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Eco-friendly spectrophotometric evaluation of triple-combination therapies in the treatment strategy of patients suffering from hypertension during coronavirus pandemic – Spectralprint recognition study

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HIGHLIGHTS

• Innovative, eco-friendly spectrophotometric evaluation of triple antihypertensive combination therapy.
• Spectralprint recognition study of a single-pill of valsartan (VAL), amlodipine besylate (AML) and hydrochlorothiazide (HCT) in challengeable ratio.
• Full elimination of hazardous solvents, tiresome manipulation and expensive instruments.
• Declaration of method sustainability through the most common and advanced green assessment tools.
• Validation and confirmation of all suggested methods was conducted in accordance with ICH guidelines.
• Successful assay of the studied drugs in their combined formulations without any excipients’ interference.

ABSTRACT

Recent studies have reported that using certain antihypertensive therapies such as angiotensin II receptor blockers (ARBs) and calcium channel blocker (CCBs) is associated with reduction of fatal outcomes and improving clinical characteristics of patients suffering from hypertension during coronavirus pandemic. Thus, in the current work an effective, innovative and eco-friendly spectrophotometric manner namely, parent spectrum extraction (PSE) was established for evaluation of recommended triple antihypertensive combination therapies incorporate valsartan (VAL) as ARBs, amlodipine besylate as CCBs (AML) and hydrochlorothiazide (HCT) as diuretic into single-pill in challengeable ratio. PSE manner composed of two complementary steps, auxiliary resolution coupled with data analysis resolution (DAR) and it is characterized by resolving the spectral bands of the drugs and extraction of their discrete parent spectra (D0). Accordingly, enabling determination of each analyte at its $\lambda_{\text{max}}$. Auxiliary resolution of AML in triple mixture was applied to decrease complexity of overlapped spectra via constant multiplication (CM) followed by spectrum subtraction (SS) to obtain resolved mixture of
1. Introduction

Cardiovascular disease is considered all over the world, as one of the most prevalent reasons of morbidity and mortality. Management and control of cardiovascular disease is performed by utilizing a combination of drugs, whereas each drug functions with a different and unique mechanism of action to succeed and accomplish the therapy’s goal. Hypertension is a major worldwide problem that affects about 30.0% of the world’s population. Hypertension is the cause of more than 12.8% of total deaths annually. The incidence of hypertension is growing globally as a result of ageing of the population and it increases in exposure to risk factors of lifestyle containing unhealthy diets (i.e. highly consumption of sodium and lower potassium diet) and also lacking of physical activity. Nevertheless, hypertension treatment was improved and enhanced significantly over the last two decades because of multiple combination therapies [1,2].

Patients with high blood pressure possess a two-fold greater risk and probability of death from the coronavirus COVID-19 in comparison to other patients lacking the condition. It further detected that hypertensive patients who were not on medicines to manage their condition had even increased probability and risk of mortality [3].

Valsartan (VAL) (Fig. 1a), is chemically designated as (2S)-3-methyl-2-[pentanoyl-[[4-[2-(2Htetrazol-5-yl) phenyl] phenyl] methyl] amino] butanoic acid [4]. Valsartan (VAL) is angiotensin II receptor blockers (ARBs) [5]. Various recent studies have reported that the use of available angiotensin receptor 1 (AT1R) blockers, such as VAL, can reduce the aggression and mortality associated with SARS-CoV-2 virus infection. This idea is relied on the observation that ACE2 is one of the enzymes that involved in renin-angiotensin system (RAS) cascade. Moreover, the virus spike protein binds and attaches to ACE2 to make a complex which is appropriate for cellular internalization. The ACE2 down regulation produces excessive angiotensin II accumulation, and it has been observed that the stimulation of the angiotensin II type 1a receptor (AT1R) leading to increase permeability of the pulmonary vascular, clarifying the increased lung pathology when ACE2 activity is decreased. At present, available AT1R blockers (ARBs) like valsartan, possess the potential to hinder and block this pathological process caused by angiotensin II. Currently, there are two complementary proposed mechanisms: 1) ARBs can block the excessive amount of angiotensin mediated AT1R activation, while 2) they upregulate ACE2, which lead to production of angiotensin II concentrations and increasing the formation of angiotensin 1-7 protective vasodilator. In a nut-shell, ARBs may prevent the development of acute respiratory distress syndrome (ARDS) and eschew morbidity (admittance to intensive care unit (ICU) and mechanical ventilation) and mortality [6–9], as shown in Diagram 1.

Amlodipine besylate (AML) (Fig. 1b), is one of calcium channel blocker CCBs, chemically designated as; benzene sulfonic acid;3-O-ethyl 5-O-methyl 2-(2-aminooethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate [4]. It is utilized for hypertension and angina pectoris’ management; it exerts its effect via blocking the influx transmembrane of calcium ions into cardiac and vascular smooth muscles [5]. Recent research focus on the vasodilatation effects of CCBs in the pulmonary and systemic vasculature which could palliate the effects of hyper coagulation, edema, local vasoconstriction, and inflammation produced as a response to the infection with SARS-CoV-2, thus enabling oxygen delivery and existence of host cells [10–12].

Hydrochlorothiazide (HCT) (Fig. 1c) is diuretic, chemically designated as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine7-sulfonamide1,1-dioxide [4]. It is a thiazide diuretic utilized to control and manage hypertension, and it is also effective for the treatment of edema which coupled with mild heart failure and with hepatic and renal disorders [5].

Single-pill combinations, which incorporate two antihypertensive drugs or more into one pill is recommended to achieve blood pressure goals, including significant reductions in cardiovascular morbidity and mortality without increasing the risk of adverse events. For the effective

![Fig. 1. The chemical structures of the proposed drugs: (a) Valsartan, (b) Amlodipine besylate and (C) Hydrochlorothiazide.](image-url)
pharmacological action of the proposed drugs, they are co-formulated in the dosage form (Exforge HCT® tablet) with two formulas with different ratios, the first one is 32.0:1.0:2.5 and for the second one is 16.0:1.0:2.5, of VAL, AML and HCT, respectively. Nowadays, these triple antihypertensive medicines attract the attention of researchers due to its dual pharmacological effects including the drug’s capability to reduce levels of the blood pressure (BP), which is the main goal factor to evade cardiovascular complications in these patients as well as lowering risk of coronavirus hospitalization and reduce the fatal outcomes of patients suffering from hypertension.

Literatures review for these triple antihypertensive formulations including spectrophotometry [13-20], chromatography [16,17,21-33] and electrochemical methods [34-35].

Mathematical spectrophotometric methods using built in spectrophotometer’s software are now being utilized more frequently for evaluation of drugs in pharmaceutical formulations [36-49] owing to their inherent benefits such as simplicity, accuracy, reduction of expensive reagents and eschewing of time consuming or special software. The objective of the current study was to develop a simple, precise, eco-friendly and economical parent spectrum resolution (PSR) manner for concurrent evaluation of VAL, AML and HCT in their triple antihypertensive medicines with trade name Exforge HCT® tablets with critical ratios so, sample enrichment technique [47,50] should be applied to increase the concentration of the minor component. The innovative PSR manner including novel methods namely, absorbance extraction (AE), peak-amplitude extraction (PE) and ratio extraction (RE) coupled with well-established constant multiplication (CM) and spectrum subtraction methods (SS) allowing the extraction of the parent absorption spectra (\( D^0 \)) of the studied drugs, separately. In addition, a comparable study has been conducted between the results attained by the proposed methods versus those attained by official and reported methods to ascertain the efficiency. Purity evaluation via spectralprint recognition index is a potential interpretation of the absorbance data obtained by methods in PSR manner using extracted parent spectrum of each drug in the combination which added superior advantages regarding human safety. Introducing of the developed methods’ greenness evaluation was performed in this study to avoid any hazardous and risky exposure to the environment or analyst while using and applying the proposed methods.

2. Theoretical background

2.1. Concept of parent spectrum extraction (PSE) manner

Parent spectrum extraction (PSE) manner focusing on the use of the recorded data (constant value) or mathematically analyzed data (analyzed absorbance, analyzed peak-amplitude and amplitude difference) of the studied mixture to extract the parent spectrum (zero order (\( D^0 \))) of each of co-formulated drug in the mixture. The proposed methods based on this concept using constant value [constant multiplication method (CM)] as well as absorbance [absorbance extraction method (AE)], peak-amplitude [peak-amplitude extraction method (PE)] and amplitude difference [ratio extraction method (RE)] are applied to evaluate each drug in the mixture via their maxima with superlative accuracy and precision. In addition, the extracted parent spectrum (\( D^0 \)) acts as the spectralprint of each component of interest which affirms its purity. Upon analysis of ternary mixtures, two complementary resolution steps namely, auxiliary resolution (using constant value) followed by data analysis resolution (DAR) through utilizing (analyzed absorbance, analyzed peak-amplitude, and amplitude difference) are applied for as follows:

2.1.1. Auxiliary resolution of ternary mixture

This auxiliary resolution is a powerful tool utilized in spectral analysis for resolving ternary mixture, where one of its components showing extension, to eliminate the extended one and get the resolved binary mixture in zero order or ratio spectra which can be analyzed by less complicated spectrophotometric methods with minimum requirement and limitation.

For analysis of X, Y and Z ternary mixture, upon dividing the mixture with a concentration of pure Z as a divisor so, component Z showing a constant all over the curve and parallel to the wavelength axis and its value in the mixture could be determined in the extended region. The constant value of each mixture is subtracted from its corresponding gross ratio spectrum of ternary mixture to get the ratio spectrum of the resolved binary mixture \([X + Y] / Z\) and saved in the computer. The constant value of Z is multiplied by\( D^0\) spectrum of Z divisor using constant multiplication method (CM) to restore \( D^0\) of Z followed by subtraction from its corresponding \( D^0\) gross spectrum of ternary mixture to get the \( D^0\) spectrum of the resolved binary mixture \([X + Y]\) and saved in the computer.

2.1.2. Data analysis resolution (DAR) scheme of the resolved binary mixture

This resolution scheme based on using the data of the resolved binary mixture to extract thier parent spectra. Thus, the analyst can use resolution tools as numerical factor or mathematic difference to eliminate the spectral influence of disturbing co-formulated component and calculate response value representing the target component. This extracted response is multiplied by factorized spectrum of drug of interest to get its corresponding parent spectrum either directly or via further manipulation. For extracting co-formulated component’s parent spectrum; spectrum subtraction of the extracted parent spectrum from the equivalent mixture is performed. Finally, the concentrations of the components in the binary mixture \([X + Y]\) can be calculated, separately via utilizing their regression equations at their maxima.

The data analysis resolution (DAR) for the resolved binary mixture can be performed via different methods based on the extent of
overlapping as follows:

2.1.2.1. Absorbance extraction method (AE). **Principle:**
This novel method can be utilized for the analysis of a saved resolved mixture of D\(\lambda\) of the two analytes (X and Y) which possessing partially overlapped spectra where D\(\lambda\) of Y is extended over that of X. By selection discrete pair of wavelengths (\(\lambda_1, \lambda_2\)) where X and Y having absorbance at (\(\lambda_1\)) while X doesn’t display any contribution at (\(\lambda_2\)).

**Resolution tools:**

1. Factorized D\(\lambda\) spectrum at (\(\lambda_1\)) (FSA\(\lambda_1\)): The scanned D\(\lambda\) of pure analyte X is divided by the value of its recorded absorbance at (\(\lambda_1\)) using spectrophotometer’s software.

Factorized D\(\lambda\) spectrum of Y = \(Y/A_{\lambda_1}\) (1)

2. Numerical absorbance factor: This factor representing the average of the calculated ratio between the absorbance values of different concentrations of pure component Y at (\(\lambda_1\)) (A\(\lambda_1\)) to those at (\(\lambda_2\)) (A\(\lambda_2\)).

**Manipulation steps of mixture:**

For analysis of resolved D\(\lambda\) spectrum of binary mixture via data analysis scheme, the absorbance of the mixture at (\(\lambda_1\)) while X doesn’t display any contribution at (\(\lambda_2\)).

Actual absorbance of Y in the mixture at (\(\lambda_1\)) = \(A_{\lambda_1} = A_{\lambda_2} \times A_{\lambda_2}(X + Y)\) (2)

where \(\frac{A_{\lambda_1}}{A_{\lambda_2}}\) is the absorbance factor of different concentrations of pure Y at (\(\lambda_1\)) and (\(\lambda_2\)) and \(A_{\lambda_2}(X + Y)\) is the mixture’s absorbance at (\(\lambda_2\)).

Multiplication of the obtained actual absorbance value (A\(\lambda_1\)) of Y in each mixture by the factorized spectrum of Y (Y/\(A_{\lambda_1}\)) was performed to restore its parent spectrum (D\(\lambda\)). For extracting the parent spectrum of X; spectrum subtraction of the extracted parent spectrum Y and equivalent mixture (X + Y) is carried out.

2.1.2.2. Peak-amplitude extraction method (PE). **Principle:**
This novel method used for the saved resolved ratio spectra of mixture composed of the two analytes (\((X + Y) / Z\) ) which possessing partially overlapping spectra where ratio spectrum of Y/Z’ is extended over that of X/Z’. By selection discrete pair of wavelengths (\(\lambda_1, \lambda_2\)) where X and Y having amplitudes at (\(\lambda_1\)) while X doesn’t display any contribution at (\(\lambda_2\)).

**Resolution tools:**

1. Factorized ratio spectrum of pure Y using P\(\lambda_1\) (FSP\(\lambda_1\)): The scanned D\(\lambda\) of pure component Y is divided by proper divisor of Z to attain ratio spectra Y/Z’. Afterwards, division of the attained ratio spectrum by the recorded amplitude at (\(\lambda_1\)) was carried out using spectrophotometer’s software.

Factorized ratio spectrum of Y = \((Y/Z')/P_{\lambda_1}\) (3)

2. Numerical amplitude factor: This factor representing the average of the calculated ratio between the amplitudes values of different concentrations of pure component Y at (\(\lambda_1\)) to those at (\(\lambda_2\)) (P\(\lambda_2\)).

Factorized ratio spectrum of X = \((X/Y')/\Delta P\) (5)

**Manipulation steps of mixture:**

For analysis of resolved ratio spectrum of binary mixture \((X + Y)/Z\) after auxiliary resolution of Z in the ratio spectrum, the amplitude of the mixture at (\(\lambda_2\)) is recorded, then the actual amplitude value of Y at (\(\lambda_1\)) can be calculated via the numerical amplitude factor equation as follows:

Actual amplitude of Y at P\(\lambda_1\) = \(P_{\lambda_1} \times P_2(X + Y)\) (4)

where \(\frac{P_{\lambda_1}}{P_2}\) is the amplitude factor of different concentrations of pure Y at (\(\lambda_1\)) and (\(\lambda_2\)) and \(P_2(X + Y)\) is the mixture’s amplitude at (\(\lambda_2\)).

Multiplication of the obtained actual amplitude value P\(\lambda_1\) of Y in each mixture by the factorized spectrum of Y \(\left(\frac{Y}{Z'}\right)\) was performed to restore its ratio spectrum Y/Z’ then multiplied this ratio spectrum by the divisor Z ‘to restore parent D\(\lambda\) of Y. For extracting the parent spectrum of X; spectrum subtraction of the extracted parent spectrum Y and equivalent mixture (X + Y) is carried out.

2.1.2.3. Ratio extraction method (RE). **Principle:**
This novel method for analyzing resolved D\(\lambda\) of mixture of the two analytes (X, Y) which possessing completely overlapped spectra then, the ratio spectrum is originated using Y‘ as divisor. By selection discrete pair of wavelengths (\(\lambda_1, \lambda_2\)) where X show equal amplitude values at these wavelengths while component of Y has amplitude value so cancellation of the contribution of X component will be achieved with nil difference.

**Resolution tools:**

- Factorized ratio spectrum of X using \(\Delta P\) (FS\(\Delta P\)): is prepared via the ratio spectrum of pure component of X using D\(\lambda\) of Y as a divisor. Afterwards, the obtained ratio spectrum is divided by the calculated amplitude difference between the two selected wavelengths (\(\lambda_1\) and (\(\lambda_2\)).

Factorized ratio spectrum of X = \((X/Y')/\Delta P\) (5)

**Manipulation steps of mixture:**

For analysis of mixture of X and Y, the ratio spectra of each mixture are obtained using Y as a divisor which is previously used in the preparation of (FS\(\Delta P\)). The amplitude difference at the two selected wavelengths (\(\lambda_1\) and (\(\lambda_2\)) is calculated then multiplication of this difference by the previously prepared factorized ratio spectrum of X representing \(\Delta P\). \(\frac{\Delta P}{P_{\lambda_1}}\) was performed to get the ratio spectrum of X/Y’ originally then multiplied this ratio spectrum by the divisor Y ‘to restore parent spectrum of X. For extracting the parent spectrum of Y; spectrum subtraction of the extracted parent spectrum X and equivalent mixture (X + Y) is carried out.

3. Experimental

3.1. Apparatus and operational system:
Spectrophotometric measurements were conducted via using double beam spectrophotometer; Shimadzu (UV-1800, Japan) using quartz cells; 1.00 cm. Scans were accomplished from 200 to 400 nm at 0.1 nm intervals. By using Shimadzu UV-Probe 2.43 system software, spectra were automatically obtained.

3.2. Materials

3.2.1. Authentic materials
Pure VAL, AML (as amlodipine besylate) and HCT samples were supplied kindly by Egyptian International Pharmaceutical Industries
and made by dissolving 100.0 mg of each of the proposed drugs, separately in ethanol in 100-mL volumetric flask, afterwards adjusting the volume to the mark was conducted through utilizing the same solvent for all the proposed drugs. The purchased mentioned pharmaceutical formulations was performed from local market.

3.2.3. Chemicals and solvents

Ethanol of spectroscopic analytical grade was obtained from Sigma-Aldrich, Darmstadt-Germany.

3.3. Standard solutions

3.3.1. Standard stock solutions

Stock solutions (1000.0 µg/mL) of all proposed drugs were prepared and made by dissolving 100.0 mg of each of the proposed drugs, separately in ethanol in 100-mL volumetric flask, afterwards adjusting the volume to the mark was conducted through utilizing the same solvent and it was remained at the refrigerator at 4 °C.

3.3.2. Working solutions

Preparation of freshly prepared working solutions was carried out through dilution from the stock solutions to attain a concentration 100.0 µg/mL for all the proposed drugs.

3.3.3. Synthetic 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. Scanning of previously prepared mixtures’ spectra were performed at wavelength region (200.0–400.0 nm) and being saved at the computer.

3.4. Procedure

Into three individual sets of volumetric flasks (10-mL), preparation of various standard solutions in ethanol was conducted over a concentration range of 4.0–44.0 µg/mL for VAL, 4.0–40.0 µg/mL for AML and 20.0–240.0 µg/mL for HCT. Spectrophotometrically scanning of the solutions in the range of 200.0 nm – 400.0 nm was performed using ethanol as blank and saved at the computer.

4.1. Spectral characteristic

The scanned standard solutions of VAL (16.0 µg/mL), AML (6.0 µg/mL) and HCT (2.5 µg/mL) were overlaid spectrophotometrically and being saved at the computer.

4.2. Calibration graphs’ construction

Utilizing the average of three experiments, calibration graphs were performed. Construction of calibration graphs of (VAL), (AML) and (HCT) was conducted through plotting their absorbance at their maxima 250.0 nm, 237.5 nm and 270.5 nm, respectively against their correlative concentrations, afterwards accurate computation of regression equations was performed.

4.3. Resolving tools

4.3.1. Factorized spectra (FS)

- Factorized D⁰ spectrum of HCT for (AE) method:

Zero order absorption spectra (D⁰) of concentration of pure HCT within its linearity range was divided by the recorded value of the absorbance at 270.5 nm using arithmetic function in spectrophotometer’s software and saved on the computer.

- Factorized ratio spectrum of HCT using AML as a divisor for (PE) method:

The obtained ratio spectrum of concentration of pure HCT within its linearity range utilizing pure AML (28.0 µg/mL) as a divisor is divided by the value of the recorded amplitude at 274.5 nm using arithmetic function in spectrophotometer’s software and saved on the computer.

- Factorized ratio spectrum of HCT using VAL as a divisor for (RE) method:

The obtained ratio spectrum of concentration of pure HCT within its linearity range utilizing pure VAL (28.0 µg/mL) as a divisor is divided by the aforementioned recorded amplitude difference (ΔP 275.0–250.0 nm) using arithmetic function in spectrophotometer’s software and saved on the computer.

4.3.2. Mathematical numerical factors

- Absorbance factor for (AE) method: it was calculated using the average of the calculated numerical values of recorded absorbance of D⁰ of various concentrations of pure HCT within its linearity range at 270.5 nm and 330.0 nm (A270.5 nm/A330.0 nm).

- Amplitude factor for (PE) method: it was calculated using the average of the calculated numerical amplitude values of different ratio spectra of various concentrations of pure HCT within its linearity range using pure AML (28.0 µg/mL) as a divisor at 274.5 nm and 330.0 nm (P274.5 nm/P330.0 nm).

4.4. Analysis of synthetic mixtures of ternary mixture (VAL, AML and HCT):

Division of D⁰ of each synthetic mixture of the ternary mixture (VAL, AML and HCT) by D⁰ of standard solution of AML (28.0 µg/mL) was performed. Afterwards, the constant of each mixture was accurately measured in the plateau region from (380.0 nm – 400.0 nm).

Determination and elimination of AML: The measured constant’s value was multiplied by the divisor’s spectrum (AML 28.0 µg/mL) to get parent spectrum of AML (constant multiplication method).

Auxiliary resolution of ternary mixture (VAL, AML and HCT) to get D⁰ resolved binary mixture (VAL-HCT): Subtraction the extracted spectrum D⁰ of AML from its corresponding ternary mixture was conducted to get resolved D⁰ of binary mixture (VAL + HCT), then saved in the computer then data analysis resolution (DAR) was applied on resolved D⁰ of VAL-HCT mixture as follows:

Absorbance extraction (AE) method: the absorbance at 270.5 nm and 330.0 nm was recorded for each mixture. The absorbance of HCT at 270.5 nm was calculated using the absorbance factor of pure HCT (A270.5 nm/A330.0 nm). The parent spectrum of HCT in the mixture could be extracted by multiplication of the calculated A270.5 nm by the previously prepared factorized D⁰ spectrum of HCT. The parent spectrum of VAL could be extracted after spectrum subtraction of obtained HCT from the obtained resolved binary mixture.

Auxiliary resolution of ternary mixture (VAL, AML and HCT) to get resolved ratio binary mixture (VAL-HCT): Subtraction of the...
previously measured constant’s value from its corresponding ratio spectrum of the ternary mixture (VAL, AML and HCT) using AML (28.0 µg/mL) as a divisor was conducted to get the resolved ratio spectrum of binary mixture of VAL and HCT/AML, then saved in the computer then data analysis resolution (DAR) was applied on resolved ratio of VAL-HCT mixture as follows:

(I) Peak-amplitude extraction (PE) method: The amplitudes at 274.5 nm and 330.0 nm were recorded for each mixture. The amplitude of HCT at 274.5 nm was calculated using the amplitude factor of pure HCT (P274.5 nm/P330.0 nm). The parent spectrum of HCT in the mixture could be extracted by multiplication of the calculated P274.5 nm by the previously prepared factorized ratio of (HCT/AML) then finally, multiplied by the divisor’s D0 spectrum AML (28.0 µg/mL). Then parent spectrum of VAL could be extracted by spectrum subtraction of the attained parent spectrum of HCT from the resolved D0 of binary mixture (VAL and HCT).

(II) Ratio extraction (RE) method: The resolved ratio spectrum of binary mixture of (VAL + HCT)/ AML was multiplied by D0 of AML (28.0 µg/mL) to get D0 of binary mixture of VAL and HCT followed by division by D0 VAL (28.0 µg/mL) to get ratio spectrum of (VAL + HCT)/ VAL, then saved in the computer then data analysis resolution (DAR) was applied on resolved ratio. The amplitudes at 275.0 nm and 250.0 nm were recorded for each mixture followed by calculation the difference between them. The parent spectrum of HCT could be extracted by multiplication of the calculated amplitude difference (ΔP275.0,250.0nm) by the previously prepared factorized ratio HCT to get (HCT/VAL) in the mixture then finally, multiplied by the divisor’s spectrum (VAL (28.0 µg/mL). The parent spectrum of VAL could be extracted after spectrum subtraction of the attained D0 of HCT from the resolved D0 of binary mixture (VAL and HCT).

For all of the proposed methods, the concentrations of VAL, AML and HCT in each mixture were calculated through substituting in the correlating regression equations at their maxima 250.0 nm, 237.5 nm and 270.5 nm, respectively.

4.5. Pharmaceutical formulations’ assessment:

4.5.1. Exforge HCT® tablets (Two formulas)

Accurately weighing of ten tablets followed by grinding was performed. An accurate weight of the mixed sample equivalent to one tablet from each of the two formulas, (formula I comprising 160.0 mg VAL, 5.0 mg AML free base and 12.5 mg HCT per tablet while formula II comprising, 160.0 mg VAL, 10.0 mg AML free base and 25.0 mg HCT per tablet) was separately transferred into two beakers, 50-mLethanol were added with continuing magnetic stirring for about 10 min. Each solution was, separately, filtered through a 0.45 µm membrane filter paper into two 100-mL volumetric flasks using ethanol. Accurately transferring of 2.0 mL of formula I solution and 1.0 mL of formula II into two separate 100-mL volumetric flasks was performed then AML was enriched for each formula with 500 µg standard of AML from its standard solution (100 µg/mL), then complete to the mark with ethanol to get solution with final concentration claimed to be 32.0 µg/mL, 6.0 µg/mL and 2.5 µg/mL of VAL, AML free base and HCT regarding formula I and 16.0 µg/ mL, 6.0 µg/mL and 2.5 µg/mL of VAL, AML free base and HCT regarding formula II, respectively. Analysis and determination of the proposed drugs in their pharmaceutical formulations were achieved by using the presented methods under analysis of synthetic mixtures. The concentrations of VAL, AML and HCT in enriched sample with AML added concentration were calculated through substituting in the correlating regression equations at their maxima 250.0 nm, 237.5 nm and 270.5 nm, respectively. Calculation of AML claimed concentration in each mixture was carried out after subtraction of AML added concentration which prepared and analyzed separately using the same experimental procedures.

4.5.2. Spectralprint recognition index

The above stated and described procedures were performed for each entire tablet (10 tablets were utilized) to extract the parent spectra of VAL, AML and HCT. Recording the absorbance value at wavelengths pairs 250.0 nm and 280.0 nm for VAL, 234.5 nm and 360.0 nm for AML and 270.5 nm and 257.6 nm for HCT for extracted spectrum of pharmaceutical formulations and authentic spectrum of the raw drugs was performed using the proposed methods.

5. Results and discussion

Investigations of pharmaceuticals analysis field and their quality control depend upon analytical manner/methods which have excellent implementation characteristics, where an analyst can choose the most suitable one for analysis. The major objective is to develop validated innovative resolution manner namely, parent spectrum resolution (PSR) including two complementary steps, auxiliary resolution (AR)via CM coupled with SS to decrease complexity of the ternary mixture and data analysis resolution(DAR) via novel spectrophotometric methods using factorized spectrum of cited drugs as a resolving spectrum. This PSR manner is able to obtain the parent spectra of each drug which considered as their spectralprint and the concentration of each component is obtained via constructed regression equation at their λmax. with maximum accuracy and precision. Validation of the developed spectrophotometric methods as stated by ICH guidelines [52], was the main purpose of the present work. The linearity ranges according to beer’s law of the studied drugs were applied at their maxima and found to be 4.0–44.0 µg/mL, 4.0–40.0 µg/mL and 2.0–24.0 µg/mL for VAL, AML and HCT, respectively.

5.1. Experimental optimization

To illustrate the power of this innovative PSR manner, the triple antihypertensive medicines consisting of VAL, AML and HCT in their challenge ratios, 32.0:1.0:2.5 and 16.0:1.0:2.5 µg/mL for VAL, AML and HCT, respectively were taken as models. By scanning standard solutions of the proposed drugs in these studied ratios within 200–400 nm, it was found that AML is out of its linearity range (4.0–40.0 µg/mL) and it show extension at wavelength region (351.0–400.0 nm) without any contribution of VAL and HCT while HCT is partially overlapped with VAL and show extension over VAL at wavelength region (330.0–350.0 nm). Thus, pre-analyzed sample should be enriched via spiking using 5.0 µg/mL of pure AML before applying proposed procedures, Fig. 2. Upon analysis of synthetic mixtures containing higher concentration of VAL > 40 µg/mL, its spectrum is completely interfering with HCT and AML spectra in wavelength region up to 379 nm thus wavelength region (380.0–400.0 nm) is the suitable region for analysis AML alone without any contribution of VAL and HCT.

This overlapping prevented the direct resolution of the studied triple drugs so advanced mathematical manner is used composed of two complementary resolution steps namely, auxiliary resolution(AR) and data analysis resolution (DAR). The auxiliary resolution applied on ternary mixture to eliminate extended drug via constant multiplication coupled with spectrum subtraction (CM-SS) method to decrease complexity of the mixture while data analysis resolution performed for the resolved binary mixture including three novel methods namely, absorbance extraction (AE), peak-amplitude extraction (PE) and ratio extraction (RE).

During PE and RE methods development, it was found that, divisor concentration was one of the major parameters which can impact the shape of the ratio spectra of the proposed drugs. The chosen divisor should achieve compromising between maximum sensitivity and minimal noise. The divisor concentrations were tested within linearity range
Fig. 2. Parent zero order absorption spectra of VAL (——) 16.0 µg/mL, AML (.....) 6.0 µg/mL and HCT (—) 2.5 µg/mL.

Fig. 3. Resolved zero order absorption spectra of the binary mixture of VAL (——) 16.0 µg/mL and HCT (—) 2.5 µg/mL showing absorbance factor using $\lambda_1$ (270.5 nm) and $\lambda_2$ (330 nm).
of AML in case of (CM-SS) method and VAL in case of RE method. As the concentration of the divisor increased or decreased, there was a correlative decrease or increase in the produced ratio values, respectively. Nevertheless, the positions of the peaks and troughs remain not affected. Using a divisor concentration of 28.0 \( \mu \)g/mL for both AML and VAL gave the best results concerning accuracy, reproducibility and recovery in synthetic mixtures and various pharmaceutical formulations.

5.2. Analysis of synthetic mixtures of ternary mixture (VAL, AML and HCT):

Division of \( D^0 \) of each synthetic mixture of triple drugs by \( D^0 \) of standard solution of AML (28.0 \( \mu \)g/mL) was performed. Afterwards, the constant of each mixture was accurately measured in the plateau region from (380.0–400.0 nm).

5.2.1. Determination of AML via CM method:

This method has the capability to restore the \( D^0 \) of AML and analysed via its maxima. The measured constant’s value was multiplied by the divisor’s spectrum (AML 28.0 \( \mu \)g/mL) to get parent spectrum of AML. The concentration of AML in each mixture is calculated by utilizing its correlating regression equation which is representing linear relationship amongst the absorbance values of AML at 237.5 nm against their correlating concentrations in the range (4.0–40.0 mg/mL).

5.2.2. PSR - manner

5.2.2.1. PSR – Manner via \( D^0 \) spectrum. Step I: Auxiliary resolution (AR)

Auxiliary resolution was applied to resolve this complicated mixture to get resolved \( D^0 \) spectrum of binary mixture of (VAL + HCT) with partially overlapped spectra via subtraction of extracted \( D^0 \) of AML from its corresponding \( D^0 \) of ternary mixture using spectrum subtraction method, Fig. 3.

**Step II: Data Analysis Resolution via absorbance extraction method (DAR-AE)**

This novel resolution method based on coupling numerical absorbance factor with the factorized \( D^0 \) spectrum of one of the proposed drugs. AE method starts with a simple mathematical manipulation to calculate numerical factor for HCT. The two selected wavelengths for numerical factor calculation were optimized and they found to be 270.5 nm and 330.0 nm without any contribution from VAL up to 40 \( \mu \)g/mL.

Absorbance factor was calculated representing the average of absorbance value of various concentrations of pure HCT at 270.5 nm and 330.0 nm (\( \text{abs}_{270.5\text{nm}} / \text{abs}_{330.0\text{nm}} \)), Fig. 3 and it found to be equal 9.5.

The actual absorbance of HCT in the mixture at 270.5 nm was attained as illustrated by the following equation:

\[
A_{(270.5\text{nm})} = \frac{\text{abs}_{270.5\text{nm}}}{\text{abs}_{330.0\text{nm}}} \times \text{abs}_{330.0\text{nm}(\text{mixture})}
\]

where \( A_{(270.5\text{nm})} \), is the actual absorbance of HCT in the mixture at 270.5 nm, \( \text{abs}_{270.5\text{nm}} / \text{abs}_{330.0\text{nm}} \), is the absorbance factor of various concentrations of pure HCT at 270.5 nm to 330.0 nm and \( \text{abs}_{330.0\text{nm}(\text{mixture})} \), is the absorbance of the mixture at 330.0 nm.

Thus, parent \( D^0 \) of HCT could be obtained by multiplication of the obtained \( A_{(270.5\text{nm})} \) value by the previously prepared factorized \( D^0 \) spectrum of pure HCT which prepared by using the same experimental specification. Consequently, \( D^0 \) of VAL could be successfully resolved by utilizing the method of spectrum subtraction, whereas the extracted \( D^0 \) of HCT in each mixture was subtracted from the corresponding resolved binary mixture’s \( D^0 \) absorption spectrum.

VAL and HCT concentrations were calculated by utilizing their correlating regression equations representing linear relationship amongst their absorbance values at 250.0 nm for VAL and 270.5 nm for

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*Fig. 4. Resolved ratio spectrum of the binary mixture of VAL (——) 16.0 \( \mu \)g/mL and HCT (—) 2.5 \( \mu \)g/mL using AML 28.0 \( \mu \)g/mL as a divisor showing amplitude factor using \( \lambda_1 (274.5\text{nm}) \) and \( \lambda_2 (330\text{nm}) \).*
HCT against their correlating concentrations in the range (4.0–44.0 µg/mL) and (2.0–24.0 µg/mL) for VAL and HCT respectively.

5.2.2.2. PSR - manner via ratio spectrum of ternary mixture using AML as a divisor. Step I: Auxiliary resolution (AR)

The ratio spectra of VAL + HCT/AML of each synthetic mixture’s ratio spectrum could be attained by subtraction of the previously recorded constant value of (AML/AML)’ for each mixture from its corresponding ratio spectra of gross spectra of the ternary mixture to get resolved ratio spectrum of (VAL + HCT/ AML), Fig. 4.

Step II: Data analysis resolution via peak-amplitude extraction method (DAR-PE)

This novel resolution method based on coupling numerical amplitude factor with the factorized ratio spectrum of one of the proposed drugs. PE method starts with a simple mathematical manipulation to calculate numerical factor for HCT. The two selected wavelengths for numerical factor calculation were optimized and they found to be 274.5 nm and 330.0 nm for PE where 330.0 nm showed no contribution from VAL up to 40 µg/mL, Fig. 4.

Amplitude factor is calculated representing the average of amplitude value of various concentrations of pure HCT at 274.5 nm and 330.0 nm [P/(274.5nm)/P330.0nm]. Calculation of the amplitude factor was performed, and it found to be equal 16.

The actual amplitude of HCT in the mixture at 274.5 nm was attained as illustrated by the following equation: 

\[ P(274.5 \text{ nm}) = \frac{P(274.5 \text{ nm})}{P(330.0 \text{ nm})} \times P(330.0 \text{ nm/mixture}) \]

where \( P(274.5 \text{ nm}) \) is the amplitude of HCT/AML in the mixture at \( P(274.5 \text{ nm}) \) and \( P(330.0 \text{ nm}) \) is the amplitude factor of various concentrations of pure HCT/AML at 274.5 nm to 330.0 nm and \( P(330.0 \text{ nm/mixture}) \) is the amplitude of the mixture at \( P(330.0 \text{ nm/mixture}) \).

Ratio spectrum of HCT/AML could be obtained by multiplying the obtained \( P(274.5 \text{ nm}) \) by the previously prepared factorized HCT prepared by using the same experimental specification. Then, by multiplying the ratio spectrum of HCT/AML by the AML divisor, parent \( D^0 \) of HCT was obtained. Consequently, \( D^0 \) of VAL could be successfully resolved by utilizing the method of spectrum subtraction, whereas the extracted \( D^0 \) of HCT in each mixture was subtracted from the corresponding mixture’s \( D^0 \) absorption spectrum of VAL + HCT.

VAL and HCT concentrations were calculated by utilizing their correlating regression equations which are representing and offering a linear relationship amongst their absorbance values at 250.0 nm for VAL and 270.5 nm for HCT against their correlating concentrations in the range (4.0–44.0 mg/mL) and (2.0–24.0 mg/mL) for VAL and HCT respectively.

5.2.2.3. PSR-manner via ratio spectrum of ternary mixture using AML and VAL as divisors. Step I: Auxiliary resolution (AR)

Auxiliary resolution was applied to get resolved \( D^0 \) spectrum of binary mixture of (VAL + HCT) with partially overlapped spectrum via subtraction of extracted \( D^0 \) of AML from its corresponding \( D^0 \) of ternary mixture using spectrum subtraction method, Fig. 3.

Step II: Data analysis resolution via ratio extraction method (DAR-RE)

This novel resolution method based on coupling amplitude difference with the factorized ratio spectrum of target component. This amplitude difference is applied at two selected wavelengths \( \lambda_1 \& \lambda_2 \) where the ratio spectrum of the co-formulated component displays the

| Drug   | VAL         | AML         | HCT         |
|-------|-------------|-------------|-------------|
| Method | \( D^0 \)   | \( D^0 \)   | \( D^0 \)   |
|       | \( \lambda_{250.0} \) | \( \lambda_{237.5} \) | \( \lambda_{270.5} \) |
| Linearity range | 4.0–44.0 | 4.0–40.0 | 2.0–24.0 |

Regression equation Parameters

- Slope: 0.0318, 0.0366, 0.068
- Intercept: 0.0112, 0.002, 0.0062
- Correlation Coefficient (r): 1.0000, 1.0000, 0.9999
- Accuracy: 100.19 ± 0.59, 99.67 ± 0.59, 100.03 ± 0.89 (Mean ± SD)
- Precision (%RSD): 0.482, 0.585, 0.660
- Repeatability: 0.654, 0.850, 0.896
- Intermediate precision: 0.682, 0.595, 0.660

Accuracy was checked using concentrations (6.0 µg/mL, 24.0 µg/mL and 40.0 µg/mL), (6.0 µg/mL, 25.0 µg/mL and 38.0 µg/mL) and (4.0 µg/mL, 16.0 µg/mL and 23.0 µg/mL) for, VAL, AML and HCT respectively.

a and b are repeatability and intermediate precision respectively (n = 9) relative standard deviation of three different concentrations in triplicate (8.0 µg/mL, 28.0 µg/mL and 36.0 µg/mL) for both VAL and AML, while (6.0 µg/mL, 14.0 µg/mL and 22.0 µg/mL) for HCT.

Fig. 5. Resolved ratio spectrum of the binary mixture of VAL (——) 16.0 µg/mL and HCT (—) 2.5 µg/mL using VAL 28.0 µg/mL as a divisor showing the selected \( \lambda_1 \) (275 nm) and \( \lambda_2 \) (250 nm).
same amplitudes (constant) and the component of interest displays significant amplitude difference. This method is valid for determination of all mixtures containing VAL up to 44.0 µg/mL.

The resolved $D^0$ of the resolved binary mixture of (VAL + HCT) is divided by $D^0$ VAL (28.0 µg/mL) to get ratio spectrum of (VAL + HCT) / VAL represents $\frac{HCT}{VAL} +$ constant $(\frac{D^0}{D^0})$. By selection two wavelengths for HCT ratio spectra and applying subtraction of these two amplitudes $(\Delta P_{275.0-250.0nm})$, cancellation of the constant will be achieved, and the divisor analyte’s interference will be omitted while the other analyte’s interference will be directly related to the calculated difference. The optimum wavelength pair was studied and found to be Maximum $\Delta P$ values were using peak maxima at 275.0 nm and peak trough at 250.0 nm, Fig. 5.

The ratio spectrum of HCT using VAL as a divisor in each mixture was obtained by multiplication of the value of the amplitude difference $(\Delta P_{275.0-250.0nm})$ by the aforementioned prepared factorized HCT prepared by using the same experimental specification, Fig. 5.

The parent $D^0$ of HCT in each mixture was obtained by multiplication of the ratio spectrum of $\frac{HCT}{VAL}$ by VAL divisor. Consequently, $D^0$ of VAL could be successfully resolved by utilizing the method of spectrum subtraction, whereas the attained $D^0$ of HCT in each mixture was subtracted from the corresponding resolved mixture’s $D^0$ spectrum of (VAL + HCT).

VAL and HCT concentrations were calculated by utilizing their correlating regression equations which are representing a linear relationship amongst their absorbance values at 250.0 nm for VAL and 270.5 nm for HCT against their correlating concentrations in the range (4.0–44.0 mg/mL) and (2.0–24.0 mg/mL) for VAL and HCT respectively.

5.3. Method validation

Validation was conducted in accordance with the guidelines of ICH [52] as revealed in Table 1.

5.3.1. Linearity and range

Estimation of the linearity of the studied methods was conducted via analyzing various concentrations of VAL, AML and HCT, ranging from 4.0–44.0 µg/mL, 4.0–40.0 µg/mL and 2.0–24.0 µg/mL, respectively. Repetition of every concentration was carried out three times. The analysis was applied in accordance with aforementioned experimental circumstances. Demonstration of linear equations were performed, Table 1.

Calibration’s range was carried out through utilizing the practical range in accordance with Beer’s law and the concentration of the proposed drugs which existing in their combination as dosage form to provide linear, accurate, and precise results, Table 1.

5.3.2. Accuracy

Accuracy was studied and investigated through implementing the studied methods for analysis and determination of various models of the cited drugs. Attaining the proposed drugs’ concentrations were conducted utilizing the relative regression equations, Table 1.

5.3.3. Precision

5.3.3.1. Repeatability and Intermediate precision. To check the precision (Repeatability and Intermediate precision), three concentrations (8.0 µg/mL, 28.0 µg/mL and 36.0 µg/mL) for both VAL and AML while (6.0 µg/mL, 14.0 µg/mL and 22.0 µg/mL) for HCT were separately examined and investigated three times intra-daily and inter-daily on three various days utilizing the studied methods. Computation of the correlated standard deviations which is associated to each concentration was performed, Table 1.

5.3.4. Specificity

Specificity was ascertained and checked through using various synthetic mixtures’ analysis comprising of studied drugs with different ratio within the range of linearity. Good results were revealed in Table 2.

5.4. Application of the developed methods for assaying Exforge HCT® tablets

Determination of the proposed drugs’ concentrations in Exforge HCT® tablets was carried out utilizing the proposed spectrophotometric methods and all proposed methods showed acceptable percentage recoveries. As a consequence, this permit their utilizing for proposed drugs’ regular analysis and determination in their combined formulation, Table 3. The accuracy of pharmaceutical formulations was certified by making comparison between the obtained results from studied methods and those of the reported method [16], Table 4.

5.5. Statistical analysis

Statistical comparison based on student t-test and f-test was performed between the results obtained by developed methods and those obtained after applying the official methods [51] and reported method [16]. It was found that at p-value 0.05, computed t and F values were less than their relative theoretical ones revealing insignificant difference between the developed methods and official and reported ones with

Table 2

| Ternary Mixture | Recovery% ± SD |
|-----------------|----------------|
| VAL:AML: HCT   |                |
| Ratio (µg/mL)   | SS of $D^0$ HCT via AE | SS of $D^0$ HCT via PE | SS of $D^0$ HCT via RE | AML($D^0)$ | HCT($D^0)$  |
| (32:0.6:0.2:5) ** | 98.86 | 100.23 | 99.80 | 100.24 | 100.51 | 100.36 | 99.26 |
| (16:0.6:0.2:5) ** | 100.36 | 99.45 | 99.28 | 99.38 | 99.46 | 99.52 | 99.84 |
| (10:0:10:0)     | 99.57 | 100.68 | 100.25 | 100.35 | 100.82 | 99.87 | 100.41 |
| (2.0:7:0:14:0)  | —    | —    | 100.44 | 99.44 | —    | 100.25 | —    |
| (20:0:10:0:20:0) | 100.44 | 100.55 | 98.56 | 99.58 | 100.23 | 100.24 | 99.56 |
| Mean ± SD      | 99.81 ± 0.74 | 100.23 ± 0.55 | 99.67 ± 0.76 | 99.80 ± 0.46 | 100.26 ± 0.58 | 100.00 ± 0.38 | 99.86 ± 0.48 |

a Average of 3 replicates.

** Ratio as dosage forms enriched with 5.0 µg/mL AML.
drugs in the two studied pharmaceutical formulations. It is vantageous due to its capability to assess and evaluate the sample purity. 5.6. Spectralprint recognition index (SRI)

The figures in parenthesis are the corresponding theoretical values at $P = 0.05$. All the statistical comparisons were performed using one way ANOVA test.

respect to accuracy and precision as shown in Table 4 and Table 5. To compare the capability of the developed methods for assaying each component in the ternary mixture of (VAL, AML and HCT), statistical analysis was implemented between the results of developed methods and those of official methods [51] using one way ANOVA test. The results attained from ANOVA analysis’s revealing insignificant difference between the developed methods for authentic samples and the official methods as shown in Table 6.

5.6. Spectralprint recognition index (SRI)

Using advanced spectrophotometric methods based on extraction of parent spectrum of each cited drugs which act as spectralprint is advantageous due to its capability to assess and evaluate the sample purity. Assessment of purity is greatly critical respecting compounds have a biological activity as impurities’ traces with either no or great potency can probably cause undesirable action [53]. This (SRI) is a measure of the purity of the component in the pharmaceutical formulations and it is applied using discrete pairs of wavelengths all over the spectrum of parent extracted spectrum and its scanned spectrum. To assess (SRI) of the cited drugs, the ratio of the absorbance at two chosen wavelengths for the identical concentration of the extracted spectrum from the parent extracted spectrum and its scanned spectrum. To assess (SRI) of VAL, AML and HCT in each individual Exforge HCT tablet (utilizing 10 tablets). The purity profile was calculated via utilizing their extracted D$_{0}$HCT, D$_{0}$AML and D$_{0}$HCT via PE, and its scanned spectrum. To assess (SRI) of VAL, AML and HCT in each individual Exforge HCT tablet (utilizing 10 tablets). The purity profile was calculated via utilizing their extracted D$_{0}$HCT, D$_{0}$AML and D$_{0}$HCT via PE, and its scanned spectrum. To assess (SRI) of VAL, AML and HCT in each individual Exforge HCT tablet (utilizing 10 tablets). The purity profile was calculated via utilizing their extracted D$_{0}$HCT, D$_{0}$AML and D$_{0}$HCT via PE, and its scanned spectrum.
Table 5
Statistical analysis for the results obtained by the proposed methods and the official HPLC methods for analysis of the cited drugs in their pure forms.

| Drug parameters | VAL | AML | HCT |
|-----------------|-----|-----|-----|
| Mean            | 100.08 | 99.51 | 100.21 |
| SD              | 0.62 | 0.55 | 0.52 |
| Variance        | 0.3844 | 0.3025 | 0.1521 |
| Student’s t-test (tab.) | 2.228 | 2.228 | 2.228 |
| F (cal.)        | 1.27 | 1.78 | 0.433 |
| F (tab.)        | 5.05 | 5.05 | 4.95 |
| Mean            | 99.95 | 99.74 | 99.95 |
| SD              | 0.68 | 0.52 | 0.68 |
| Variance        | 0.8424 | 0.2704 | 0.4225 |
| Student’s t-test (cal.) | 2.201 | 2.201 | 2.201 |
| F (cal.)        | 0.65 | 0.433 | 0.433 |
| Mean            | 99.79 | 99.74 | 99.79 |
| SD              | 0.65 | 0.52 | 0.65 |
| Variance        | 0.4225 | 0.4225 | 0.4225 |

- Official USP methods for determination of VAL, AML and HCT (RP-HPLC).
- The figures in parenthesis are the corresponding theoretical values at P = 0.05.

Table 6
Results of ANOVA (single factor) for comparison of the results obtained by the proposed methods and the official methods for determination of the proposed drugs in their pure powder forms.

| Source of variation | Sum of squares | DF | Mean Square | F value | P value | F crit |
|---------------------|----------------|----|-------------|---------|---------|--------|
| VAL                 |                |    |             |         |         |        |
| Between exp.        | 0.9747         | 1  | 0.9747      | 2.85783 | 0.121807| 4.964603|
| Within exp.         | 3.410566667    | 10 | 0.341,057   |         |         |        |
| Total               | 4.385266667    | 11 |             |         |         |        |
| AML                 |                |    |             |         |         |        |
| Between exp.        | 0.6627         | 1  | 0.6627      | 3.170207| 0.10534 | 4.964603|
| Within exp.         | 2.0904         | 10 | 0.20904     |         |         |        |
| Total               | 2.7531         | 11 |             |         |         |        |
| HCT                 |                |    |             |         |         |        |
| Between exp.        | 0.087958       | 1  | 0.087958    | 0.19628 | 0.666327| 4.844336|
| Within exp.         | 4.92935        | 11 | 0.448123    |         |         |        |
| Total               | 5.017308       | 12 |             |         |         |        |

- At the 0.05 level.
- The population means are not significantly different.

Table 7
Spectralprint recognition index for Valsartan (VAL), Amlodipine (AML) and Hydrochlorothiazide (HCT) in Exforge HCT® tablets.

| Tablet No. | Exforge HCT® Tablets (Formula I) | Exforge HCT® Tablets (Formula II) |
|------------|---------------------------------|---------------------------------|
|            | VAL                             | AML                             | HCT                             | VAL                             | AML                             | HCT                             |
|            | Spectralprint Index              | Spectralprint Index              |                                 |                                 |                                 |                                 |
|            | SS of ΔD^0_HCT via PE            | SS of ΔD^0_HCT via RE           | SS of ΔD^0_HCT via AE           | CM AE PE RE                     | SS of ΔD^0_HCT via AE           | CM AE PE RE                     |
| Tablet 1   | 0.991                           | 0.991                           | 0.999                           | 0.998                           | 0.998                           | 0.996                           |
| Tablet 2   | 0.996                           | 0.998                           | 0.994                           | 0.994                           | 0.999                           | 0.996                           |
| Tablet 3   | 0.998                           | 0.996                           | 0.992                           | 0.999                           | 0.999                           | 0.997                           |
| Tablet 4   | 0.997                           | 0.997                           | 0.995                           | 0.996                           | 0.998                           | 0.998                           |
| Tablet 5   | 0.992                           | 0.993                           | 0.995                           | 0.996                           | 0.997                           | 0.996                           |
| Tablet 6   | 0.985                           | 0.998                           | 0.999                           | 0.996                           | 0.997                           | 0.996                           |
| Tablet 7   | 0.988                           | 0.991                           | 0.998                           | 0.998                           | 0.999                           | 0.996                           |
| Tablet 8   | 0.999                           | 0.993                           | 0.994                           | 0.994                           | 0.999                           | 0.996                           |
| Tablet 9   | 0.996                           | 0.998                           | 0.995                           | 0.997                           | 0.998                           | 0.996                           |
| Tablet 10  | 0.998                           | 0.995                           | 0.994                           | 0.998                           | 0.997                           | 0.996                           |
| Mean       | 0.990                           | 0.997                           | 0.998                           | 0.993                           | 0.996                           | 0.990                           |
| SD         | 0.002                           | 0.002                           | 0.001                           | 0.002                           | 0.002                           | 0.001                           |

5.7. Greenness evaluation of the proposed methods

The intent for replacing conventional pharmaceutical analysis techniques that rely on high volume consumption of several toxic and organic solvents, by eco-friendly green ones with comparable fulfilment characteristics, has developed considering deeply assure the compliance with the requirements of the green chemistry and the importance of environmental protection. In this study, using four various greenness evaluation techniques was performed to evaluate the proposed spectrophotometric methods’ ecofriendly nature.

5.7.1. National environmental methods Index (NEMI)

From the first used qualitative tools for prompting the greenness of an analytical methodology is NEMI approach [54]. It is applied via using a simple expression called pictogram by means of circle composing of four quadrants. Each quarter shows specific criteria, namely, the usage of PBT chemicals (persistent, bioaccumulative and toxic), consumption of hazardous chemical, corrosive criteria and the amount of waste generated [55]. Fortunately, NEMI pictograms were all green for the
proposed methods and fulfilled the four quadrants’ acceptance criteria, Table 8, since ethanol was the used solvent which is safe in terms of safety, health, and environment hazards, it has been described by the US Environmental Protection Agency as neither hazardous nor PBT [56] in addition, The pH of the study is non-corrosive (pH is not 12) and the amount of waste released is less than 50 g / sample.

5.7.2. Analytical Eco-Scale system

Another scoring tool associated with the penalty point system was presented as a semi-quantitative approach to assess the environmental impact and greenness of analytical methods [57]. The penalty points are given for each parameter of the analytical process that may affect the excellent green analysis, for instance the hazardousness and amount of the used reagents, waste generation, energy consumption, types of waste treatment and any probable occupational risks. They are subtracted from the base value of 100 for the ideal green method, above 75 for the analytical procedure is considered to be ecofriendly. The score obtained from the proposed methods was more than 75 which affirms the excellent green performs of proposed methods, Table 8. The total of penalty points for the developed methods was 7. Accordingly, the eco-scale score for the developed spectrophotometric methods was 93 which indicate the greenness of the developed methods.

5.7.3. Green analytical procedure Index (GAPI)

(GAPI) is approach composing of hybrid of both NEMI and Eco-Scale. It has been presented to assess the whole analytical procedures’ green character [58]. This tool utilizes a certain pictogram with five pentagrams for assessing and measuring it is color-coded extending from green through yellow to red, indicating low, medium, and high environmental impact for each level. The GAPI tool provides information regarding 15 different parameters including sample preparation and its collection, instrumentation, health and safety impact of reagents and compounds utilized, as well as waste treatment. The proposed spectrophotometric methods reveal GAPI pictograms with a greater number of shaded green and yellow sections than red shaded ones, showing the greenness of the developed methods.

5.7.4. Analytical greenness metric (AGREE)

AGREE tool [59] is a software which could be downloaded producing a twelve sections colored pictogram. It covers twelve principles and standards of green analytical chemistry. Every section is colored and shaded from deep green to deep red according to its environmental impact and the total score assessment is displayed in the middle of the circular pictogram. The score value of AGREE pictogram for the proposed methods was found to be 0.71, Table 8.

To summarize greenness evaluation of the developed methods, the success of the developed methods in decreasing the consumption of solvents and energy of instrument in addition, the ethanol utilization were obviously reflected by the high values of analytical eco-score and AGREE score with the prevalence of green color in pictograms of NEMI and GAPI. Therefore, the developed methods are considered “green methods” which can be safely applied without any vulnerable harm to either the analyst or the environment.

5.8. Comparative study of the developed methods along with the reported methods

The criteria for applying the proposed PSE manner including three spectrophotometric methods (AE, PE and RE) to evaluate multi component medicines, are the coupling of two complementary resolution manner namely, auxiliary and data analysis which successfully has the ability to extract the full parent spectra (D°)of the studied drugs allowing the determination of studied drugs’ concentrations at their maxima with maximum accuracy and precision using only one divisor in case of AE and PE and two divisors in case of RE with minimum manipulation steps via built-in spectrophotometric software. PE has advantage over AE since the calculated numerical factor at the two chosen wavelengths has a higher value with more accurate determination of VAL and HCT, so minimize the instrumental error. RE gave a satisfactory results for all synthetic mixtures with VAL concentration over (4.0 µg/mL- 44.0 µg/mL) while AE and PE fail to give good results for mixtures containing VAL more than 40 µg/mL thus RE is advantageous since it has no limitation in data analysis resolution (DAR). The proposed methods offer advantages over the other reported spectrophotometric methods [13–20] namely, successive ratio subtraction coupled with constant multiplication method (SS-CM) and successive derivative subtraction (SDS) which needs the availability of the two pure drugs as divisors to recover their D° spectra (in case of CM-SS) and D¹ spectra (in case SDS)as well as the extension of each spectrum over the other one. In addition, absorbance subtraction (AS), amplitude modulation (AM) and advanced amplitude centering(AAC) which based on multistep progressive resolution of iso- points responses (in case of AS and AM) and amplitude maxima(in case of AAC) to get their concentrations with multi-manipulation step using unified regression equation for determination three components. Finally, these proposed methods are applicable for the determination of spectralprint recognition index (SRI) as a measure of purity of the target components.

6. Conclusion

Findings about antihypertensive drugs and their relation with COVID-19 encouraged a statement from different medical societies counselling antihypertensive patients suffering from corona virus to continue on their current antihypertensive therapies especially those containing ARB and CCB, additionally they are considered as a good alternative for other classes of antihypertensive drugs during this pandemic. The superiority of this work is due to its ability to implement quantitative, accurate, precise, and easily used spectrophotometric methods for progressive determination and assaying of the proposed drugs in their pure powdered form, synthetic mixtures, and pharmaceutical formulations. Moreover, the proposed methods are advantageous owing to their capability of overcoming the overlapping spectra between the mentioned drugs via utilizing various manipulation steps for their measurement by restoring their parent spectra which acts as

| Table 8 | Greenness assessment of the two proposed spectrophotometric methods according to Eco-Scale, NEMI, GAPI and AGREE tools. |
| Parameters | Penalty points | NEMI tool | GAPI tool | AGREE tool |
| --- | --- | --- | --- | --- |
| Reagents | Ethanol* | 4 |  |  |
| Instrument (spectrophotometer) | Energy consumption | 0 |  |  |
| Occupational hazards | 0 |  |  |
| Waste | 3 |  |  |
| Total penalty points (PP) | 7 |  |  |
| Analytical Eco-Scale total score | 93 |  |  |
| Comment | Excellent green analysis |  |  |  |
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