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Sticking - pulling strategy for assessment of combined medicine for management of tough symptoms in COVID-19 Pandemic using different windows of spectrophotometric Platform-Counterfeit products’ detection

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HIGHLIGHTS

- Environmentally green UV-spectrophotometric power for analyzing ternary mixture containing aceclofenac (ACE), paracetamol (PAR) and rabeprazole (RAB) in combined medicine for tough symptoms in the COVID-19 Pandemic present in critical ratios via different windows of spectrophotometric platform; window I; based on manipulation of the data of (D0) of the mixture using novel extended absorbance difference (EAD-SS) and absorbance difference (AD-SS) methods. Window III; based on manipulation of the data of ratio spectra via constant value coupled with constant subtraction (CV-CS) and novel induced dual amplitude difference (IDAD-SS) method. Window IV; based on manipulation of the ratio spectra; DD1 via novel factorized derivative ratio null contribution (FDD-NC) and factorized unlimited derivative ratio (FUDD-SS) methods.
- Sticking - pulling strategy (SPS) by applying successive In-Silico sample enrichment for ACE and RAB as minor components for resolving the ternary mixture.
- Ascertaining the developed spectrophotometric methods’ power to extract the drugs’ spectrum for detecting counterfeit medicine via calculating spectral similarity index (SSI).
- Guarantying methods’ greenness profile through analytical greenness profile.

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A B S T R A C T

This study presents a comprehensive comparative study of different green spectrophotometric approaches without any physical separation on processing a ternary mixture of Aceclofenac (ACE), Paracetamol (PAR) and Rabeprazole (RAB) in combined medicine for managing tough symptoms in the COVID-19 Pandemic. The different univariate complementary resolutions according to the response used for the assay of the cited drugs after applying the processing steps were implemented using successive in-silico sample enrichment for resolving the ternary mixture via different windows of spectrophotometric platform using sticking – pulling strategy (SPS). Window I; based on manipulation of the data of zero order absorption spectrum of the mixture using novel Extended absorbance difference (EAD) and Absorbance difference (AD) methods coupled with corresponding spectrum subtraction method (SS). Window II; based on manipulation of the data of ratio spectra via Constant value coupled with constant subtraction (CV-CS) and novel Induced dual amplitude difference (IDAD) method coupled with corresponding spectrum subtraction method (SS). Finally, window IV; based on manipulation of the data of derivative of the ratio spectrum of the mixture via novel Factorized derivative ratio null contribution (FDD-NC) and Factorized unlimited derivative ratio (FUDD) methods coupled with corresponding spectrum subtraction method (SS). Synthetic mixtures and commercial medicine were constructively analyzed using the proposed methods while maintaining calibration graphs to be linear over ranges; 4.0–40.0 μg/mL for ACE, 2.0–14.0 μg/mL for PAR and 4.0–30.0 μg/mL for RAB. Moreover, methods’ validation was confirmed via performing exhaustive statistical treatment of the experimental findings. The proposed methodologies can be used for the routine analysis of the cited drugs in quality control laboratories. Additionally, Spectral Similarity Index (SSI) was calculated to detect counterfeit products and methods’ greenness profile was finally guaranteed through analytical greenness (AGREE) metric assessment tool.

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1. Introduction

Aceclofenac (ACE), [(2-[(2,6-Dichlorophenyl) amino] phenyl) acetyl] oxy] acetic acid, a non-steroidal anti-inflammatory drug having anti-rheumatic and analgesic activity [1,2]. Literature survey publicized that ACE’s determination was applied through titrimetric [3], spectrophotometric [4], colorimetric [5,6] and spectrofluorimetric methods [5].

Paracetamol (PAR), N-(4-hydroxyphenyl) acetamide with analgesic and antipyretic activity. PAR’s assessment was achieved via titrimetric[7], spectrophotometric [8–10], fluorimetric [11], HPTLC [12] and HPLC [13,14] methods.

Rabeprazole (RAB), 2-[[4-(3-Methoxypropoxy)-3-methylpyridin-2-yl] methyl sulfinyl]1H-benzimidazole used in peptic ulcer disease, excess stomach acid production and gastro esophageal reflux disease where, its pharmacological effect is similar to other proton pump inhibitors (PPIs) [15]. Spectrophotometric [16,17], fluorimetric [18], HPTLC [19,20] and HPLC [21,22] methods were successfully applied for RAB’s estimation. The chemical structures for the three drugs; ACE, PAR and RAB are shown in Fig. 1.

It was previously proposed that SARS-CoV induced lymphopenia is likely to be caused by the elevated cortisol levels that occurs as part of the body stress response to the severe respiratory viral infection or by an iatrogenic effect of glucocorticoids used to manage those patients. Noteworthy, NSAIDs deserve their enrollment in the early management of COVID-19, trying to lessen the suggested inflammatory process leading to lymphopenia and immunosuppression. Theoretically, NSAIDs when used as early as possible during the clinical course of COVID-19 might prevent disease progression or even reverse lymphocytopenia. Therefore, it is humbly suggested to be added to the newly suggested nitazoxanide/azithromycin protocol for early management of COVID-19 [23]. Moreover, a recently published clinical study [24] has proven the superiority of NSAIDs over the currently used analgesic/antipyretic; Paracetamol not only regarding their analgesic and anti-pyretic effect but also in significantly improving the lymphocytic count in COVID-19 patients, enhancing their immune response as well as recovery mostly in five days [24].

ACE- Proxyvon™ tablet; is a pain-relieving medicine containing ACE, PAR and RAB in critical ratio. Taken together, both PAR and the NSAID; ACE in COVID-19 patients can surprisingly show an obvious synergistic pharmacological effect for managing the painful symptoms and limiting the possibility of occurrence of lymphocytopenia the patients are prone to without any accompanied drug induced stomach irritation due to the incorporation of proton pump inhibitor; RAB. Consequently, this will increase the patients’ compliance and save more lives.

Determination of ACE has been publicized by several analytical methods either in combination with PAR [25,26] or RAB [27,28] but only one HPTLC [29], HPLC [30] and a sole conventional first derivative spectrophotometric [17] method have been reported for the analysis of the ternary mixture comprising ACE, PAR and RAB.

Microcomputers coupled with the spectrophotometer succeeded in engendering derivative and ratio spectra via data set manipulation as well as arithmetic manipulation easily, quickly and reproducibly. The accompanied properties of UV-spectrophotometric technique in saving cost and time if compared to the HPLC technique gave it a great chance to gain an important rank in pharmacopoeias and to its widespread usage in the pharmaceutical dosage forms’ analysis due to the excellent results obtained for accuracy and precision. Therefore, the trend used
nowadays by specialized analysts is to make use of spectrophotometric methods based on the recorded absorbance or amplitude responses of either the scanned or manipulated spectra [31,32] to analyze the studied drugs each according to the overlaid spectral configuration.

According to the Food and Drug Administration (FDA), a counterfeit medicine is an unsafe falsified medicine as it can be contaminated, contain the wrong or no active ingredient, or could contain the right active ingredient but at a wrong dose, or with toxic or harmful ingredients. Unfortunately, these medicines are usually packaged to look like the real ones. In any of these scenarios, patient’s safety is bargained. It’s clear out that there is an urgent need to innovate a detection system to filter out counterfeit and expired products for safeguarding patients against potentially threatening pharmaceuticals. There are a variety of currently used analytical methods to detect counterfeit medicines, including spectroscopic and chromatographic methods. Counterfeit medicines’ detection by customs officials usually arises because of intelligence or random checks, after which suspect medicines are sent away for laboratory-based analysis [33,34].

Over many years, separation techniques (chromatographic methods) were the most popular and widely used techniques for resolving multi component medicines which could be used in counterfeit products’ detection. However, several perceived drawbacks were associated with the separation methods such as a probability of the incomplete separation and the procedure’s expensive and time consumption. Overcoming the complicated problems of analytical chemistry, spectrophotometric technique was mainly described and applied as an alternative resolution, minimizing the previously mentioned separation obstacles and reducing analysis time and expense. Spectrophotometric platform is classified into four different windows according to the applied manipulation steps namely, window I (zero order spectra), window II (derivative spectra), window III (ratio spectra) and finally window IV (manipulation on ratio spectra; derivatization, mean centering, ... etc). In recent years, extracting parent spectrum of each component in combined medicine through applying mathematical manipulation using different windows of spectrophotometric platform caused a major leap in the field of analytical chemistry. Detection of counterfeit products was easily achieved via data processing and mathematical manipulation of the recorded data using spectrophotometer platform offering an accurate and selective possibility to develop quantitative methods. The rate of counterfeiting medicines is the highest specially in developing countries and considered to be the most frightening issue as it can lead to therapeutic failure or drug resistance.

Due to the pharmacological prominence of the studied dosage form; ACE-Proxyvon™ tablets as a pain-reliever, this work focused on coupling the well-established environmentally green methods with factorized spectra as a new resolution tool, as well as creating novel approaches. Thus, the aim of this study was focused on a clear stepwise explanation of different resolution windows of spectrophotometric platform using windows I (zero order), III (ratio) and IV (derivatization of ratio spectra) as well as illustrating their
limitations and advantages. The proposed methods were exploited for assaying the cited drugs in the combined medicine. Methods' validation was accomplished through following the ICH guidelines [35]. Moreover, to detect counterfeit medicines, the spectral similarity index (SSI) was calculated for each cited drug's extracted spectrum relative to that of the authentic analyte. Finally, environmental and health impacts of the proposed methods were qualitatively and quantitatively appraised via an analytical greenness (AGREE) metric assessment tool [36,37].

2. Theoretical background

2.1. Sticking and pulling strategy

Sticking and pulling strategy for ternary mixture is based on addition – subtraction manipulation via spectrophotometer's software where it is applied when the spectral bands in (D0) of the individual components: X, Y and Z in the studied ternary mixture are overlaid to a large extent, and component Z is extended over X and Y with poor response at the extension region, subsequently, hindering the accurate determination of the proposed mixture using spectrophotometric manipulation steps.

Two complementary steps are applied in this strategy; the first one is enrichment (sticking) then pulling (decreasing complexity). Where, enrichment of the extended spectrum; Z is performed via spectrum addition method [38] using the spectrophotometric software's addition function to confirm its accurate elimination from the ternary mixture under the same manipulated window. The spectrum of an accurately known concentration (Cadded) of Z within its linearity range is used and stuck to Z spectrum (Cclaimed) to get the augmented spectrum of Z. Recovering the targeting component's spectrum in the mixture is attained via multiplying pinpoint response value of Z by the factorized spectrum using the specified mathematical processing.

The second step is decreasing mixture's complexity by pulling of the recovered augmented Z spectrum from the ternary mixture's gross spectrum via the subtraction function under the same manipulated window (zero, ratio and derivative ratio), getting the less complicated resolved binary mixture of X and Y which can be analyzed via simple signals processing procedures.

2.2. Resolution pattern using different windows of spectrophotometric platform

This resolution technique is used for separation as well as identification based on factorized spectrum which is accomplished through dividing of the proposed spectrum at the specified window (zero, ratio or derivative ratio) of one of the scanned spectra within its linearity range by its recorded or modified response (s) at the selected wavelength (s).

Consider a mixture of three compounds X, Y and Z. The studied drugs' spectra are scanned taking in consideration the shape, characteristic features of the proposed spectra and the degree of overlapping either partially or completely, as well as if one or more spectra is showing extension. Pinpointing the response value (s) is achieved by defining a value which corresponds to one of the targeting analytes with no contribution from the other component(s) in the studied mixture using either its zero, ratio or derivative ratio window. This response value (s) at the specified window such as absorbance difference (ΔA), amplitude difference of ratio or derivative ratio spectrum (ΔP) can be applied for recorded response or for modified response calculated via simple mathematical steps utilizing the mathematical factor as equality factor at two wavelength pairs [39,40]. Finally, recovering the targeting component's spectrum in the mixture is attained via multiplying the response value of targeting component by the corresponding factorized spectrum to get the full spectrum of it and measuring using regression equation at response maxima either absorbance, amplitude or peak to peak in case of D0, ratio or derivative ratio, respectively.

2.2.1. Window I: Based on processing data of the mixture's absorption spectrum

The aim of this resolution technique is to recover the parent (D0) spectra of the three drugs in the ternary mixture X, Y and Z, separately via two successive steps and to calculate their concentrations using their regression equations representing the absorbance value at their maxima (Amax) versus their corresponding concentrations. These steps are summarized as follows:

2.2.1.1. Step I: Extended absorbance difference coupled with spectrum subtraction method (EAD-SS). Innovative method for resolution of one of the components in ternary mixtures (X, Y and Z) where the spectrum of interest; Z shows an extension over the other interfering components. This method aims to recover the parent (D0) of component Z using the absorbance difference at the extended wavelength region. The factorized spectrum of Z (C claimed) is prepared via computer's software by dividing the (D0) of any concentration of pure Z within Beer's law by the absorbance difference at the two selected wavelengths at the extended region where the other components showing no contribution.

For laboratory mixtures: The absorbance values are recorded at the two selected wavelengths at the extended region. The calculated absorbance difference (ΔA) is multiplied by the proper factorized spectrum of pure drug of interest.

\[ \Delta A \frac{Z(D)}{\Delta A} = \text{Recovered (D)} \text{ of Z} \quad (1) \]

While, the resolved mixture (X + Y) is achieved via subtracting the obtained (D0) of Z from the equivalent mixture's spectrum; X + Y + Z by spectrum subtraction method [37,41].

2.2.1.2. Step II: Absorbance difference coupled with spectrum subtraction method (AD-SS). This method is applied for resolved binary mixture composed of two spectra; X and Y, where X has equal absorbance at selected wavelengths and component Y has a value, thus cancelling the contribution of X. Factorized spectrum of Y (Ay/CΔA) is prepared via dividing the (D0) of any concentration of pure Y within Beer's law by the absorbance values at the previously chosen wavelengths.

To extract the (D0) of Y from the binary mixture; the absorbance difference (ΔA) of the mixture is calculated at the specified wavelengths then multiplied by the corresponding FS of Y : [Ay/CΔA].

For pulling the (D0) of co-formulated component Y; spectrum subtraction of the recovered (D0) of Y from the gross (D0) of the equivalent mixture is carried out obtaining (D0) of X.

\[ |Ay + Ax| - Ay = \text{Recovered (D)} \text{ of } X \quad (2) \]

2.2.2. Window III: Based on processing data of the mixture's ratio spectrum

The aim of this resolution technique is to recover the ratio spectra of the three drugs in the ternary mixture, separately via two successive steps and to calculate their concentrations using their regression equations representing the amplitude value at maxima (Pmax) versus their concentrations. These steps are summarized as follows:

2.2.2.1. Step 1: Constant value coupled with constant subtraction method (CV-CS). This method can be applied on a ternary mixtures'
ratio spectrum composed of X, Y and Z using Z as a divisor where the spectrum of interest; Z shows an extension over the other interfering components represented as a straight line in the extended region; Z/Z thus, the constant value is recorded. Upon subtracting this constant value from the corresponding ratio spectrum of the mixture, the resolved ratio spectrum of (X + Y) will be obtained.

2.2.2.2. Step 2: Induced dual amplitude difference coupled with spectrum subtraction method (IDAD-SS). A novel method which can be applied on a resolved binary mixtures' ratio spectrum composed of \( \frac{F_x}{F_y} \) using two selected wavelengths where component X has a significant amplitude difference while the amplitude difference of Y between those two wavelengths aren't equal (amplitude difference does not equal zero). This method is a modification of dual amplitude difference method [38].

To cancel the effect of Y at the two selected wavelengths, the equality factor of pure ratio spectra of Y at these wavelengths \((F_Y)\) is calculated which is the average of amplitude ratio \( \frac{F_x}{F_y} \) (where \( F \geq 1 \) or \( < 1 \)) between the two recorded amplitudes of different concentrations of Y at the chosen wavelength pair of X using Z as a divisor. By calculating the difference, Y will be cancelled. So, \( \Delta P(Pm_1 - FyPm_2) \) is related to X only.

Meanwhile, a factorized induced ratio spectrum of X : \( \frac{x}{x+ρ} \) is prepared via dividing the ratio spectrum of any concentration of pure X (throughout all the scanned wavelengths) using Z as a divisor by the calculated amplitude difference (recorded amplitude difference value at the chosen wavelengths after multiplying by the calculated equality factor of pure Y).

The multiplication of the mixture’s induced numerical amplitude value; \( \Delta P.Fy \) and the stored factorized induced ratio spectrum of X; \( \frac{x}{x+ρ} \) will produce ratio spectrum; \( \frac{x}{x+ρ} \) of X in the mixture as follows:

\[
\Delta P.Fy \times \frac{x}{x+ρ} = \frac{x}{Z} \]

The ratio spectrum of component Y could be then obtained by subtracting the obtained ratio spectrum of X from the gross binary mixture's ratio spectrum by spectrum subtraction method [42].

Induced dual amplitude difference (IDAD) using factorized induced ratio spectrum approach has the advantages of minimum data analysis of signal output with high accuracy where the cited component is determined at its \( P_{max} \). In addition, minimization of the random error by the usage of factorized induced ratio spectrum in the manipulation since its preparation via spectrophotometer software based on the response only despite normalized spectrum which is based on concentration.

2.2.2.3. Window IV: Based on processing data of the mixture's derivative ratio spectrum

The aim of this resolution technique is to recover the derivative ratio spectra of the three drugs in the ternary mixture, separately and to analyze each one using its regression equation representing the algebraic sum of the amplitude values using graphical representation: peak to peak (\( P_{max} \) to \( P_{min} \)) (irrespective to the sign) (for maximum sensitivity) versus their corresponding concentration of each component in the mixture. The steps are summarized as follows:

2.2.2.3.1. Step 1: Factorized derivative ratio null contribution method (FDD-NC). This novel approach is used for determination of one of the components in ternary mixture using derivative ratio technique. Consider a ternary mixture of X, Y and Z, component Z is determined in the derivative ratio spectra using Y as a divisor via selecting two wavelengths at which the spectrum of Y shows maxima and minima peaks while the spectrum of X has two minima of \( \frac{F_x}{F_y} \) possessing equal amplitudes. Thus, the peak amplitude (maxima) of \( \frac{F_x}{F_y} \) is diminished by minima of \( \frac{F_x}{F_y} \) at the first wavelength; \( \lambda_1 \) while the peak amplitude (minima) of \( \frac{F_x}{F_y} \) is furnished by minima of \( \frac{F_x}{F_y} \) at the second wavelength; \( \lambda_2 \). The diminish and furnish effects are equal since \( PX_1 = PX_2 \) keeping the total of the maxima and minima of \( Pz \) the same with no effect of X (null impact of \( Pz \)). Thus, the total of amplitudes of graphical representation of \( Z/Y \) at those selected wavelengths will be dependent on the concentration of Z only (the sign is ignored). Generally, this method could be applied also using equality factor \( \text{Eq} \) (where \( F \geq 1 \) or \( < 1 \)) of pure X in case \( PX_1 \neq PX_2 \) where \( Fx \) is the ratio of peak amplitudes of different concentrations of X at the chosen maxima and minima.

\[
Pm_1 \equiv (A = πr^2Pz_1 - Px_1) + (FxPz_2 + FxPx_2)
\]

Where,

\[
PX_1 = FxPz_2(\text{where} F \geq 1 \text{or} \leq 1) = (-Pz_1) + (FxPz_2)
\]

For ternary mixture X, Y and Z, the contribution of one of interfering components in the mixture possessing equal response at two selected wavelengths is ignored. For determination of Z, the ratio spectra derivative method involves dividing the spectrum of mixture by the normalized spectra of Y and deriving the ratio to obtain a spectrum that is independent of the concentration of analyte used as a divisor; Y while component X has equal amplitudes at the selected wavelength pair.

\[
Pm = -Px + Py + Pz
\]

The first derivative of these ratio spectra is traced with the interval of \( \Delta \lambda = 4 \text{ nm} \) and scaling factor 10.

The concentration of Z in the mixture is calculated by multiplying the amplitude summation at the selected wavelength pair by the factorized derivative of ratio spectrum of Z to get the derivative ratio spectrum of Z in the mixture (this factorized spectrum is prepared by dividing the derivative ratio spectrum of pure drug of interest within its Beer’s law limit using the same experimental condition by the calculated amplitude summation at the selected wavelengths).

The derivative ratio null contribution has advantage over derivative ratio zero contribution that it can eliminate the contribution of the interfering component via simple mathematic analysis with maximum sensitivity using graphical representation maxima to minima so, the sensitivity is enhanced.

2.2.2.3.2. Step 2: Factorized unlimited derivative ratio coupled with spectrum subtraction method (FUDD-SS). This novel approach deals with the resolution of ternary mixture where the interfering component lacks two equal amplitudes at the selected wavelengths of drug of interest. The method is a novel approach of unlimited derivative ratio method [40] depending on obtaining the difference between two wavelengths to ignore the contribution of one of interfering components in the mixtures lacking equal response at these selected wavelengths.

For determination of X in mixtures of X, Y and Z, two wavelengths; \( \lambda_1 \) and \( \lambda_2 \) were selected in the derivative ratio spectra of the ternary mixture using Z as a divisor where, X/Z and Y/Z have peaks on the same graphical representation of those of target drug X while Z has no contribution (derivative of constant is zero).

A significant amplitude difference between the two selected wavelengths (\( \lambda_1 \) and \( \lambda_2 \)) in the derivative ratio spectrum of the mixture is calculated where \( Py_1 \neq Py_2 \):

\[
Pm_1 = Px_1 + Py_1\at \lambda_1 \text{where} Pz_1 = 0
\]
To cancel the effect of Y at the two selected wavelengths, the equality factor of pure derivative ratio spectra of Y at these wavelengths (Fy) is calculated which is the average of amplitude ratio 

\[ Fy = \frac{Py}{Py2} \] (where \( F \geq 1 \) or \( \leq 1 \)) between the two recorded amplitudes of different concentrations of Y at the chosen wavelength pair of X (\( z_1 \) and \( z_2 \)) using the same derivative order of its ratio spectra using Z as a divisor. Thus, the response difference of the derivative ratio spectra of the ternary mixture is dependent only on X and is independent on Y:

\[ \Delta P(\text{Pm1} – \text{PyPm2}) = \text{Pm1} – \text{PyPm2} \] (9)

Thus, the induced amplitude difference at wavelength pair is multiplied by the factorized induced derivative ratio spectrum of (X) \( \frac{\text{D}(\text{Pm})}{\text{D}(\text{P0})} \) to get the recovered derivative ratio spectrum of X in the mixture.

The derivative ratio spectrum of Y/Z is obtained after subtracting the recovered derivative ratio spectrum of X/Z via spectrum subtraction method from the ternary mixture's derivative ratio spectrum using Z as a divisor (X + Y + Z)/Z where Z/Z equal zero.

3. Experimental

3.1. Apparatus and software

Spectrophotometric measurements were performed in quartz cell; 1.00 cm using double beam spectrophotometer; Shimadzu (UV-1800, Japan). Scans were carried out from 200 to 400 nm at 0.1 nm intervals and the spectra were attained by Shimadzu UV-Probe 2.43 system software automatically.

3.2. Samples and solvents

3.2.1. Pure samples

Aceclofenac was thankfully offered by Al-Amriya Pharmaceutical Industries, Al-Amriya, Alexandria, while, pure samples of Paracetamol and Rabeprazole were offered by Global Napi Industries, Al-Amriya, Alexandria, while, pure samples of Paracetamol and Rabeprazole were offered by Global Napi Pharmaceutical Industries, 6th of October, Egypt. Their purity was tested and found to be 99.76 ± 1.44, 99.07 ± 1.33 and 99.86 ± 1.22, respectively according to their reported methods, respectively [14,27].

3.2.2. Market sample

ACE- Proxyvon tablets dosage form, batch number (174103) embracing 100.0 mg of ACE, 500.0 mg of PAR and 10.0 mg of RAB, was manufactured by Wockhardt Pharmaceutical Industries, India and purchased from the Indian market.

3.2.3. Solvents

Spectroscopic analytical grade; ethanol was obtained from El-NASR Pharmaceutical Chemicals Co., Cairo, Egypt.

3.3. Standard solutions

3.3.1. Standard stock solutions

100.0 μg/mL stock standard solutions for each of ACE, PAR and RAB were prepared separately in ethanol.

3.3.2. Laboratory-prepared mixtures

Different mixtures were prepared via transferring accurate portions with different ratios of the studied drugs from their standard solutions and transferring them to a series of 10- mL volumetric flasks using ethanol as a solvent.

4. Procedure

4.1. Spectral characteristics

The studied drugs' (D0) spectra were scanned at region 200.0–400.0 nm against ethanol as a blank.

4.2. Construction of calibration graphs and resolution spectrum

Preparation of calibration standards over concentration ranges (4.0–40.0 μg/mL) of ACE, (2.0–14.0 μg/mL) of PAR and (4.0–30.0 μg/mL) of RAB was done in 10-ml volumetric flasks' separate series using ethanol as a solvent. Spectral scanning was carried out and the obtained (D0) spectra were saved on the computer. Each proposed method's calibration graph was constructed using the average of three experiments as follows:

4.2.1. Window I

The stored (D0) maximum absorbance values of ACE, PAR and RAB at 277.2 nm, 250.0 nm and 284.5 nm were constructed against their corresponding concentrations, respectively and the regression equations were computed. Factorized extended absorbance difference spectrum for ACE was obtained via dividing the (D0) of any pure ACE's concentration within its linearity range by the numerical value of absorbance difference (ΔA) at 316.0 nm and 320.0 nm using spectrophotometric software.

Paracetamol's factorized absorbance difference spectrum was obtained via dividing the (D0) of any PAR's concentration within its linearity range by the numerical value of absorbance difference (ΔA) at 250.0 nm and 298.0 nm using spectrophotometric software.

4.2.2. Window III

Constant value amplitudes of ACE's ratio spectra using ACE's normalized spectrum as a divisor in the region 316.0 nm–320.0 nm and peak amplitudes at P249.8 and P250.8 of PAR's and RAB's ratio spectra using ACE's normalized spectrum as a divisor were constructed versus their corresponding concentrations, respectively then computing their regression equations. Equality factor of pure RAB's ratio spectra using ACE's normalized spectrum as a divisor was prepared by getting the average of amplitude ratios at 249.8 nm (P249.8) and 292.4 nm (P292.4).

Factorized induced ratio spectrum for PAR was prepared via dividing the ratio spectrum of any PAR's concentration within its linearity range using ACE's normalized spectrum as a divisor by the numerical value of amplitude difference at 249.8 nm and 292.4 nm, after multiplying the latter by F: \((P\text{249.8-F292.4})\).

4.2.3. Window IV

The calculated peak-to-peak amplitude measurements at 306.5 nm and 315.9 nm (P2315.9) for (D0) of ACE using PAR's (10.0 μg/mL) normalized spectrum as a divisor and at 243.4 nm and 256.0 nm (P243.4-P256.0) for (D0) of PAR and at 245.1 nm and 257.8 nm (P245.1-P257.8) for (D0) of RAB using ACE's (10.0 μg/mL) normalized spectrum as a divisor were constructed against their corresponding concentrations, respectively then their regression equations were computed. Equality factor of pure RAB's (D0) using ACE's normalized spectrum as a divisor was prepared by getting the average of amplitude ratios at 299.5 nm (P299.5) and 256.0 nm (P256.0).

Factorized derivative ratio spectrum for ACE was prepared via dividing (D0) of any ACE's concentration within its linearity range using Δλ = 4 and scaling factor = 10.0 using PAR's normalized spectrum as a divisor by the numerical value of amplitude summation at 305.8 nm and 316.8 nm.
Factorized induced derivative ratio spectrum for PAR was obtained via dividing the (DD^1) of any PAR's concentration within its linearity range using \( \Delta \lambda = 4 \) and scaling factor = 10.0 using ACE's normalized spectrum as a divisor by the numerical value of amplitude difference at 256.0 nm and 299.5 nm, after multiplying the former by \( F: (P_{256.0} - P_{299.5}) \).

### 4.3. Analysis of laboratory-prepared mixtures

Solutions containing diverse ratios of ACE, PAR, and RAB were prepared to test the specificity of the proposed spectrophotometric methods. Recording each mixtures' spectra at 200.0–400.0 nm was performed and stored in the computer. Stepwise sample enrichment was applied via spectrum addition (In Silico) technique for laboratory prepared mixtures containing low concentrations of ACE and RAB out of their linearity range using the scanned spectrum of 5.0 \( \mu \)g/mL of ACE's pure standard and 15.0 \( \mu \)g/mL of RAB's pure standard which were added to each recorded mixture's spectrum using the spectrophotometer's software before applying the corresponding manipulating steps for each method.

#### 4.3.1. Window I

For each stored (D^0) of ternary laboratory prepared mixture containing different ratios of ACE, PAR, and RAB enriched with 5.0 \( \mu \)g/mL of pure standard of ACE via spectrum addition, the absorbance difference at 316.0 nm and 320.0 nm was recorded and multiplied by ACE's factorized extended absorbance difference spectrum to get the pulled parent (D^0) of ACE. The binary mixture of PAR and RAB was obtained via subtracting ACE's pulled parent spectrum from the ternary mixture. The resolved binary mixture containing PAR and RAB was then enriched with 15.0 \( \mu \)g/mL of RAB's pure standard. Obtaining the parent (D^0) of PAR was achieved by recording the absorbance difference at 250.0 nm and 298.0 nm and multiplying it by PAR's factorized absorbance difference spectrum. Finally, RAB's parent spectrum was obtained after subtracting the previously recovered (D^0) of PAR from the binary mixture. The concentrations of the cited drugs were calculated via regression equation constructed at the corresponding maxima of each drug. The actual concentrations of ACE and RAB in each mixture were calculated after eliminating the added concentrations.

#### 4.3.2. Window III

The stored (D^0) of each ternary laboratory prepared mixture containing different ratios of ACE, PAR, and RAB enriched with 5.0 \( \mu \)g/mL of pure standard of ACE via spectrum addition was divided by the normalized pure ACE's spectrum to obtain its corresponding ratio spectrum. The constant value amplitude of the mixture in the region 316.0–320.0 nm was recorded. ACE's concentration was calculated via regression equation constructed at the corresponding ACE's constant values at the selected wavelength region. A resolved binary mixture comprising PAR's and RAB's ratio spectrum will be obtained via subtracting the constant value of ACE from the ternary laboratory prepared mixture's ratio spectrum by constant subtraction method. This resolved binary mixture's ratio spectrum was enriched with RAB's (15.0 \( \mu \)g/mL) ratio spectrum via spectrum addition. The calculated amplitude difference (AP) of each mixture at 249.8 nm and 292.4 nm, after multiplying the latter by RAB's equality factor (F) was multiplied by the previously generated PAR's factorized induced ratio spectrum obtaining a new ratio spectrum representing PAR/ACE' in the mixture. Finally, subtracting the generated PAR/ACE' ratio spectrum from that of the binary mixture will generate the ratio spectrum of RAB/ACE'. The concentrations of PAR and RAB were calculated via regression equation constructed at the corresponding amplitude maxima of each drug. The actual concentrations of ACE and RAB in each mixture were calculated after eliminating the added concentrations.

#### 4.3.3. Window IV

For ACE, the stored (D^0) of each ternary laboratory prepared mixture enriched with 5.0 \( \mu \)g/mL of pure standard of ACE via spectrum addition was divided by PAR's normalized spectrum and then the first derivative (DD^1) of the obtained spectrum was manipulated using \( \Delta \lambda = 4 \) nm and scaling factor = 10.0. Amplitude summation at 305.8 nm and 316.6 nm was calculated then the total value was multiplied by the previously prepared ACE's factorized derivative of ratio spectrum obtaining a recovered (DD^1) representing ACE/PAR.

For PAR and RAB, the stored (D^0) spectrum of each ternary laboratory prepared mixture enriched with 15.0 \( \mu \)g/mL of pure standard RAB via spectrum addition was divided by ACE's normalized spectrum then the (DD^1) of the obtained spectrum was manipulated using \( \Delta \lambda = 4 \) nm and scaling factor = 10.0. The calculated amplitude difference at 256.0 nm and 299.5 nm, after multiplying the former by RAB's equality factor (F) was then multiplied by the previously prepared PAR's factorized induced derivative of ratio spectrum obtaining a new (DD^1) representing PAR/ACE' in the mixture. RAB/ACE (DD^1) was obtained after subtracting the generated (DD^1) of PAR/ACE' from that of the ternary mixture. The concentrations of the cited drugs were calculated via regression equation constructed at the corresponding amplitudes (P_max-P_min) of each drug. The actual concentrations of ACE and RAB in each mixture were calculated after eliminating the added concentrations.

#### 4.4. Pharmaceutical formulation's assay

Ten ACE- Proxyvon® tablets were weighed accurately and a quantity of tablet powder equivalent to 2.0 mg of ACE, 10.0 mg of PAR, and 0.2 mg of RAB was transferred into 100-mL beaker, 30 mL of ethanol was added, the solution was sonicated for 15 min, filtered through Whatmann filter paper, Grade 1 into a 100-mL volumetric flask and then volume was completed to mark with same solvent. A solution claimed to contain 2.4 \( \mu \)g/mL of ACE, 12.0 \( \mu \)g/mL of PAR and 0.24 \( \mu \)g/mL of RAB was prepared by an appropriate dilution. Analysis of each studied drug was achieved by applying the proposed methods previously mentioned under the analysis of laboratory prepared mixtures. The corresponding regression equations were employed for calculating the cited drugs' concentrations then the mean recoveries were calculated.

#### 4.5. Application of spectral similarity index (SSI)

#### 4.5.1. Window I

The ratio of absorbance differences (\( \Delta A \)) was calculated for the same concentration of the (D^0) spectrum of each proposed drug extracted from either laboratory mixture's or dosage form's analysis and its authentic spectrum at (A_{277.2} - A_{316.0}) for ACE, (A_{250.0} - A_{298.0}) for PAR and (A_{284.5} - A_{310.0}) for RAB.

#### 4.5.2. Window III

Amplitude differences' ratio (\( \Delta P \)) was calculated for the same concentration of the ratio spectrum of each proposed drug extracted from either laboratory mixture's or dosage form's analysis and its authentic ratio spectrum at (P_{251.0} - P_{220.0}) for ACE, (P_{249.8} - P_{276.0}) for PAR and (P_{250.8} - P_{292.8}) for RAB.

#### 4.5.3. Window IV

Amplitude differences' ratio (\( \Delta P \)) was calculated for the same concentration of the (DD^1) spectrum of each proposed drug.
extracted from either laboratory mixture’s or dosage form’s analysis and its authentic (DD) at (P_{206.5-P_{215.5}}) for ACE, (P_{243.4-P_{256.0}}) for PAR and (P_{245.1-P_{257.8}}) for RAB.

5. Results and discussion

An ongoing global pandemic of COVID-19 has spread with alarming speed, infecting millions and bringing economic activity to a near-standstill as countries imposed tight restrictions on movement to halt the spread of the virus. As the health and human toll grows, the economic damage is already evident and represents the largest economic shock in the world. For that unfortunate reason, many obstacles and challenges have been hardly faced during the analysis of pharmaceutical preparations in many quality control laboratories as there is an urgent need nowadays to apply impeccable, simple, accurate and time-consuming analytical methods, meanwhile, taking into consideration to be economical trying to save the huge analytical expenses during drugs’ analysis. A great opportunity has been offered for the mathematical spectrophotometric techniques with their simple, economical, accurate and time saving characteristics to take the leading pathway over the other hyphenated techniques as HPLC or capillary electrophoresis, which permanently demand former dreary set up measures and extensive expenses. Additionally, the newly developed mathematical spectrophotometric techniques have proven their credit over the chemometric ones which require purchasing highly expensive and special software which is not commensurate with the problematic catastrophe facing the whole world nowadays specially in the developing countries.

Accordingly, all the pre-mentioned inspirations should be carefully noted during the quantification of complex binary or ternary mixtures with overlapping spectra utilizing newly developed methods.

Through scrutiny, ACE, PAR and RAB’s absorption spectra spectacle high overlapping which unfortunately led to many difficulties and challenges during the studied drugs’ simultaneous determination. Moreover, exacerbation of this spectral obstacle was clearly noticed as zero order, ratio or derivative of ratio forms was the main target during studying the newly developed methods to successfully restore typical absorption, ratio or derivative spectra of the analytes under investigation from their laboratory prepared mixtures and their matrix in dosage form, thus, confirming the spectral profile of each analyte with comparing the requirements and limitations of each window using spectrophotometric platform via different windows (zero, ratio and derivative ratio). Furthermore, the extracted spectra of the analytes can be used for assessing their spectral similarity index in their formulation when compared with the original spectra acquired from the authentic analytes using the same spectrophotometric platform thus offering a beneficial counterfeit product’s detection.

5.1. Window 1

As presented in Fig. 2.a, ACE, PAR and RAB showed an overlapped spectra within a wavelength range of 200.0 nm – 315.5 nm with ACE’s slight extension over the rest of the components at 316.0 nm–320.0 nm in the presence of up to 15.0 µg/mL RAB.

5.1.1. Extended absorbance difference coupled with spectrum subtraction method (EAD-SS) for ACE

For prepared mixture’s (D) scanned spectrum comprising ACE, PAR and RAB, some mixtures are containing very low concentrations of ACE (out of its linearity range). Hence, in silico sample enrichment was employed via spectrum addition using pure standard ACE’s spectrum (5.0 µg/mL) to get ACE’s augmented spectrum (claimed and added). The mixture’s absorbance difference (ΔA) value was calculated at the extended wavelength region at 316.0 nm–320.0 nm then this value was multiplied by the previously prepared ACE’s factorized extended absorbance difference spectrum to extract parent (D) of ACE; Fig. 2.b.

Actual concentration of ACE in the mixture was calculated via subtracting ACE’s added concentration from its augmented concentration calculated by substituting in corresponding regression equation relating absorbance values of ACE at its λ_{max} 277.2 nm and its corresponding concentrations in the range (4.0–40.0 µg/mL), Table 1.

The extracted parent (D) of ACE was pulled from the corresponding ternary mixture’s gross spectrum via spectrum subtraction to get the resolved binary mixture of PAR and RAB which was then enriched via spectrum addition using pure standard RAB’s spectrum (15.0 µg/mL); Fig. 2.c.

5.1.2. Absorbance difference coupled with spectrum subtraction method (AD-SS) for PAR and RAB

This proposed method was applied for PAR’s analysis in each resolved (D) binary mixture enriched with 15.0 µg/mL of pure standard RAB. Two wavelengths were selected; 250.0 nm and 298.0 nm which correspond to PAR, meanwhile, opposing two equal absorbance readings for RAB, Fig. 2.c.

The parent (D) of PAR was reproduced from each mixture; Fig. 2.d by recording the mixture’s (ΔA) value at 250.0 nm and 298.0 nm and its multiplication by the previously prepared PAR’s factorized absorbance difference spectrum. The concentration of the extracted PAR from each mixture was obtained by substituting the recorded absorbance value in the regression equation relating absorbance values of pure PAR at its λ_{max} 250.0 nm and the corresponding concentrations in the range (2.0–14.0 µg/mL), Table 1.

Subtracting PAR’s (D) spectrum from the (D) of the binary mixture will produce RAB’s parent (D) spectrum; Fig. 2.e. By substituting the recorded absorbance value in the regression equation relating absorbance values of RAB at its λ_{max} 284.5 nm and the...
corresponding concentrations in the range (4.0–30.0 μg/mL); Table 1, the augmented concentration of RAB in each mixture (claimed and added) was calculated then subtracted from the added pure standard RAB’s concentration obtaining the claimed concentration of RAB in each mixture.

5.2. Window III

An overlapped ratio spectra were surveyed for ACE, PAR and RAB within a wavelength range of 200.0 nm – 315.5 nm with ACE’s slight extension over the rest of the components at 316.0 nm-320.0 nm in the presence of up to 15.0 μg/ml RAB.

5.2.1. Constant value coupled with constant subtraction method (CV-CS) for ACE

In mixtures with ACE’s concentration out of linearity range, a constant value was recorded at the extended part; 316.0 nm-320.0 nm; Fig. 3.a. in ratio spectrum comprising ACE, PAR and RAB enriched with ACE pure standard spectrum addition (5.0 μg/mL) using ACE’s normalized spectrum as a divisor.

This constant value has been effectively utilized in the determination of ACE in its corresponding mixture; Fig. 3.b. The augmented concentration of ACE (actual + added) was calculated via its regression equation representing linear relationship between ACE’s constant value amplitudes in the region 316.0 nm-320.0 nm and the corresponding concentrations in the range (4.0–40.0 μg/mL), Table 1.

The actual concentration of ACE found in the mixture could be then calculated by subtracting the added concentration of ACE from the augmented concentration.

The resolved PAR’s and RAB’s ratio spectrum could be obtained via subtracting ACE’s constant value from the ternary mixture’s ratio spectrum. This resolved binary mixture was then enriched with pure RAB’s ratio spectrum (15.0 μg/mL) using normalized ACE as a divisor; Fig. 3.c.

5.2.2. Induced dual amplitude difference coupled with spectrum subtraction method (IDAD-RS) for PAR and RAB

Two wavelengths; 249.8 nm and 292.4 nm were selected on PAR’s ratio spectrum. The equality factor (F); which is the average

Fig. 2. Spectral scheme of resolution of 7.4 μg/mL (2.4 + 5.0 μg/mL) of ACE ( ), 12.0 μg/mL PAR ( - - - ) and 15.24 μg/mL (0.24 + 15.0 μg/mL) of RAB ( ) in their dosage form’s ratio using spectrophotometric platform window I.
Table 1
Assay parameters and results of determination of pure samples of ACE, PAR and RAB by the proposed methods.

| Parameter | Window I | Window III | Window IV |
|-----------|----------|------------|-----------|
|           | EAD-SS   | AD-SS      | CV-CS     | IDAD-SS   | FDD-NC   | FUDD-SS   |           |
| Range (µg/ml) | 4.0–40.0 | 2.0–14.0  | 4.0–30.0  | 4.0–40.0  | 2.0–14.0 | 4.0–30.0  | 4.0–40.0  |
| Linearity |          |           |           |           |           |           |           |
| Intercept | 0.3775   | 0.0982    | 0.0406    | 0.9705    | 7.7282   | 1.5267    | 5.1514    |
| Slope    | 0.0091   | -0.0044   | 0.0025    | 0.3282    | -0.3668  | 0.1288    | 5.2121    |
| Correlation coefficient (r) | 0.9999 | 0.9998 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 0.9999 |
| Mean ± SD | 100.17 ± 0.67 | 100.01 ± 1.16 | 99.46 ± 0.82 | 99.89 ± 0.92 | 99.73 ± 1.43 | 100.36 ± 0.66 | 100.03 ± 1.54 | 99.84 ± 0.73 | 100.21 ± 1.00 |
| Accuracy |          |           |           |           |           |           |           |
| Mean ± SD | 100.09 ± 0.26 | 100.49 ± 0.37 | 100.38 ± 0.81 | 98.29 ± 1.44 | 100.39 ± 1.10 | 100.32 ± 1.02 | 99.84 ± 1.01 | 100.88 ± 1.12 | 99.95 ± 0.71 |
| Precision |          |           |           |           |           |           |           |
| RSD% a | 1.200 | 1.122 | 0.998 | 0.956 | 1.158 | 1.222 | 1.200 |
| RSD% b | 1.302 | 1.256 | 1.304 | 1.321 | 1.362 | 1.403 | 1.401 |

Window I: Applied on zero-order spectrum.
Window III: Applied on ratio spectrum.
Window IV: Applied on derivative of ratio spectrum.
RSD% a, RSD% b: Repeatability & intermediate precision, respectively (n = 3) relative standard deviation of concentrations (10.0, 15.0, 25.0 µg/ml for ACE, 4.0, 8.0, 13.0 µg/ml for PAR and 6.0, 12.0, 25.0 µg/ml for RAB.

Fig. 3. Spectral scheme of resolution of 7.4 µg/mL (2.4 + 5.0 µg/mL) of ACE ( ), 12.0 µg/mL PAR (- - - ) and 15.24 µg/mL (0.24 + 15.0 µg/mL) of RAB ( ) in their dosage form’s ratio using spectrophotometric platform window III.
of the ratio of amplitudes for different concentrations of RAB's ratio spectra using ACE's normalized spectrum as a divisor at the two selected wavelengths (249.8 nm and 292.4 nm); \[ \frac{P_{249.8}}{P_{292.4}} \] was calculated and was found to be equal 1.17. This factor prospered to equalize the amplitudes of the interfering substance; RAB at the two selected wavelengths, while PAR's amplitudes were different, Fig. 3.c.

For each resolved binary mixture, the \( D_P \) of the mixture's ratio spectrum at 249.8 nm and 292.4 nm, after multiplying the later by \( F \) was calculated then this value was multiplied by the previously prepared PAR's factorized induced ratio spectrum to obtain a new spectrum representing the ratio spectrum of PAR/ACE' in the mixture, Fig. 3.d.

The concentration of PAR was calculated from the regression equation representing linear relationship between the amplitude values of PAR at its \( P_{\text{max}} \); 249.8 nm and its corresponding concentrations in the range (2.0–14.0 \( \mu \text{g/mL} \), Table 1.

Subtracting the obtained PAR's ratio spectrum from that of the resolved binary mixture successfully produced RAB's ratio spectrum; Fig. 3.e. By substituting the recorded amplitude value in the regression equation relating amplitude values of RAB's ratio spectra at its \( P_{\text{max}} \); 250.8 nm using ACE as a divisor and the corresponding concentrations in the range (4.0–30.0 \( \mu \text{g/mL} \); Table 1, the augmented concentration of RAB in each mixture (actual and added) was calculated then subtracted from pure standard RAB's added concentration to obtain RAB's actual concentration in each mixture.

5.3. Window IV

This applied window depended on using ACE's normalized spectrum as a divisor for the determination of PAR and RAB. As well as using PAR's normalized spectrum as a divisor for ACE's determination without any restrictions limiting RAB's concentration.

5.3.1. Factorized derivative ratio null contribution (FDD-NC) for ACE

For each ternary laboratory prepared mixture containing out of linearity ACE's range and enriched with pure standard ACE's spectrum addition (5.0 \( \mu \text{g/mL} \), the stored (\( D^0 \)) spectra was divided by...
PAR’s normalized spectrum and then the first derivative (DD$^1$) of the obtained spectra was manipulated using $\Delta \lambda = 4$ nm and scaling factor = 10.0. The amplitude sum at 305.8 nm and 316.6 nm was calculated and was related to ACE without any interference of RAB which showed equal amplitudes at the selected wavelengths, while PAR was cancelled; Fig. 4a. By multiplying the amplitudes’ summation by the previously prepared ACE’s factorized derivative ratio spectrum, a new spectrum representing the (DD$^1$) of ACE/PAR in the mixture; Fig. 4b was obtained.

A linear relationship was obtained between $P_{\text{max-min}}$ (306.5 nm and 315.9 nm) of ACE and the corresponding concentrations in the range (4.0–40.0 $\mu$g/mL), Table 1.

The added concentration of ACE was subtracted from its augmented concentration calculated by substituting in the corresponding regression equation to obtain the actual concentration of ACE found in the mixture.

5.3.2. Factorized unlimited derivative ratio coupled with spectrum subtraction method (FUDD-SS) for PAR and RAB

Compared to the application of the conventional derivative ratio methods for ternary mixtures; zero crossing, double divisor and successive derivative ratio, this novel approach offered a great vision for the analysis of the studied challengeable ternary mixture to enhance the specificity of the quantification of PAR in presence of the interfering components; ACE and RAB.

This proposed FUDD method was applied for the analysis of PAR in ternary mixtures comprising RAB out of its linearity range thus, enriched with (D$^0$) RAB’s spectrum addition (15.0 $\mu$g/mL). This novel approach implies the integrational use of zero- difference technique cancelling one of the two interfering components; ACE while the other; RAB has two equalized amplitudes at the selected wavelengths ($\lambda_1$ and $\lambda_2$). This equalization was obtained using an equality factor for RAB which represents the average of the ratio of the amplitudes of the first derivative of the ratio spectra of different concentrations of RAB using ACE’s normalized spectrum as a divisor; $[P_{299.5}/P_{256.0}]$ at $\Delta \lambda = 4$ nm and scaling factor = 10.0 and it was found to be equal 1.75. The $\Delta P$ of the mixture’s (DD$^1$) spectrum at 256.0 nm and 299.5 nm, after multiplying the former by F was calculated and was related to pure PAR; Fig. 4c. By multiplying this value by the previously prepared PAR’s factorized induced derivative of ratio spectrum, a new spectrum representing the (DD$^1$) of PAR/ACE in the mixture; Fig. 4d was obtained.

The concentration of PAR in each mixture was calculated via its regression equation representing a linear relationship between $P_{\text{max-min}}$ (243.4 nm and 256.0 nm) of PAR and the corresponding concentrations in the range (2.0–14.0 $\mu$g/mL), Table 1.

RAB’s (DD$^1$) spectrum; Fig. 4e was resolved via subtracting the obtained PAR’s (DD$^1$) spectrum from the ternary mixture’s (DD$^1$) spectrum. By substituting the recorded amplitude value of resolved RAB’s (DD$^1$) spectrum in the regression equation relating linear relationship of RAB’s amplitude values at its $P_{\text{max-min}}$ (243.1 nm and 257.8 nm) and the corresponding concentrations in the range (4.0–30.0 $\mu$g/mL); Table 1, the augmented concentration of RAB in each mixture was calculated then subtracted from the RAB’s added concentration to obtain RAB’s actual concentration in each mixture.

6. Method validation

6.1. Range and linearity

Through considerations of the studied drugs’ practical range according to adherence to Beer’s law and their concentration in the pharmaceutical preparation giving accurate, precise and linear
results, the calibration ranges for ACE (4.0–40.0 μg/mL), PAR (2.0–14.0 μg/mL) and RAB (4.0–30.0 μg/mL) were established, Table 1.

6.2. Accuracy and precision

Accuracy of pure powdered samples of ACE, PAR and RAB was listed in Table 1. The concentrations used in accuracy testing were (10.0, 15.0 and 25.0 μg/mL) of pure ACE, (4.0, 8.0 and 13.0 μg/mL) of pure PAR and (6.0, 12.0 and 25.0 μg/mL) of pure RAB.

Moreover, precision was validated through evaluating the relative standard deviation (RSD) of three concentration levels in triplicate analysis either on the same day (intraday precision) or on three different days (inter-day precision). RSD % was found to be less than 2%; Table 1.

6.3. Specificity

Assessed by analyzing different laboratory-prepared mixtures containing ACE, PAR and RAB fulfilling the dosage form’s ratio. The relative standard deviation (RSD) showed good percentage recovery with the lowest standard deviation among the other methods; Table 2.

The mixture fulfilling the dosage form’s ratio (10.0:50.0:1.0) for ACE, PAR and RAB in triplicate was chosen to check the effect of mathematical filtration manipulation steps using the three windows of spectrophotometric platform on the accuracy and precision of the obtained concentration results of each component in the mixture. The recovery studies were carried out at various concentrations in in-silico enrichment by comparing the average of individual concentration for each studied drug in the prepared mixtures (in triplicate) by the numerical claimed concentration values. The results abridged in Table 2 ensured that all methods were accurate as the percentage of error (E %); [experimental value - theoretical value] / theoretical value × 100% was less than 2. Where, E% for window I were 0.48%, 0.05% and 0.05%. While for window III, E% were 1.3 %, 0.42 % and 0.98 % and E% for window IV were 1.0%, 0.33% and 0.08% for ACE, PAR and RAB, respectively; Fig. 5. Moreover, precision of the obtained recovery percentages in triplicate for each component was checked and it was found that the results are precise since RSD% is less than 2%; 0.312 %, 0.637 %, 0.889 % for window I. While, for window III % RSD were 1.245 %, 1.163 % and 1.244 % and % RSD for window IV were 1.072 %. 0.933 % and 1.048 % for ACE, PAR and RAB, respectively. Based on the achieved results, it can be concluded that (window I) applied on regression equations using maxima (Amax) of each drug versus concentrations for calculating concentrations of components in the samples significantly increases the accuracy and precision of the obtained results due to minimum signal to noise ratio.

Drugs’ determination in ACE- Proxyvon® tablets was successfully achieved through applying the proposed methods and methods’ validity was further assessed by applying the standard addition technique. The results of tablets obtained in good agreement with the labeled claim as apparent from the satisfactory values of recovery and SD; Table 3. Further, the recovery studies of standard addition indicate that all the methods are practically free from interference due to tablets additives.

7. Solutions’ stability

No practical changes were noted in the concentration as evident from spectrophotometric measurements. Where, the stock and calibration solutions of the studied drugs in ethanol remained unaffected for a minimum period of 2 weeks when stored at 4°C.

8. Statistical analysis

The calculated student’s t and F values showed lower values than the theoretical ones through the statistical assessment of the proposed methods and comparing the obtained results with that of the reported methods of ACE, PAR and RAB in pure powdered form, respectively which proves that there was no considerable difference between the proposed and reported HPLC methods [30] regarding both accuracy and precision, Table 4.

9. Spectral similarity index

The developed spectrophotometric methods’ power to extract the spectrum is well ascertained through calculating SSI which is based on comparing the resolved drug with the authentic one through calculating the ratio of the absorbance difference or amplitudes difference at two selected wavelengths (λ1 and λ2) for the same concentration of the extracted raw spectrum and authentic spectrum of the proposed drug ([A1,A2] of extracted spectrum / [A1,A2] of authentic spectrum) in case of window I or ([P1,P2] of extracted spectrum / [P1,P2] of authentic spectrum) in case of window III or ([P1,P2] of extracted spectrum / [P1,P2] of authentic spectrum) in case of window IV and it should be around one for confirming spectral similarity. The efficiency of SSI was approved through its application on laboratory mixtures where, satisfactory results were obtained; 0.998–1.000.
### Table 4
Statistical analysis of the proposed methods and the reported methods of ACE, PAR and RAB in their pure powdered form.

| Parameter | Acetaminophen (ACE) | Paracetamol (PAR) | Rabeprazole (RAB) |
|-----------|---------------------|-------------------|-------------------|
| Mean      | 100.17              | 99.46             | 100.00            |
| SD        | 0.67                | 0.92              | 1.34              |
| n         | 7                   | 7                 | 6                 |
| Variance  | 0.4489              | 0.8464            | 2.3716            |
| Student’s t test (2.201) | 0.521            | 0.976             | 0.771             |
| F*        | (4.71)              | (4.71)            | (4.95)            |

| Parameter | EAD-SS   | CV-CS   | FDD-NC  | Reported method ** [27] |
|-----------|----------|---------|---------|-------------------------|
| ACE      | 99.89    | 99.92   | 100.03  | 99.76                   |
| PAR      | 0.92     | 1.34    | 1.44    |                         |
| RAB      | 0.8464   | 2.3716  | 2.0736  |                         |

| Parameter | IDAD-SS   | FUDD-SS  | Reported method ** [27] |
|-----------|-----------|----------|-------------------------|
| ACE      | 99.84     | 99.44    | 99.07                   |
| PAR      | 0.73      | 1.00     | 1.33                    |
| RAB      | 0.329     | 0.820    | 1.7689                  |

| F*        | (4.71)    | (4.71)   | (4.95)                |
|-----------|-----------|----------|-----------------------|
|           | 4.62      | 2.45     | 1.14                  |

* The figures in parenthesis are the corresponding theoretical values at P = 0.05.
** RP-HPLC reported method for ACE and RAB determination using C-18 column as stationary phase and methanol: acetonitrile: water (60: 10: 30 v/v/v) as the mobile phase at a flow rate of 1.0 ml/min at ambient temperature and detected at 280.0 nm.
*** RP-HPLC reported method for PAR determination using C-18 column as stationary phase and methanol: 0.01 M phosphate buffer, pH 5.0 (30: 70 v/v) as the mobile phase at a flow rate of 1.0 ml/min at ambient temperature and detected at 243.0 nm.
10. Counterfeit products’ detection

The issue of counterfeit drugs has been rising in importance worldwide. Counterfeit drugs cause significant economic loss to the pharmaceutical industry as they are taking income from consumers and drug companies [43]. Moreover, they possess adverse impacts on human lives, as they pose health hazards to patients that may unfortunately lead to death.

Detection of counterfeit drug is highly critical, the extracted spectrum of each component in the tablet is used to calculate their actual concentration via the proposed methods using the specified manipulation procedures of the window and corresponding regression equation. Moreover, the found% of pharmaceutical dosage form is also used to confirm the accurate potency of the proposed drugs. The spectral similarity index is calculated for the extracted spectrum of each component using the spectrum of the same concentration of authentic, Fig. 6. High value of spectral similarity index (far from 1) confirming counterfeiting.

11. Credit introduced by the developed methods

The main credit introduced by the proposed methods is their direct utilization for mixtures’ quantification based on applying factorized spectra which depend on the recorded response rather than concentration and spectral analytes’ resolution by means of a minimal arithmetic operation of the response in addition to minimal manipulation steps of the utilized spectrophotometer software. An additional important advantage of using the factorized spectra is that it possesses the capability of recovering the corresponding spectrum of each analyte present in the investigated mixtures separately and in consequence allowing their determination at their maxima without tedious searching of zero crossing point or zero or null contribution for the interfering analyte(s). Moreover, the extracted spectra of the analytes (zero, ratio or derivative ratio) are considered a spectral profile thus, giving the best accuracy and precision.

12. Greenness profile assessment

Green analytical chemistry focuses on developing environmentally benign analytical procedures with a high humans’ safety. The amounts and toxicity of reagents, generated waste, energy requirements, the number of procedural steps, miniaturization, and automation are just a few of the multitude of criteria considered when assessing an analytical methodology’s greenness. The use of greenness assessment criteria requires dedicated tools. Thus, the greenness profile of the proposed spectrophotometric method was appraised via the recently published AGREE tool which is a comprehensive, flexible, and straightforward downloadable software creating a colored pictogram with twelve sections that cover the twelve principles of green analytical chemistry (SIGNIFICANCE) [36,37]. Each section is colored ranging from deep green to deep red based on its environmental impact while the overall assessment score appears in the middle of the circular pictogram, Fig. 7.
illustrates the obtained AGREE pictograms of the proposed spectrophotometric method and the reported HPLC methods used for the proposed drugs’ analysis where, it was found that the score of the spectrophotometric methods; 0.74 was greener than the reported ones; 0.56 for PAR [14] and 0.53 for ACE/ RAB [27] which was attributed to the fulfilling of the 12 required principles for greenness evaluation. In a nutshell, the successfulness of the proposed methods in reducing solvent consumption and instrumental energy as well as the utilization of ethanol were obviously reflected by the high AGREE score.

13. Conclusion

The developed methods for the selective determination of ACE, PAR and RAB using different windows of spectrophotometric platform are simple, accurate and precise. Furthermore, it does not require specific software for complex mathematical treatment of the data which is a prerequisite for chemometric methods. Window I; applied on (D0) spectrum of the ternary mixture was able to determine the three components of the mixture at their maxima. While, window III was applied on ratio spectrum of the mixture for analyzing the target drugs via simple procedures using one divisor only. Finally, the procedure of derivative ratio; window IV is comprised of two successive procedures, which included a division step followed by derivatization using two divisors. DD1 spectra offer several maxima and minima which aid in determining active components in the presence of other components. Besides, Windows I and IV provide better resolution of the components using automated manipulation of the spectral data and help in minimizing mutual interference between the drugs compared to the window III. Additionally, it is worth noted that in silicon sample enrichment is proven to be more beneficial than in lab enrichment due to the application of stepwise spectrum addition and this was obvious in both windows; I and III as there was an urgent need for ACE’s extension over the rest of the components. While in window IV, ACE was determined using different divisor than that needed for PAR’s and RAB’s determination without being affected by RAB’s interference. Additionally, based on the overall performance of these methods, they can be readily applied to detect counterfeit products without the need to use sophisticated instruments like reported HPLC. Finally, the greenness profile of the proposed methods was thoroughly assured via the recently published (AGREE) assessment tool. The distinctive simplicity, accuracy and reliability of the proposed methods along with their high greenness profile promoted their applicability in quality control laboratories.

CRediT authorship contribution statement

Dina A. Ahmed: Conceptualization, Methodology, Software. Data curation, Software, Validation, Writing – original draft, Writing – review & editing. Hayam M. Lotfy: Visualization, Methodology, Investigation, Software, Data curation, 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.

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