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Eco-friendly spectrophotometric methods for determination of remdesivir and favipiravir; the recently approved antivirals for COVID-19 treatment

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HIGHLIGHTS

• Direct spectrophotometric methods for determination of REM and FAV.
• Application to Remdesivir® vials and Avipiravir tablets.
• Four methods for simultaneous quantitation of REM and FAV in human plasma.
• Derivative, dual wavelength, ratio subtraction, ratio derivative methods were used.
• All methods were applied to determine REM and FAV in human plasma samples.

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ABSTRACT

Remdesivir (REM) and Favipiravir (FAV) are recently approved antivirals prescribed in severely ill COVID-19 patients. Therefore, development of new, simple, rapid, sensitive, and selective methods for analysis of such drugs in their pharmaceutical formulations will be highly advantageous. Herein, we have developed different spectrophotometric methods for analysis of the studied analytes. Method I is based on direct spectrophotometric analysis of REM and FAV in ethanol at $\lambda_{\text{max}}$ 244 and 323 nm, respectively. For simultaneous quantitation of REM and FAV, methods II-V were followed. Method II is based on derivative spectrophotometry in which REM was determined in second-order derivative spectra at 248 nm (the zero-crossing wavelength for FAV), while FAV was measured in first-order derivative spectra at 337 nm (the zero-crossing point for REM). Method III is the dual-wavelength method in which spectral intensities were subtracted at 244–207 nm for REM and at 330–400 nm for FAV. Method IV is the ratio subtraction in which ratio spectra were obtained by a suitable divisor followed by subtraction of intensities at 272–340 nm and 335–222 nm for REM and FAV, respectively. Method V is the derivative ratio method in which the obtained ratio spectra in method IV were converted to first-order derivative spectra at 280 and 340 nm, respectively. Calibration graphs were linear in the ranges of 1–10 µg/mL for REM through all methods and 1–20 µg/mL for FAV in methods I and II, and 2–20 µg/mL by the other methods. The evolved methods were applied to pharmaceutical dosage forms of REM and FAV. All the proposed methods were further applied to human plasma samples containing both drugs with acceptable mean recoveries.

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1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus that started as a fast-spreading pandemic, claiming a huge number of lives worldwide [1]. Up to date, more than 200 million cases have been infected with SARS-CoV-2 with about 5 million confirmed deaths in 223 countries according to WHO [2]. Therefore, many countries decided to start lockdown to control the global outbreak, and hence, the global economy suffered from recession after lockdown [3].

The medical sector was shocked by the new fast spreading pandemic and several therapeutic options have been investigated to counteract or even minimize the clinical symptoms of COVID-19 patients. Currently, there are no specific drugs for COVID-19, but several national agencies authorized many antivirals and antimalarials to be used in the treatment protocol [4-8].

Amongst broad spectrum antivirals, remdesivir (REM), a drug in question in this study, is an experimental antiviral agent developed by Gilead Sciences for treatment of Ebola virus infection [9] and has been approved by the FDA for treatment of SARS-CoV-2 [10]. REM is an adenosine analog and monophosphoramidate prodrug. Its antiviral mechanism of action is through a delayed chain cessation of growing viral RNA [11].

REM, 2-ethylbutyl (2S)-2-[[2R,3S,4R,5R]-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl]methoxy-phenoxy phosphonyl]amino)propanoate, is shown in Fig. 1. Up to date, the literature survey reveals only one spectrophotometric method [12] having a linearity range of 10-60 μg/mL using deionized water as diluting solvent, spectrofluorometry [13], UHPLC in a gradient elution program [14], and HPLC methods [12,15-17] for REM determination.

In addition, the Science and Technology Ministry in China, stated that Favipiravir (FAV) was clearly effective in the treatment of Covid-19 [18]. Favipiravir is a nucleoside analogue (purine base) that is converted intracellularly to the active form, FAV-ribofuranosyl-5B-triphosphate, by a phosphoribosylation process. FAV selectively inhibits RNA-dependent RNA polymerase which is the core enzyme in SARS-CoV-2 replication process. FAV was the antiviral of choice for the current study as it showed rapid viral clearance in comparison to ritonavir and lopinavir in addition to superior rate of recovery compared to umifenovir. Additionally, FAV is incorporated recently in COVID-19 treatment protocols in some countries such as India [19].

FAV, 5-fluoro-2-oxo-1H-pyrazine-3-carboxamide is shown in Fig. 1. The literature was screened for analytical procedures that can be applied for determination of FAV, a spectrophotometric method [20] having a range of 10-60 μg/mL, two spectrophotometric techniques based on utilization of its conventional fluorescence spectra [21,22], electrochemical [23-25] techniques and HPLC [20,22,26] methods were reported. Reported proofs tested the drug-drug interactions involving REM and FAV and revealed that such co-administered developed an enhanced antiviral activity [27]. Co-administering of FAV and REM -after metabolism- suppressed viral load in hamster lungs more efficiently than did each drug alone. The co-administration efficiently inhibited lung inflammation, virus transmission in the infected model. Hence, it may help combat COVID-19 outbreak [28]. Such antiviral combination was previously assessed by first derivative synchronous spectrophotofluorimetry [29].

The objective of this study was to overcome complexity of the reported HPLC methods and poor sensitivity of reported spectrophotometric methods for assessment of REM and FAV (10 μg·mL⁻¹) [12,20] by developing and validating spectrophotometric measures to ensure best measurement conditions for both drugs in their single dosage forms and human plasma. Besides, the study aimed to develop procedures for simultaneous estimation of FAV and REM followed by application to spiked human plasma samples. The supposed studies have the merits of being cheap, rapid, direct, and sensitive as well. The cost-friendly and robustness benefits of spectrophotometry were utilized during our work. The present work proposed four alternative methods for the simultaneous quantitation of FAV and REM in plasma samples.

2. Experimental

2.1. Equipment

A Shimadzu UV-1601 PC (Kyoto, Japan), UV–Visible double-beam spectrophotometer was used to perform spectrophotometric analysis with matched 1 cm path-length quartz cells and 1 nm slit width.

2.2. Reagents and materials

All the solvents were of HPLC grade, and the chemicals used were of analytical grade.

Raw materials of REM and FAV were kindly supplied by Rameda for pharmaceutical industries, Cairo, Egypt.

The investigated dosages of remdesivir® vial (100 mg remdesivir/20-mL vial for intravenous infusion), and avipiravir® film coated tablets (200 mg favipiravir per tablet) are both produced by Eva-Pharma, Egypt. Remdesivir® vials were kindly gifted by Eva-Pharma in cooperation with Mansoura University for research objectives, while avipiravir® film coated tablets were purchased from a local pharmacy.

Methanol, acetoniitrile, and ethanol were purchased from Sigma-Aldrich, Missouri, United States.

Britton–Robinson buffer (2.0-12.0) was used for optimization of pH throughout methods development.

Human plasma was obtained from Mansoura University Hospitals, Mansoura, Egypt and kept frozen (-5°C) until used after gentle thawing.

2.3. Standard solutions

Stock standard solutions (100 μg/mL) of REM and FAV were prepared using ethanol as a solvent. Working concentrations were attained by proper dilution of the prepared stock.
2.4.1. Calibration graphs

Method I.
Two series of 10-mL volumetric flasks (IA and IB) were arranged, where IA set is for REM and IB for FAV. The final concentration range of 1–10 µg/mL was obtained by transferring precisely measured aliquots of REM or FAV standard solutions into the flask sets, completing with ethanol and mixing. Afterwards, absorbance values were recorded at 245 nm for series IA and 323 nm for IB series against ethanol blank.

Methods II and III.
Into a series of 10-mL volumetric flasks, precisely measured aliquots of REM and FAV stock solutions (100 µg/mL for each) were placed, simultaneously. Then, flasks were completed to the final volume with ethanol and mixed well, so that the final concentration ranges stated in Table 1 were obtained. Ultraviolet spectrophotometric spectra were recorded in the scale of 190–400 nm. For method II, absorbance spectra were converted to their first and second order derivatives. REM was determined in second order derivative spectra at 248 nm as a zero-crossing wavelength for FAV, while FAV was calibrated at 337 nm in first order derivative spectra as a zero-crossing point for REM. In method III, the difference in absorbance intensities was performed at 244–207 nm for REM and 330–400 nm for FAV. Either derivative amplitudes or absorbance difference were plotted versus the corresponding drug concentration to get calibration graphs performed. Alternatively, the corresponding regression equations were derived.

Method IV.
The recorded spectrophotometric spectra by the previous procedure were used. To determine FAV, the spectra were divided by the spectrum corresponding regression equations were derived. Alternatively, the absorbance difference were plotted versus the corresponding derivative spectra as a zero-crossing point for REM. In method IV, the difference in absorbance intensities was performed at 248 nm as a zero-crossing point for REM. In method V, the absorbance spectra were subjected to zero-crossing point for REM by 2nd derivative spectra as a zero-crossing wavelength for FAV, while FAV was calibrated at 337 nm in first order derivative spectra as a zero-crossing point for REM. In method V, the difference in absorbance intensities was performed at 248 nm as a zero-crossing point for REM. In method V, the absorbance spectra were subjected to zero-crossing point for REM by 1st derivative spectra as a zero-crossing wavelength for FAV, while FAV was calibrated at 337 nm in first order derivative spectra as a zero-crossing point for REM.

Table 1

Proposed spectrophotometric methods' analytical performance data.

| Method Parameter | Method I | Method II | Method III | Method IV | Method V |
|------------------|---------|----------|------------|-----------|---------|
| Wavelength (nm)  | 244     | 323      | 248        | 337.8     | 244–207.3 |
| Concentration    | 1–10    | 1–20     | 1–10       | 2–20      | 1–10    |
| Range (µg/mL)    | 0.05    | 0.27     | 0.14       | 0.21      | 0.20    |
| LOD (µg/mL)      | 0.16    | 0.82     | 0.43       | 0.63      | 0.60    |
| LOQ (µg/mL)      | 0.9999  | 0.9999   | 0.9999     | 0.9999    | 0.9997  |
| Correlation      | 0.0000  | 0.0000   | 0.0000     | 0.0000    | 0.0000  |
| Slope            | 0.08    | 0.06     | 1 × 10^-3  | 15 × 10^-3 | 41 × 10^-3 |
| Intercept        | 0.06    | -0.03    | 9 × 10^-3  | -0.01     | 6 × 10^-3 |
| s_x              | 3 × 10^-3 | 9 × 10^-4 | 8 × 10^-4  | 18 × 10^-4 | 41 × 10^-4 |
| s_b              | 1.1 × 10^-3 | 5 × 10^-4 | 5 × 10^-4  | 9 × 10^-4  | 25 × 10^-4 |
| % Error          | 0.45    | 0.53     | 0.45       | 0.54      | 0.60    |
| %RSD             | 1.10    | 1.41     | 1.00       | 1.32      | 1.34    |
| Regression equation | -0.078x | y = 0 - 0.06x | y = 1 × 10^-5x | y = 15 × 10^-6x | y = 41 × 10^-2x |
| Mean found (%)    | 99.75 ± 1.10 | 99.67 ± 1.40 | 99.67 ± 1.00 | 99.78 ± 1.32 | 99.66 ± 1.33 |
| ± S.D.           | 99.8 ± 1.00 | 99.72 ± 1.40 | 99.71 ± 1.00 | 99.87 ± 1.32 | 99.90 ± 1.33 |

3. Method application

3.1. Analysis of REM in its vial dosage form

The vial is labelled to contain 100 mg REM/2 mL and should be stored at 5 °C. Aliquots of the vial were serially diluted to cover the concentration ranges 1–10 µg/mL using method I. Subsequently, the procedures explained under “2.4.1. Calibration graphs” were followed (Table 3).

3.2. Average content determination of Avipiravir® tablets

For this application, commercial tablets were obtained. Contents of 5 identical tablets were mixed well by mortar trituration. Accurate amounts of tablets powder equivalent to 10.0 mg FAV were transferred into 50 mL volumetric flask. About half volume of flasks were filled with ethanol, then the flasks were sonicated for 30 min and then completed to final marks with ethanol. The solution flask was then filtered discarding first few milliliters. Further dilutions were performed with ethanol in order to obtain working standard solutions to be analyzed following the discussed procedure under “2.4.1. Calibration graphs.” The nominal contents were calculated using corresponding regression equations or already plotted calibration graphs for each method (Table 3). Average nominal content = mean of %recoveries × the labelled content = 199.52 mg FAV/tablet.

3.3. Estimation of REM and FAV in spiked human plasma samples

Choosing a suitable extracting solvent is a key step upon dealing with biological samples to ensure complete precipitation of plasma proteins. Ethanol, the optimum diluting solvent for the proposed methods witnessed high extraction efficiency for both analytes. The following steps were cautiously followed: 1.0 mL aliquots of human plasma were transferred into a series of centrifugation tubes and spiked with different concentrations of REM or FAV for method I or both REM and FAV for methods II–V to cover the final ranges stated in Table 1. All tubes were subjected to vortex mixing for 30 s and completed to 5 mL with ethanol. For the next 30 min, all tubes were centrifuged at 3600 rotation per minute (rpm) to assure efficient separation of the studied analytes from other human plasma components. Then, 1 mL of supernatant–from each centrifuged tube–was filtered by syringe disc filter to ensure separation of any interfering particles, and transferred to 10 mL volumetric flasks, subsequently the procedures under “2.4.1” were applied.
Table 2
Spectrophotometric methods’ application for REM and FAV raw materials.

| Parameter | % Found | Drug   |
|-----------|---------|--------|
|           |         | REM    |
| Method/Conc taken (µg.mL⁻¹) | I | II | III | IV | V |
| 1.0       | 98.00  | 99.00 | 98.10 | 98.40 | 101.30 |
| 2.0       | 99.20  | 98.75 | 99.65 | 100.15 | 99.50 |
| 4.0       | 100.63 | –     | –     | 100.80 | 100.65 |
| 6.0       | 101.02 | 101.05 | 99.88 | 101.15 | 100.35 |
| 8.0       | 100.19 | 100.40 | 101.7 | 98.08 | 98.04 |
| 10.0      | 99.44  | 99.17 | 98.97 | 100.69 | 101.05 |
| Mean found (%) ± S. D. | 99.87 ± 0.99 | 97.72 ± 0.66 | 99.66 ± 0.67 | 99.75 ± 0.75 |
|           |        |        |        |        |        |
|           |         | FAV   |
| Conc. Taken (µg.mL⁻¹) | I | II | III | IV | V |
| 1.0       | 99.48  | –    | –    | 98.60 | –    |
| 2.0       | 98.52  | 98.50 | 98.10 | 98.35 | 102  |
| 3.0       | 99.36  | 98.27 | 98.00 | 98.25 | 99.38 |
| 5.0       | 98.04  | 99.68 | 100.08 | 100.44 | 100.9 |
| 10.0      | 101.43 | 101.30 | 101.78 | 100.71 | 99.15 |
| 15.0      | 101.75 | 101.32 | 101.77 | 100.81 | 100.39 |
| 20.0      | 99.14  | 99.58 | 98.87 | 99.42 | 99.95 |
| Mean found (%) ± S. D. | 100.45 ± 1.21 | 1.31 | 1.33 | 1.00 | 1.10 |
|           |        |        |        |        |        |
|           |         |        |        |        |        |

Values between brackets are the tabulated values of t and F tests [34].

Table 3
Application of the proposed method I to remdesivir® and avipiravir® dosage forms.

| Parameter | Proposed method I | Comparison methods [13,22] |
|-----------|-------------------|---------------------------|
| Conc. taken (µg.mL⁻¹) | % Found | % Found | % Found |
| Remdesivir® | 2.0 | 98.47 | 101.72 | 100.34 |
| (Remdesivir, 100 mg/20 mL vial) | 4.0 | 99.69 | 100.34 | 100.19 |
| Mean ± S.D. | 99.12 ± 0.61 | 100.41 ± 1.27 | 1.39 (2.78) |
| Student’s t test | 1.59 (2.78) | 4.25 (19.00) |
| Variance ratio F test | 98.97 |
| Avipiravir® | 5.0 | 99.12 | 98.97 |
| (Favipiravir, 200 mg/tab) | 10.0 | 99.43 | 99.13 |
| Mean ± S.D. | 99.76 ± 0.86 | 99.60 ± 0.29 |
| Student’s t test | 0.31 (2.47) |
| Variance ratio F test | 1.72 (6.94) |

Values between brackets are the tabulated values of t and F tests [34].

Table 4
Methods’ application for determination of REM and FAV in spiked human plasma.

| Parameter | REM | FAV |
|-----------|-----|-----|
| Conc. taken (µg.mL⁻¹) | Conc. Found (µg.mL⁻¹) | % Recovery | Conc. taken (µg.mL⁻¹) | Conc. Found (µg.mL⁻¹) | % Recovery |
| I         | 1.0 | 0.9100 | 91.00 | 2.0 | 1.6540 | 82.70 |
| II        | 3.0 | 2.9980 | 99.93 | 5.0 | 5.2410 | 104.82 |
| III       | 5.0 | 5.2410 | 104.82 | 10.0 | 10.3890 | 103.89 |
| IV        | 7.0 | 7.1300 | 101.86 | 15.0 | 15.1980 | 101.32 |
| V         | 10.0 | 9.7970 | 97.97 | 20.0 | 19.6890 | 98.45 |
| I         | 1.0 | 0.871 | 87.1 | 2.0 | 2.000 | 100.00 |
| II        | 3.0 | 3.132 | 104.4 | 5.0 | 5.167 | 103.34 |
| III       | 5.0 | 5.167 | 103.34 | 10.0 | 10.3850 | 103.85 |
| IV        | 7.0 | 7.136 | 101.94 | 15.0 | 15.9940 | 100.63 |
| V         | 1.0 | 9.74 | 97.4 | 20.0 | 19.8130 | 99.07 |
| I         | 1.0 | 0.9164 | 91.64 | 2.0 | 2.0182 | 100.91 |
| II        | 3.0 | 2.9907 | 99.69 | 5.0 | 5.2972 | 105.94 |
| III       | 5.0 | 5.2972 | 105.94 | 10.0 | 10.4727 | 104.73 |
| IV        | 7.0 | 6.7833 | 96.90 | 15.0 | 16.5636 | 110.42 |
| V         | 10.0 | 10.0186 | 100.19 | 20.0 | 18.8455 | 93.73 |
| I         | 1.0 | 1.0200 | 102.00 | 2.0 | 1.9880 | 99.40 |
| II        | 3.0 | 2.8800 | 96.00 | 5.0 | 5.1850 | 103.70 |
| III       | 5.0 | 4.2700 | 85.40 | 10.0 | 10.4950 | 99.59 |
| IV        | 7.0 | 6.6210 | 94.59 | 15.0 | 15.6530 | 97.69 |
| V         | 10.0 | 10.5730 | 105.73 | 20.0 | 20.2360 | 101.18 |
| I         | 1.0 | 1.024 | 102.4 | 2.0 | 2.021 | 101.05 |
| II        | 3.0 | 2.772 | 92.4 | 5.0 | 5.059 | 101.18 |
| III       | 5.0 | 5.138 | 102.76 | 10.0 | 9.553 | 95.53 |
| IV        | 7.0 | 7.293 | 104.19 | 15.0 | 15.653 | 104.35 |
| V         | 10.0 | 9.787 | 97.87 | 20.0 | 19.718 | 98.59 |

Values between brackets are the tabulated values of t and F tests [34].

Table 4 summarizes % recoveries of the spiked human plasma samples by the proposed methods.

4. Results and discussion

COVID-19 is the pandemic of this era and health care specialists always modify the treatment regimen based on the findings. Recently, studies revealed the enhanced efficacy upon co-administering REM and FAV [27]. Spectrophotometric technique was of choice as it is superior in multi-component analysis without need for tedious separation or clean-up steps [30].

As REM and FAV have single dosage forms, a direct spectrophotometric determination was developed at \( \lambda_{\text{max}} \) of 244 and 323 nm for REM and FAV, respectively (methods I), as shown in Fig. 2.

REM is reported to have human plasma concentrations of 4.38 and 2.23 µg.mL⁻¹ following intravenous injection of 200 mg and 100 mg doses, respectively [31]. While FAV is reported to have plasma maximum levels of 4.43 µg.mL⁻¹ in critically ill COVID-19 patients [32].
Due to the satisfying sensitivity of the proposed methods as shown in Table 1, and relatively high plasma concentration of the studied drugs, different spectrophotometric methods were investigated to simultaneously determine the drugs in question. As shown in Fig. 2, spectral overlap is clearly observed for both antivirals, then different mathematical methods were set to resolve these spectra.

Derivative spectrophotometry (method II).
It is characterized by converting the main zero spectra to a more characteristic profile with new maxima and minima with better detectability and specificity [30]. REM and FAV were converted to their first and second-order derivatives by differentiating their absorbance with respect to wavelength. As shown in Fig. 3 FAV was determined in first order-derivative at 337 nm a zero crossing for REM. While REM was determined in the second order at 248 nm a zero crossing with FAV Fig. 4, Fig. 6.

Dual wavelength method (method III).
The utility of dual wavelength data processing program is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest, independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration [30]. Selected wavelengths were in the range of 244–207 nm for REM and 330–400 for FAV.

Absorbance ratio spectra method (ratio subtraction, method IV).
Having a mixture of 2 components (REM and FAV), if the absorbance of the mixture is divided by the absorbance of a standard solution of REM called a divisor, a constant ratio is obtained and REM is eliminated from mixture by subtraction at two wavelengths in which the difference is proportional for FAV’s concentration [30]. Selected divisors were 6.0 µg. ml\(^{-1}\) REM and 5.0 µg. ml\(^{-1}\) FAV, then subtraction of ratio intensities was done at 272–340 nm and 335–222 nm for REM and FAV, respectively as depicted in Figs. 5 and 6.

Ratio derivative spectrophotometry (method V).
As in method IV, the ratio spectra are obtained in the same manner, while removing the interfering ingredient was performed by first order derivative [30]. As shown in Figs. 7 and 8, REM and FAV were recorded at 280 and 340 nm in their corresponding ratio first-order derivative spectra. These wavelengths were selected due to high intensities compared to other maxima.

4.1. Optimization of study conditions
Different factors that may alter spectrophotometric absorbance of REM and FAV, namely pH, and diluting solvent were carefully studied to achieve the best sensitivity and linearity with maximum stability. Each factor was carefully studied meanwhile other factors were kept constant using univariate optimization technique.

4.1.1. Effect of pH
pH was studied carefully, as a factor of a pronounced effect on analytes’ absorbance intensity. Ranges of pH 3.0–12.0 were examined.
HCl, 0.1 N NaOH, methanol, ethanol, and distilled water. 0.1 N HCl was observed to minimize REM application. Ethanol was chosen for further plasma preparation and methodation in absorbance over the studied matrices; however, it examined a bathochromic shift in aqueous solutions. Ethanol as a diluting solvent showed superior repeatability of results comparing to distilled water. Therefore, ethanol was chosen as a solvent for further spectrophotometric application as it meets green chemistry guidelines and produced sufficient sensitivity in the five methods.

4.1.2. Choice of extracting solvent

Acetonitrile, methanol, and ethanol were investigated during plasma application. Both ethanol and acetonitrile showed satisfactory precipitation of plasma proteins and high extraction recovery for both analytes. Ethanol was chosen for further plasma preparation and method application.

4.1.3. Effect of diluting matrix

Different diluting solvents were examined in our study such as 0.1 N HCl, 0.1 NaOH, methanol, ethanol, and distilled water. 0.1 N HCl was observed to minimize REM’s absorbance, while others were almost of the same absorbance value. FAV has not shown a characteristic elevation in absorbance over the studied matrices; however, it examined a

5. Methods validation

Following ICH guidelines [33], different validation parameters were estimated.

Detection and quantitation limits values were both calculated by data obtained in calibration graphs using the reported equations: LOD = $\frac{3.3 \times s_a}{m}$, and LOQ = $\frac{10 \times s_a}{m}$, where LOD is the limit of detection, LOQ is the limit of quantitation, and $s_a$ is the standard deviation of intercept. Table 1 summarized values of LOD and LOQ for FAV and REM.

To evaluate methods’ linearity and range, calibration curves were obtained by plotting absorbance intensities against the corresponding drug concentrations. Linearity ranges were observed over the concentration ranges of 1–10 μg/mL for REM through all methods and 1–20 μg/mL for FAV in methods I and II, and 2–20 μg/mL by methods III–V as shown in Table 1. By statistical analysis [34] of the data obtained, REM and FAV had the regression formula stated in Table 1.

Table 1 also states correlation coefficient (r) values obtained by further statistical analysis of REM and FAV’s calibration graphs. (r) of greater than 0.998 for both drugs assured good linearity of methods for both drugs in the selected range of analysis.

Accuracy of proposed procedures could be expressed by accepted values of Student’s t-test and variance ratio F-test when compared to comparison procedures. Obtained data and analysis values are expressed in Table 2.

FAV comparison method was based on direct spectrofluorometric analysis at 436 nm in Britton-Robinson buffer of pH 4 after excitation at 323 nm with linearity range of 20–350 ng. mL$^{-1}$ [22]. Additionally, the reported comparison method for REM analysis relied on conventional spectrofluorometric measurement at 401 nm following excitation at 244 nm using distilled water with linearity range of 1–65 ng. mL$^{-1}$ [13]. The presented methods showed superior robustness compared to the reported ones as spectrofluorometry’s elevated sensitivity is accompanied by affected robustness.

The selectivity of the proposed methods was estimated by determination of REM and FAV in their pharmaceutical dosage forms in addition to human plasma samples as complex matrices. Sufficient selectivity of the recommended procedures was expressed by good % recoveries and % RSD upon analyzing the selected medications. Then, the lack of interference from common excipients in pharmaceutical dosages was confirmed. Additionally, the obtained data following human plasma application evidenced a strong selectivity of simultaneous determination of REM and FAV in human plasma by the proposed methods without interference from endogenous matrices.

Table 5 recaps the practically obtained inter-day and intra-day precision results for the proposed methods. Low values of % RSD and % error indicated reasonable intra and inter-day precision.

6. Conclusion

Five rapid, direct, and cheap spectrophotometric methods are proposed for simultaneous determination of COVID-19 antivirals FAV and REM. Direct, derivative, dual wavelength, ratio subtraction, and ratio derivative spectrophotometric techniques are described for overcoming the spectral overlap of FAV and REM. The evolved methods guaranteed the successful simultaneous determination of both antivirals in human plasma samples without interference with plasma components with mean recoveries ranging from 85% to 98%. Moreover, the developed methods did not require sample pretreatment or tedious preparation steps, rendering them the superiority and simplicity in quantitation of
the studied analytes in their dosage forms compared to the reported chromatographic methods.

CRediT authorship contribution statement

Heba Samir Elama: Conceptualization, Methodology, Validation, Investigation, Writing – original draft. Abdullah M. Zeid: Conceptualization, Visualization, Supervision, Writing – review & editing. Shereen Mahmoud Shalan: Supervision, Formal analysis, Visualization, Writing – review & editing. Yasser El-Shabrawy: Conceptualization, Supervision, Validation, Writing – review & editing. Manal Ibrahim Eid: Validation, Project administration, Supervision, 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.

Data availability

Data will be made available on request.

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