COMPARATIVE STUDY AMONG THREE MANIPULATING RATIO SPECTRA METHODS FOR THE DETERMINATION OF MOLNUPIRAVIR IN PRESENCE OF ITS ACID-INDUCED DEGRADATION PRODUCT

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Abstract:

The main purpose of this work is to establish three simple, sensitive, accurate and precise spectrophotometric methods manipulating ratio spectra for determination of molnupiravir in bulk powder and in pharmaceutical preparation in the presence of its acid-induced degradation product. Ratio derivative, ratio difference and mean centering of ratio spectra are simple, rapid and selective methods using manipulation of ratio spectra for the determination of components having overlapping spectra. The suggested methods were validated in compliance with the ICH guidelines and were successfully applied for determination of molnupiravir in bulk powder and in pharmaceutical preparation in the presence of its acid-induced degradation product.

Keywords: UV–VIS; Spectrophotometry; Ratio derivative; Ratio difference; Mean centering; Acid-induced degradation product; Molnuprivir.

Introduction:

Molnuprivir **Figure (1)** N-Hydroxy-5'-O-isobutyryl-3,4-dihydrocytidine [(2*R*,3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-[4-(hydroxyamino)-2-oxopyrimidin-1-yl]oxolan-2-yl]methyl 2-methylpropanoate is a prodrug of the synthetic nucleoside derivative N^4-hydroxy cytidine and exerts its antiviral action by introducing copying errors during viral RNA replication, Molnupiravir is an oral broad-spectrum antiviral agent originally designed for the treatment of Alphavirus infections and now has been used for the treatment of COVID-19 disease caused by the nasopharyngeal SARS-CoV-2 infectious virus.

An emergency use authorization has been issued on December 2021 for MLP by the U. S. Food and Drug Administration (FDA) for the treatment of mild-to-moderate COVID-19 in adults with positive results of direct SARS-CoV-2 viral testing and who are at high risk for progression to severe COVID-19 and European Medicines Agency (EMA) has issued advice on the use of MLP in adults with the increased risk of developing severe COVID-19 on November 2021. It shows its antiviral effect by adding transcription errors in viral RNA replication.

If ongoing clinical trials result as expected, MLP is considered to become an important tool to counter the effects of the COVID-19 pandemic. However, the literature review revealed that few studies have estimated Molnuprivir simultaneously by UV visible Spectrophotometery (Abdelnabi, Foo et al. 2021, Amara, Penchala et al. 2021, Gouda, Marzouk et al. 2022, Reçber, Timur et al. 2022, Saraya, Deeb et al. 2022, Abdelazim, Abourehab et al. 2023, Komarov, Karnakova et al. 2023) HPLC(Annadi, El Zahar et al. 2022, Reçber, Timur et al. 2022, Afify, Ali et al. 2023, Suresh, Mamatha et al. 2023) TLC(Tekade and Patil 2022, Abdel Moneim, Kamal et al. 2023) **In the present study,** three simple and sensitive spectrophotometric methods manipulating ratio spectra were suggested for the selective quantitative determination of molnupiravir in presence of its acid-induced degradation product without previous separation.

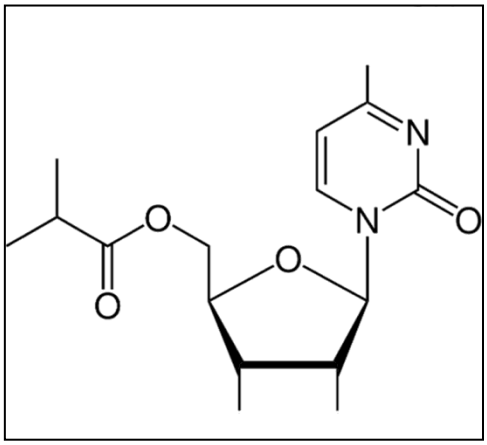


Figure (1) Chemical structure of Molnupiravir.

2**. Experimental:**

2.1. Apparatus:

- Shimadzu UV-Visible 1650 Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells. The bundled software, UV-Probe personal spectroscopy software version 2.21 (Shimadzu).

Analytical balance (Precisa125A, Switzerland).
Matlab Software version matlab r2009a
2.2. Materials:

2.2.1. Pure sample:

Pure molnupiravir (99.70%), the purity was checked by applying reported method[21], was kindly supplied by EVA Pharma Pharmaceutical Company, Tenth of Ramadan city, Egypt.

2.2.2. Pharmaceutical preparation:

Molnupiravir-EVA Pharma® labeled to contain 200 mg molnupiravir per capsule (batch number: 2202398A), manufactured by EVA Pharma Company, Tenth of Ramadan city, Egypt, purchased from local market.

2.3. Chemicals and reagents:

Methanol, analytical grade (Sigma–Aldrich, Germany).

2.4. Standard solutions:

2.4.1. Standard solution of intact molnupiravir:

Molnupiravir standard solutions (100 µg/mL), were prepared in 100-mL volumetric flasks by dissolving 10 mg of the drug powder in 50 mL of methanol and then completing to volume with the same solvent.

2.4.2. Standard solution of molnupiravir degradation product:

In a 100-mL round bottomed flask, 100 mg of pure molnupiravir powder was treated with 25 ml of 5 M HCl and the solution was heated under reflux for 5 hours. After cooling, the solution was neutralized to pH 7 - 8 using 5 M NaOH and evaporated to dryness under vacuum. The obtained residue was extracted three times with 25 ml of methanol, filtered into 100-mL volumetric flask and diluted to volume with methanol to obtain a stock solution labeled to contain degradation product with concentration 1 mg/mL of molnupiravir. Working solution of degradation product (10 µg/mL) was obtained by dilution of the stock solution with methanol.

2.5**. Procedures:**

2.5.1. General procedures:

Different aliquots of molnupiravir standard solution (100 µg/mL) ranging from (40–240) µg were transferred to 10-mL volumetric flasks and completed to volume with methanol. The absorption spectra (from 200 to ٤00 nm) of these solutions were recorded using methanol as a blank, and then divided by the spectrum of molnupiravir degradation product solution (30 µg/mL).

- Ratio derivative method: The first derivative corresponding to each ratio spectrum was recorded, using $\Delta\lambda$ = 4 nm and scaling factor = 10. The amplitude values were measured at 248 nm.
- Ratio difference method: The difference in the peak amplitudes (ΔP) at the ratio spectra was measured at 240 and 290 nm ($\Delta P_{240\text{-}290\ nm}$).
- Mean centering method: The ratio spectra (from 200 to ٤00 nm) were mean centered and the mean centered values were measured at 240 nm.

2.5.2. Optimization of experimental conditions:

To choose the best divisor concentration, different concentrations of degradation product were tried (5, 10, 15, 20, 25 and 30 µg/mL) using the general procedure under "2.5.1" for each method.

2.5.3. Validation of the procedures:

The methods were tested for linearity, range, limits of detection and quantitaion, acuracy, precision and specificity as per the ICH guidelines[27].

2.5.4. Procedure for pharmaceutical preparation:

i. Preparation of pharmaceutical sample:

Contents of 10 **Molnupiravir-EVA Pharma®** 200 mg capsules were weighted and finely powdered. Appropriate weight of powder equivalent to 10 mg of molnupiravir was accurately weighted, transferred to 100-mL volumetric flask and the volume was made up to 75 mL with methanol. The solution was shaken vigorously for 15 min then sonicated for 30 min and then filtered. The volume was completed to 100 mL with methanol to obtain a concentration of 100 µg/mL.

ii. Analysis of pharmaceutical sample:

The general procedure under "2.5.1" for each method was repeated using aliquots covering the working concentration range. The content of the capsules was determined from the corresponding regression equations.

3. **Results & discussions**

3.1. Theory of ratio derivative method:

Upon dividing the absorption spectrum of a compound by a spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. However, upon dividing the absorption spectrum of a compound (***X***) by the absorption spectrum of another compound (***Y***), a new spectrum (ratio spectrum) will result. The amplitude of the first or second derivative of the ratio spectrum at a maximum or a minimum is proportional to concentration of ***X*** without interference from ***Y*** [7].

3.2. Theory of ratio difference method:

Upon dividing the absorption spectrum of a compound by a spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. However, upon dividing the absorption spectrum of a compound (***X***) by the absorption spectrum of another compound (***Y***), a new spectrum (ratio spectrum) will result. The difference in peak amplitudes between two selected wavelengths in the ratio spectrum is proportional to concentration of ***X*** without interference from ***Y***.

Mathematically it can be explained as follows:

- In the ratio spectrum of a laboratory prepared mixture of ***X*** and ***Y*** divided by a standard spectrum of ***Y*** as a divisor (***Y′***)

$$P_1 = A_{1X}/A_{1Y'} + A_{1Y}/A_{1Y'} \quad (1)$$

$$P_2 = A_{2X}/A_{2Y'} + A_{2Y}/A_{2Y'} \quad (2)$$

Where P_1 and P_2 are the amplitudes of the mixture in the ratio spectrum at λ_1 and λ_2, respectively. A_{1X}, A_{1Y} and $A_{1Y'}$ are the absorbances of ***X***, ***Y*** and divisor (***Y′***), respectively at λ_1. A_{2X}, A_{2Y} and $A_{2Y'}$ are the absorbances of ***X***, ***Y*** and divisor (***Y′***), respectively at λ_2.

- Since $A_{1Y}/A_{1Y'}$ equal $A_{2Y}/A_{2Y'}$ (straight line of constant amplitude), therefore:

$$\Delta P_{1-2} = P_1 - P_2 = (A_{1X}/A_{1Y'}) - (A_{2X}/A_{2Y'}) \quad (3)$$

- As we use a certain concentration of ***Y*** as a divisor (***Y′***), so $A_{1Y'}$ and $A_{2Y'}$ have certain constant values and the component ***Y*** will be completely cancelled and the difference will represent the ***X*** component only.

Component ***X*** in a binary mixture can be determined from a calibration curve that relates the difference in amplitudes (ΔP_{1-2}) in the ratio spectrum at λ_1 and λ_2 using a certain concentration of ***Y*** as a divisor to the corresponding concentration of ***X***. Similarly component ***Y*** can be obtained by using certain concentration of ***X*** as a divisor[16, 17].

3.3. Theory of mean centering method:

Upon dividing the absorption spectrum of a compound by a spectrum of the same compound, a straight line of constant amplitude (parallel to the baseline) will result. However, upon dividing the absorption spectrum of a compound (***X***) by the absorption spectrum of another compound (***Y***), a new spectrum (ratio spectrum) will result.

Mathematically it can be explained as follows:

- If we have a column of a set of data (***X***) where:

$$X = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix}$$

- We center or mean center (MC) column ***X*** by subtracting the mean of the three numbers ($\bar{X}$**):**

$$\mathbf{MC}\ (X) = X - \bar{X} = \begin{bmatrix} 5 \\ 1 \\ 3 \end{bmatrix} - \begin{bmatrix} 3 \\ 3 \\ 3 \end{bmatrix} = \begin{bmatrix} +2 \\ -2 \\ 0 \end{bmatrix}$$

- If the spectrum of a mixture of two compounds *X* and *Y* is divided by a standard spectrum of ***Y*** as a divisor (***Y'***), a ratio spectrum will result and therefore:

$$P = A_X/A_{Y'} + A_Y/A_{Y'} \qquad \textbf{(1)}$$

Where, ***P*** is the amplitude of the mixture in the ratio spectrum. A_X, A_Y and $A_{Y'}$ are the absorbance values of ***X***, ***Y*** and divisor (***Y'***), respectively.

- Mean centering of equation (1) will lead to:

$$\text{MC}\ (P) = \text{MC}\ (A_X/A_{Y'}) + \text{MC}\ (A_Y/A_{Y'}) \qquad \textbf{(2)}$$

- Since $A_Y/A_{Y'}$ is a straight line of constant amplitude, so its mean centering is equal to zero and therefore:

$$\text{MC}\ (P) = \text{MC}\ (A_X/A_{Y'}) \qquad \textbf{(3)}$$

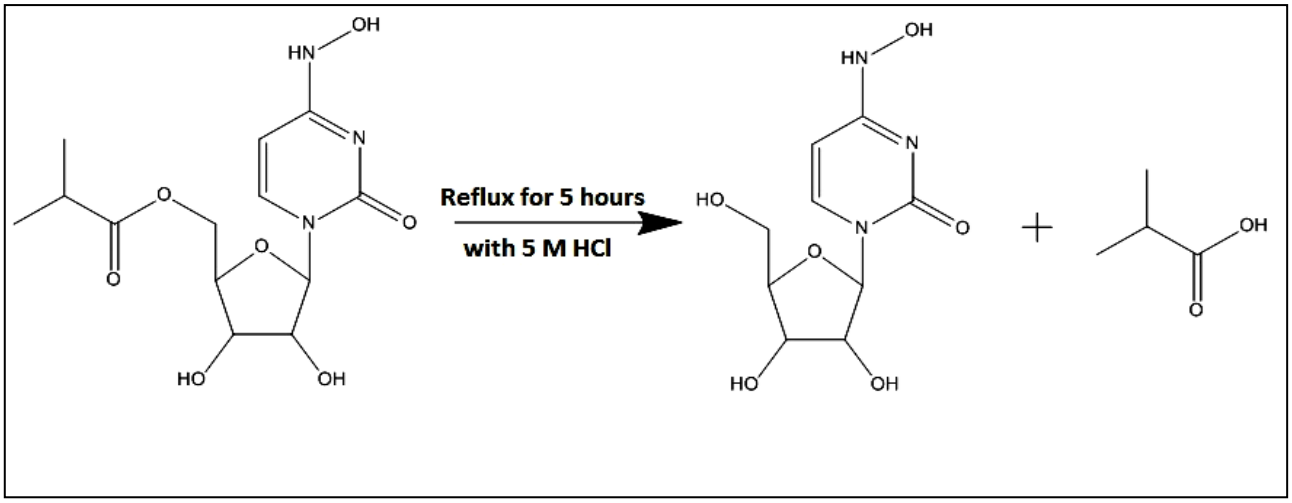
- As we use a certain known concentration of ***Y*** as a divisor (***Y'***), so component ***Y*** will be completely cancelled and the mean centering value will represent the ***X*** component only[26].

Component ***X*** in a binary mixture (***X*** and ***Y***) can be determined from a calibration curve that relates the mean centering values of the ratio spectrum using a certain concentration of ***Y*** as a divisor to the corresponding concentration of ***X***. For

more sensitivity, the mean centering values should be measured at a maximum or minimum wavelength.

Degradation of molnupiravir :

Complete degradation of molnupiravir was achieved upon heating under reflux with 5 M hydrochloric acid for 5 hours, as shown in **scheme (1)**.



Scheme (1): Suggested degradation pathway of molnupiravir.

- **Confirmation of degradation product:**
- **Confirmation of complete degradation using TLC technique:**

Time required for complete degradation was exactly determined by spotting on TLC plates every 30 minutes using mobile phase system consists of 0.3 M ammonium chloride solution : conc. ammonia solution : 1-propanol (1:1:8, by volume). Complete degradation of molnupiravir was confirmed by absence of spot in the region of the degradation product corresponds to the spot of the intact drug, where spots appear at R_f 0.47 for intact drug and 0.83 for its degredation product.

- **Confirmation of degradation product using IR spectroscopy:**

IR spectrum of the intact molnupiravir, **figure (2)**, showed peak of (C=O) of ester group (R-COOR) at 1731.98 cm^{-1}, while IR spectrum of degradation product, **figure (3)**, showed disappearance of (C=O) stretch of the ester group which indicate the cleavage of ester linkage.

- **Confirmation of degradation product using ^{1}H NMR spectroscopy:**

The ^{1}H NMR of the intact molnupiravir in dimethyl sulfoxide (DMSO), **figure (4)**, showed triplet signal of six protons of two aliphatic methyl groups (-CH_3) at 1.037 ppm, singlet signal of one proton of (-CH-) group at 2.589 ppm, singlet signal of one proton of (-NH) group at 3.873 ppm, multiplet signals of four protons in tetrahydrofuran ring at 4.088 – 4.208 ppm, two singlet signals of two protons of methylene group (-CH_2)

at 5.189 and 5.332 ppm, two multiplet signals of two protons of the two hydroxyl groups attached to the tetrahydrofuran ring at 5.549 and 5.698 ppm, doublet signal of one proton in pyrimidine ring in para position at 6.793 – 6.814 ppm, doublet signal of one proton in pyrimidine ring in meta position at 9.375 – 9.505 ppm and singlet signal of one proton of hydroxyl group adjacent to amino group at 9.985 ppm.

The ^{1}H NMR of the degradation product in dimethyl sulfoxide (DMSO), **figure(5)**, showed disappearance of triplet signal of six protons of two aliphatic methyl groups (-CH_3) at 1.037 ppm and singlet signal of one proton of (-CH-) group at 2.589 ppm indicating the cleavage of the ester linkage.

- **Confirmation of degradation product using mass spectrometry:**

Mass spectrometry was performed for the intact drug and its degradation product and molecular ion peak was obtained at m/z = 329.12 and m/z = 259.08, respectively indicating that the molecular weight of the degradation product is 259.08 as shown in **figures (6,7)**.

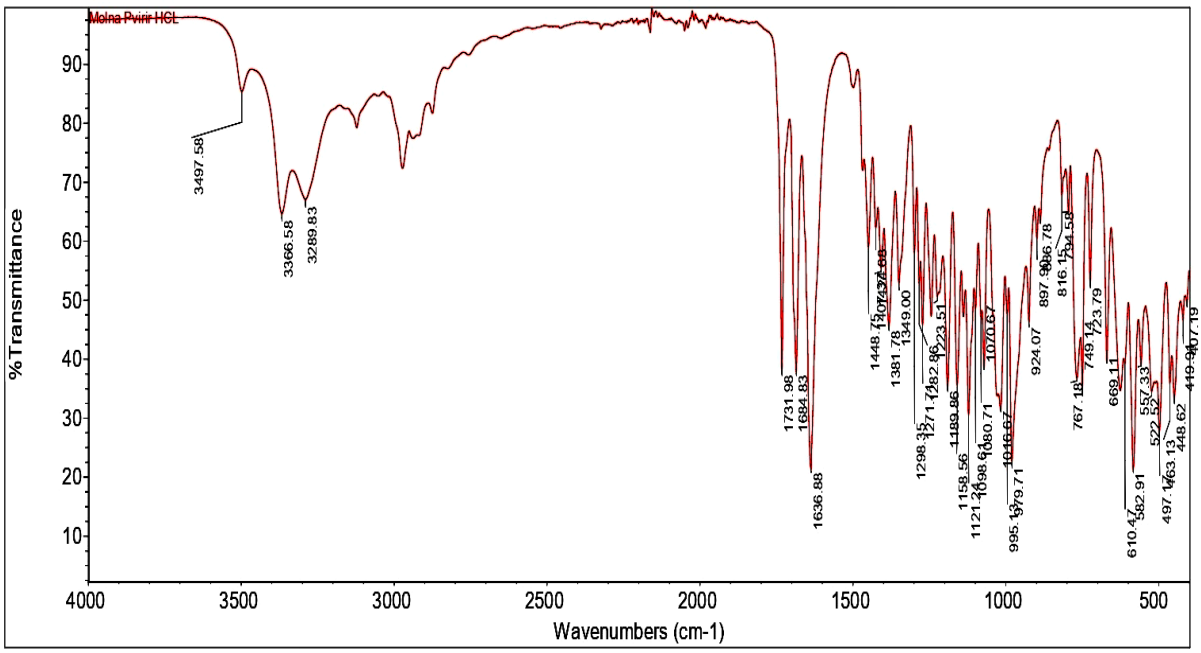


Figure (2): IR spectrum of intact molnupiravir.

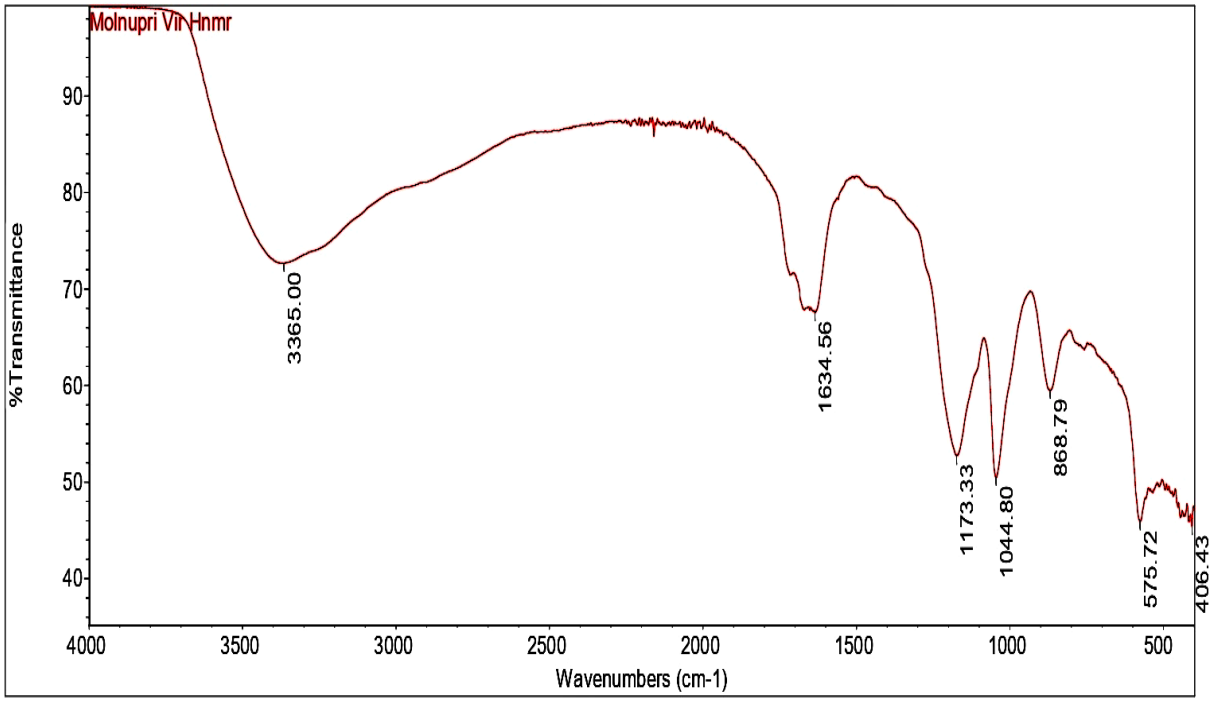


Figure (3): IR spectrum of molnupiravir degradation product.

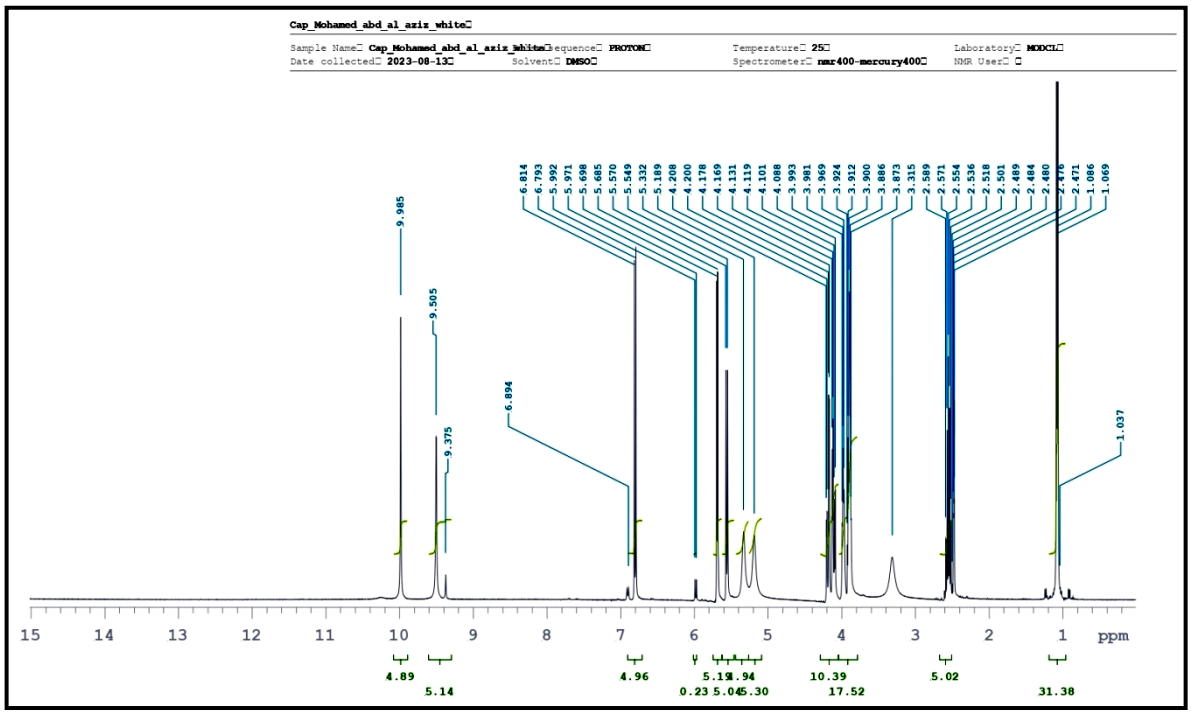


Figure (4): ^{1}H NMR spectrum of intact molnupiravir in (DMSO).

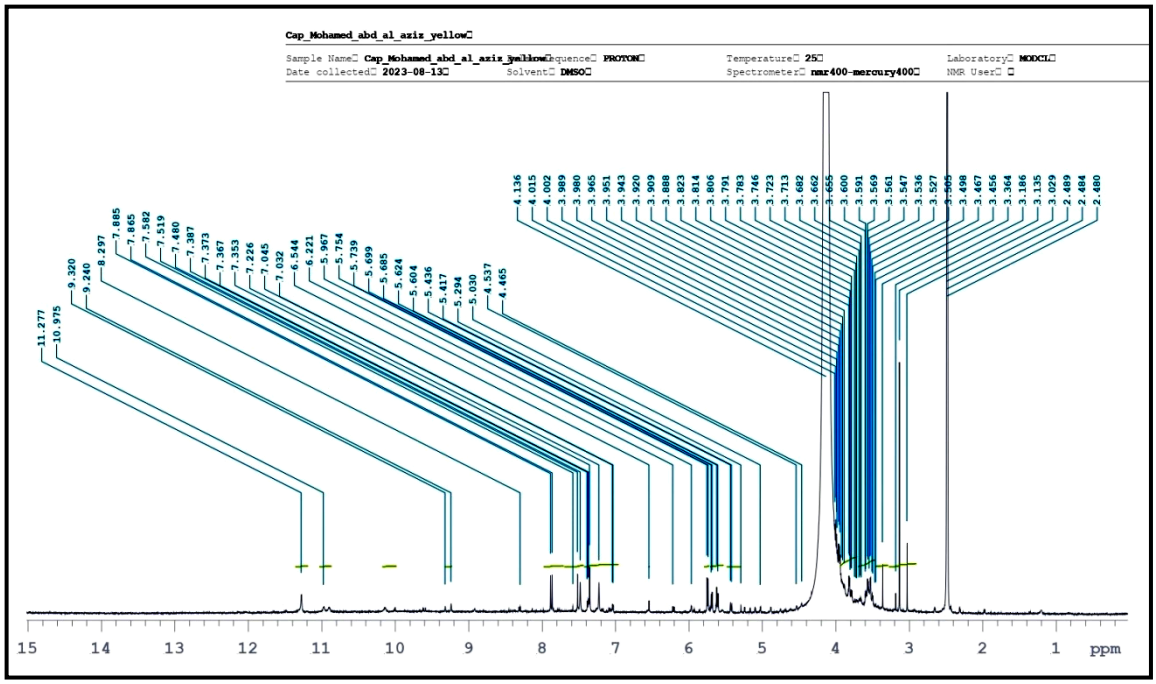


Figure (5): ^{1}H NMR spectrum of molnupiravir degradation product in (DMSO).

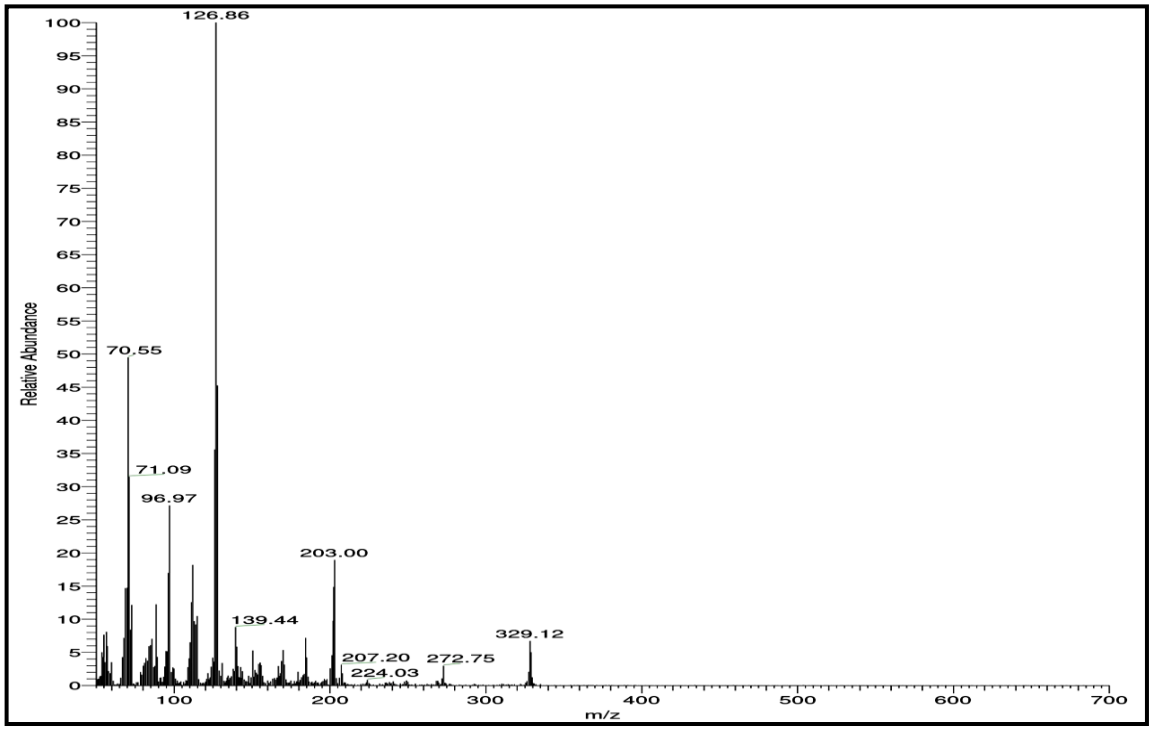


Figure (6): Mass spectrum of intact molnupiravir.

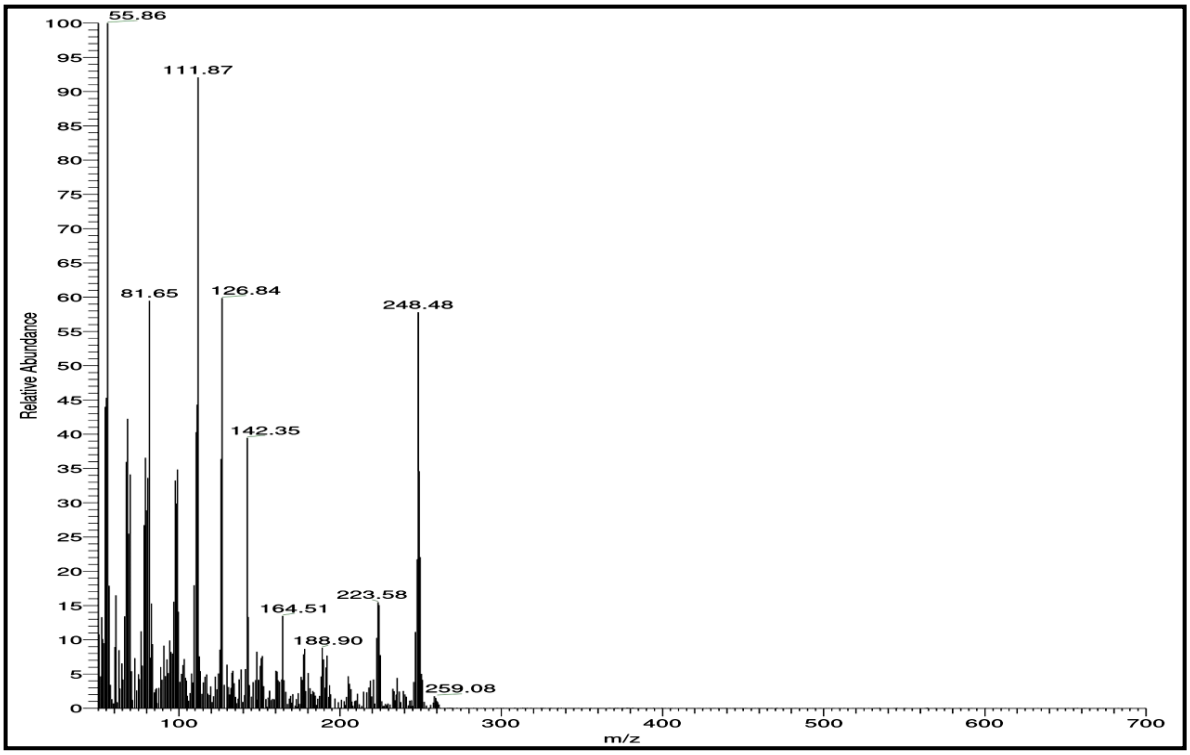


Figure (7): Mass spectrum of molnupiravir degradation product.

➢ Spectral characteristics:

The zero-order absorption spectra of molnupiravir and its degradation product, as shown in **figure (8)**, show severe overlap, which does not permit direct determination of molnupiravir in presence of its degradation product. The proposed methods are based on dividing the absorption spectra of the intact drug by the absorption spectrum of its degradation product (30 µg/mL), as a divisor, to get the ratio spectra, as shown in **figure (9)**.

- Ratio derivative method: The amplitudes of the first derivative of the ratio spectra at 248 nm are proportional to the concentrations of the drug without interference from its degradation product (divisor), as shown in **figures (10, 11)**.
- Ratio difference method: The difference in peak amplitudes between 240 and 290 nm in the ratio spectra is proportional to the concentration of the drug without interference from its degradation product (divisor).
- Mean centering method: The obtained ratio spectra were mean centered. The mean centered values at 240 nm are proportional to the concentrations of the drug without interference from its degradation product, as shown in **figure (12)**.

➢ Optimization of experimental conditions:

Careful choice of the divisor concentration was of great importance, so different concentrations of degradation product were tried as a divisor (5, 10, 15, 20, 25 and 30 µg/mL); the best one was 30 µg/mL, as it produced minimum noise and gave better results in accordance with selectivity.

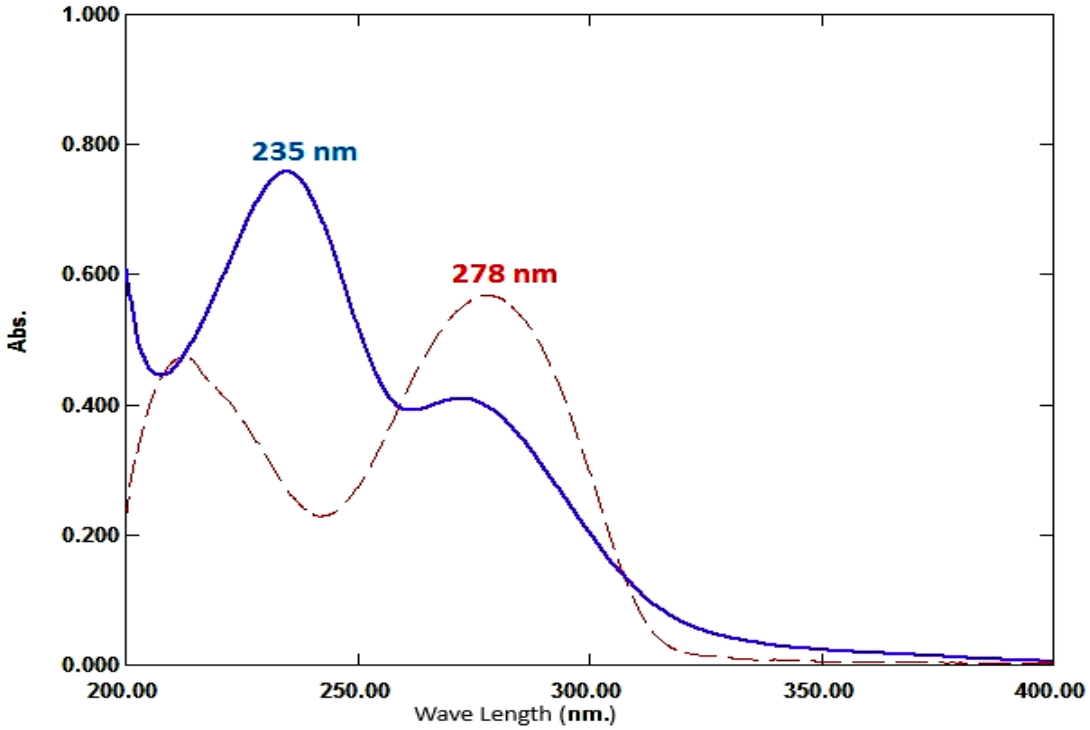


Figure (8): Zero order absorption spectra of intact molnupiravir, 16 µg/mL (– –) and its acid-induced degradation product, 15 µg/mL (—) in methanol.

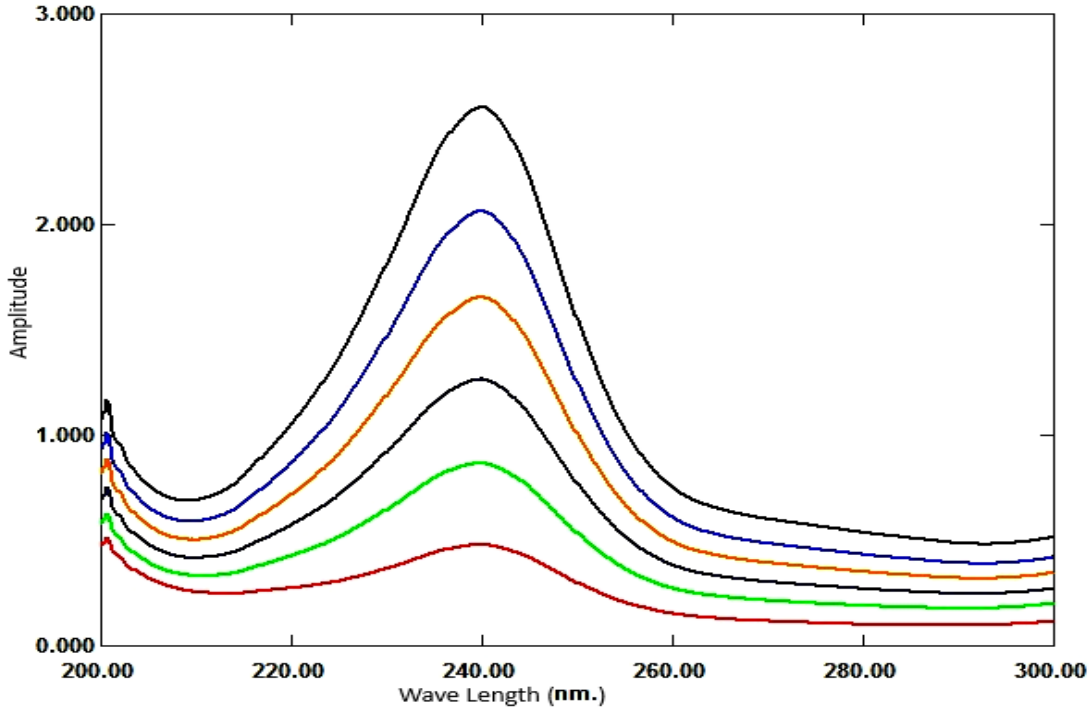


Figure (9): Ratio spectra of molnupiravir at various concentrations (4-24 µg/mL) using 30 µg/mL of its degradation product as a divisor.

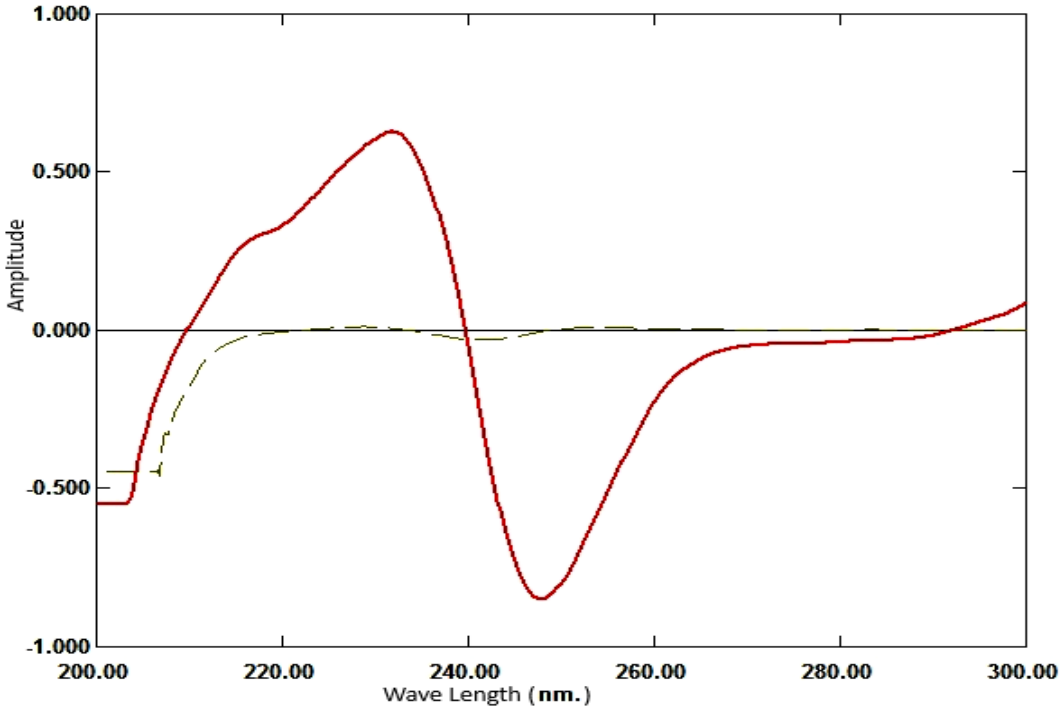


Figure (10): First derivative of the ratio spectra of molnupiravir, 16 µg/mL (—) and its degradation product, 25 µg/mL (— –) using 30 µg/mL of its degradation product as a divisor.

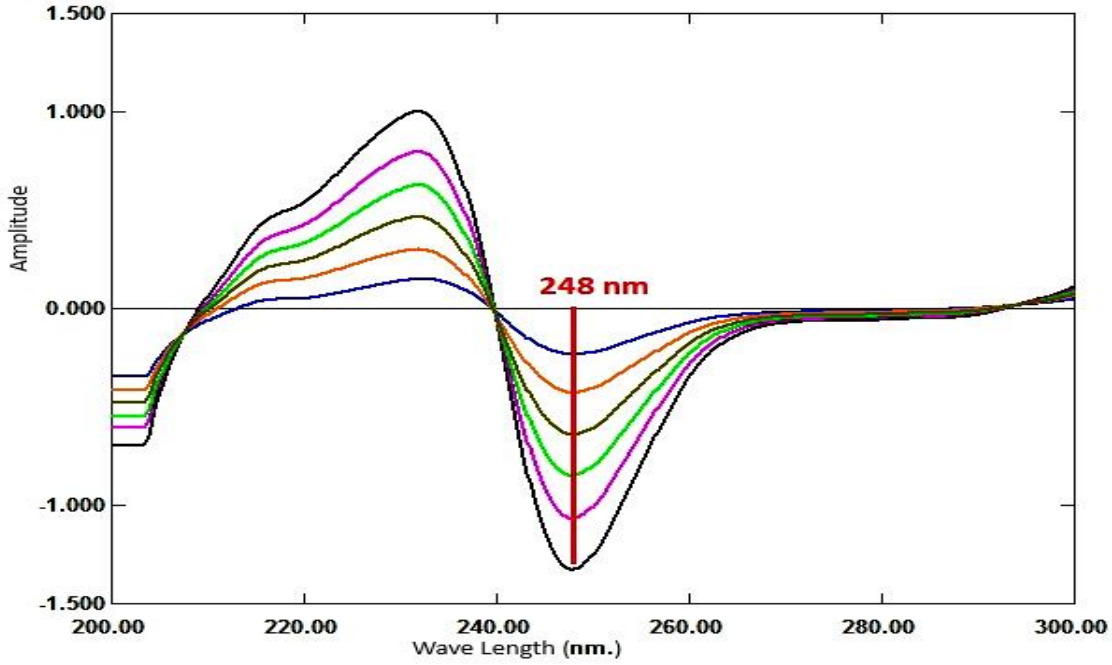


Figure (11): First derivative of the ratio spectra of molnupiravir at various concentrations (4-24 µg/mL) using 30 µg/mL of its degradation product as a divisor at 248 nm

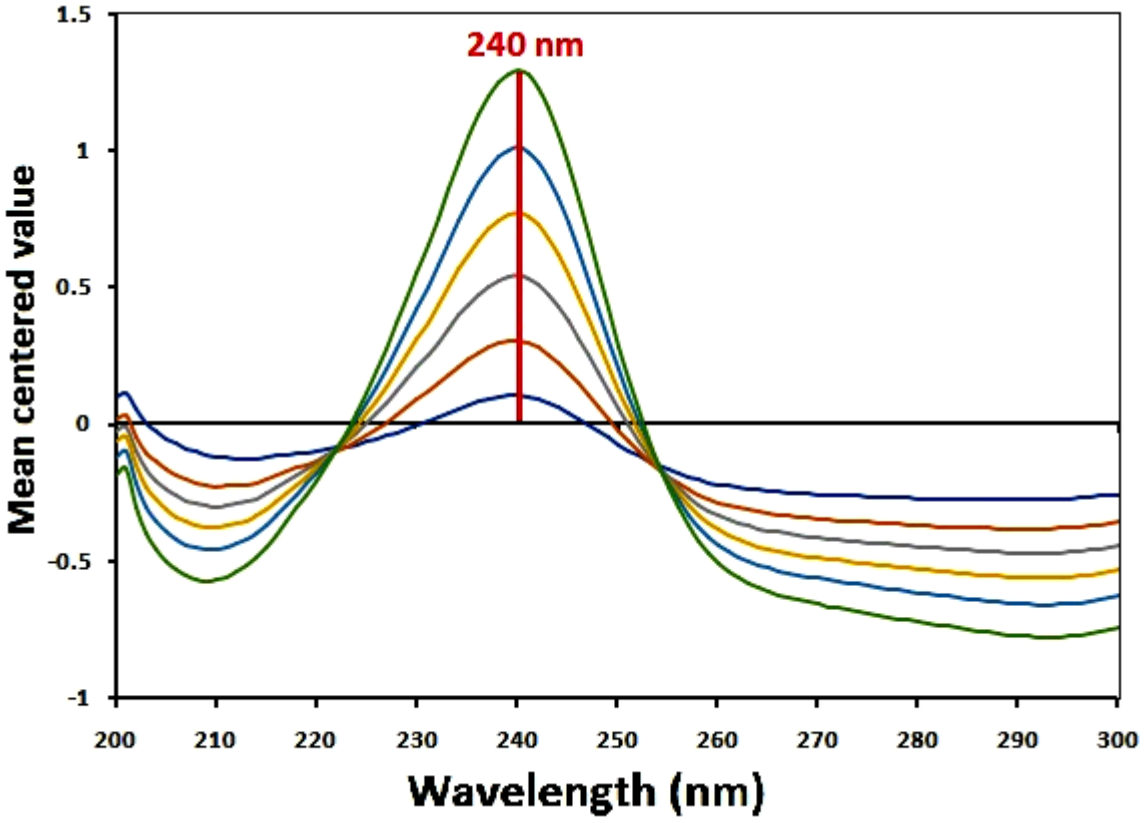


Figure (12): Mean centering of the ratio spectra of molnupiravir at various concentrations (4-24 µg/mL) using 30 µg/mL of its degradation product as a divisor at 240 nm

➢ Method validation:

- Linearity and range:

Under the described experimental conditions, the calibration graphs for the three methods were constructed by plotting the response of each method versus the drug concentrations in µg/mL. For ratio derivative method the response is the amplitudes of the first derivative of the ratio spectra at 248 nm. For ratio difference method the response is the difference in the peak amplitudes between the two selected wavelengths (240 and 290 nm) in the ratio spectra. For mean centering method the response is the mean centered values of the ratio spectra at 240 nm. The regression plots were found to be linear over the range of 4-24 µg/mL, as shown in **figures (13-15)**. The regression data were presented in **table (1)**. The values of coefficient of determination indicated the good linearity of the calibration graphs.

- Limits of detection and quantitation:

LODs and LOQs values were calculated and the obtained results indicated the sensitivity of the proposed methods for analysis of the studied drug as shown in **table (1)**.

- Accuracy and precision:

Accuracy of the methods, calculated as the mean percent recovery (%R), was assessed by applying the described procedure for triplicate determination of three concentration levels covering the linearity range of the drug (8, 12 and 16 µg mL). The results in **table (1)** indicated the accuracy of the proposed methods.

Precision of the methods, calculated as the percent relative standard deviation (%RSD), was assessed by triplicate determination of three concentration levels covering the linearity range of the drug (8, 12 and 16 µg mL) within one day for repeatability and on three successive days for inter mediate precision. The small values of %RSD indicated high precision of the method as shown in **table (1)**.

- Specificity:

Synthetic mixtures containing different ratios of the intact drug together with its degradation product were prepared and mixed well. Then, the mixtures were analyzed using the previously described procedure of each method. Good satisfactory results were obtained and listed in **table (2).**

Specificity was also determined by applying the standard addition technique where the general procedure of each method was applied using aliquots of standard molnupiravir solution (100 µg/mL) containing (40, 80, 100 and 120 µg) with aliquot of already analyzed **Molnupiravir-EVA Pharma®** 200 mg capsule solution (100 µg/mL) containing (100 µg), then the percent recovery **(%R)** of pure added concentrations were calculated, as shown in **table (3)** which indicates no matrix interference.

Pharmaceutical applications:

The proposed methods were applied to the determination of molnupiravir in **Molnupiravir-EVA Pharma®** 200 mg capsule. Satisfactory results were obtained in good agreement with the label claim, indicating no interference from excipients and additives which was confirmed by the results of standard addition technique **table (3)**. The obtained results were statistically compared to those obtained by the reported method[6]. No significant differences were found by applying student's *t*-test and *F*-test at 95 % confidence level[28], indicating good accuracy and precision of the proposed methods for the analysis of the studied drug in its pharmaceutical dosage form, as shown in **table (4)**.

Table (1): Regression and validation data for the determination of molnupiravir by the proposed methods:

Parameters	**Ratio derivative method**	**Ratio difference method**	**Mean centering method**
Wavelength (nm)	248	240 and 290	240
Linearity range (µg/mL)	4-24	4-24	4-24
LOD (µg/mL)	1.259	1.211	1.197
LOQ (µg/mL)	3.815	3.670	3.628
Regression Equation	$y^{a} = b\,x^{b} + a$	$y^{a} = b\,x^{b} + a$	$y^{a} = b\,x^{b} + a$
- Slope (*b*)	0.0506	0.0813	0.0543
- Intercept (*a*)	0.0315	0.0453	-0.0955
Coefficient of determination (r^2)	0.9997	0.9997	0.9997
Accuracy (% R)[c]	101.15	100.26	98.62
Precision[d] (% RSD)			
- Repeatability	0.687	1.124	0.697
- Intermediate precision	1.514	0.967	1.320

[a] The specific responce of each method.

[b] Concentration of of molnupiravir in µg/mL.

[c] Average of nine determinations (three concentrations repeated three times).

[d] %RSD of nine determinations (three concentrations repeated three times).

Table (2): Determination of molnupiravir in mixtures with its degradation product by the proposed methods:

Method	Intact (µg/mL)	Degradation product (µg/mL)	Degradation product (%)	Intact found (µg/mL)	%Recovery of intact
Ratio derivative	20	4	16.67	١٩.٩	99.5
	16	8	33.33	١٥.٧٢	98.25
	12	12	50	12.09	100.75
	8	16	66.67	٨.٠٩٥	101.19
	4	20	83.33	٤.٠٧٨	101.95
	Mean ± %RSD				100.33±1.457
Ratio difference	20	4	16.67	١٩.٩٥	99.75
	16	8	33.33	١٥.٧٨	98.63
	12	12	50	11.94	99.50
	8	16	66.67	٨.٠٦٤	100.81
	4	20	83.33	٤.٠٥٨	101.45
	Mean ± %RSD				100.03±1.114
Mean centering	20	4	16.67	٢٠.٢	101.00
	16	8	33.33	١٥.٩٢	99.50
	12	12	50	12.16	101.33
	8	16	66.67	٨.٠٤٤	100.56
	4	20	83.33	٤.٠٧٤	101.85
	Mean ± %RSD				100.85±0.882

Table (3): Recovery study of molnupiravir by standard addition technique using the proposed methods in Molnupiravir-EVA Pharma® 200 mg capsules:

Method	Pharmaceutical taken (µg/mL)	Pharmaceutical found[a] (µg/mL)	Pure added (µg/mL)	Pure found[b] (µg/mL)	Pure recovery (%R)
Ratio derivative	10	9.97	4	3.94	98.50
			8	8.06	100.75
			10	9.95	99.50
			12	12.22	101.83
	Mean ± %RSD				100.15±1.452
Ratio difference	10	9.91	4	3.97	99.25
			8	7.90	98.75
			10	10.13	101.30
			12	11.78	98.17
	Mean ± %RSD				99.37±1.372
Mean centering	10	10.12	4	3.96	99.00
			8	7.84	98.00
			10	10.03	100.30
			12	11.77	98.08
	Mean ± %RSD				98.85±1.083

[a] Average of five determinations.

[b] Average of three determinations.

Table (4): Determination of molnupiravir in Molnupiravir-EVA Pharma® 200 mg capsule by the proposed and reported methods:

Parameters	Ratio derivative method	Ratio difference method	Mean centering method	Reported method*[(6)]
Number of measurements	5	5	5	5
Mean % recovery	99.70	99.12	101.15	100.48
% RSD	1.239	0.886	0.902	1.314
Variance	1.526	0.771	0.832	1.743
Student's *t*-test**	0.958 (2.306)	1.914 (2.306)	0.933 (2.306)	——
F-value**	1.142 (6.388)	2.260 (6.388)	2.095 (6.388)	——

* The reported method is 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 values in parenthesis are tabulated values of "*t* " and "*F* " at (P = 0.05)

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دراسة مقارنة بين ثلاث طرق لتلاعب بنسب الطيف لتحديد مولنوبيرافير في وجود منتج تحلله الناتج عن الحمض

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الغرض الرئيسي من هذا العمل هو تأسيس ثلاث طرق طيفية بسيطة وحساسة ودقيقة ومحددة باستخدام تلاعب بنسب الطيف لتحديد مولنوبيرافير في صورتة النقية وفي المستحضر الصيدلى في وجود منتج تحلله الناتج عن الحمض. طرق مشتق النسبة، وفارق النسبة، وتمركز متوسط نسب الطيف هي طرق بسيطة وسريعة وانتقائية تستخدم تلاعب بنسب الطيف لتحديد المكونات ذات الطيف المتداخل. تم التحقق من صحة الطرق المقترحة بما يتوافق مع معايير المجلس الدولى لتنسيق المتطلبات الفنية لتسجيل الادوية للاستخدام البشرى وتم تطبيقها بنجاح لتحديد مولنوبيرافير في البودرة الخام وفي التحضير الدوائي في وجود منتج التحلل الناتج عن الحمض

الكلمات المفتاحية :الأشعة فوق البنفسجية ـ المرئية؛ طيفية الامتصاص؛ مشتق النسبة؛ فارق النسبة؛ التمركز المتوسط؛ منتج التحلل الناتج عن الحمض؛ مولنوبيرافير.