368.
[34] S.M.T. Shaikh, D.H. Manjunatha, K. Harikrishna, K.C. Ramesh, R.S. Kumar, J. Seetharamappa, Diazocoupling reaction for the spectrophotometric determination of physiologically active catecholamines in bulk and pharmaceutical preparations, J. Anal. Chem. 63 (7) (2008) 637–642.
[35] W. Fischer, J.J. Eron, W. Holman, M.S. Cohen, L. Fang, L.J. Szewczyk, T. P. Sheahan, R. Baric, K.R. Mollan, C.R. Wolfe, Molnupiravir, an oral antiviral treatment for COVID-19, MedRxiv (2021).
[36] A.K. Singh, A. Singh, R. Singh, A. Misra, Molnupiravir in COVID-19: a systematic review of literature, Diabet. Metab. Syndr.: Clin. Res. Rev. 15 (2021) 102329.
[37] F. Kabinger, C. Stiller, J. Schmitzová, C. Dienemann, G. Kokic, H.S. Hillen, C. Höbartner, P. Cramer, Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis, Nat. Struct. Mol. Biol. 28 (2021) 740–746.
[38] A. Jayk Bernal, M.M. Gomes da Silva, D.B. Musungaie, E. Kovalchuk, A. Gonzalez, V. Delos Reyes, A. Martín-Quirós, Y. Caraco, A. Williams-Diaz, M.L. Brown, Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients, N. Engl. J. Med. 386 (2022) 509–520.
[39] A.S. Gouda, H.M. Marzouk, M.R. Rezk, A.M. Salem, M.I. Morsi, E.G. Nouman, Y. M. Abdallah, A.Y. Hassan, A.M. Abdel-Megied, A validated LC-MS/MS method for determination of antiviral prodrug molnupiravir in human plasma and its application for a pharmacokinetic modeling study in healthy Egyptian volunteers, J. Chromatogr. B 1206 (2022) 123363.
[40] A. Amara, S.D. Penchala, L. Else, C. Hale, R. FitzGerald, L. Walker, R. Lyons, T. Fletcher, S. Khoo, 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. 206 (2021) 114356.
[41] T. Reçber, S.S. Timur, S.E. Kablan, F. Yalçın, T.C. Karabulut, R.N. Gürsoy, H. Eroğlu, S. Kır, E. Nemutlu, A stability indicating RP-HPLC method for determination of the COVID-19 drug molnupiravir applied using nanoformulations in permeability studies, J. Pharmaceut. Biomed. Anal. 214 (2022) 114693.
[42] M. Tobiszewski, Metrics for green analytical chemistry, Anal. Methods 8 (15) (2016) 2993–2999.
[43] L.H. Keith, L.U. Gron, J.L. Young, Green analytical methodologies, Chem. Rev. 107 (6) (2007) 2695–2708.
[44] A. Gałuszka, Z.M. Migaszewski, P. Konieczka, J. Namieśnik, Analytical eco-scale for assessing the greenness of analytical procedures, TrAC Trends Anal. Chem. 37 (2012) 61–72.
[45] J. Płotka-Wasylka, A new tool for the evaluation of the analytical procedure: green analytical procedure index, Talanta 181 (2018) 204–209.

[46] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE—analytical GREEnness metric approach and software, Anal. Chem. 92 (2020) 10076–10082.
[47] E.K. Gross, R.M. Dreizler, Density Functional Theory, Springer Science & Business Media, 2013.
[48] J.S. Renny, L.L. Tomasevich, E.H. Tallmadge, D.B. Collum, Method of continuous variations: applications of job plots to the study of molecular associations in organometallic chemistry, Angew. Chem. Int. Ed. 52 (2013) 11998–12013.
[49] D. Prat, J. Hayler, A. Wells, A survey of solvent selection guides, Green Chem. 16 (2014) 4546–4551.