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Sustainable spectrophotometric determination of antihypertensive medicines reducing COVID-19 risk via paired wavelength data processing technique - Assessment of purity, greenness and whiteness

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ABSTRACT

Recent studies have reported that using certain antihypertensive therapies such as angiotensin II receptor blockers (ARBs) is associated with mitigation of fatal outcomes and enhancing clinical features of patients having hypertension during coronavirus pandemic. Thus, in the current work an innovative, effective, white and sustainable spectrophotometric technique called paired wavelength data processing technique (PWDPT) was developed for evaluation of recommended antihypertensive combination therapies incorporating candesartan cilexetil (CAN) and hydrochlorothiazide (HCT). This technique included three methods, namely, absorbance resolution (AR), amplitude resolution (PR) and ratio extraction (RE). Linearity ranges were (5.0 μg/mL - 50.0 μg/mL) and (2.0 μg/mL - 24.0 μg/mL) for CAN and HCT, respectively. Validation and confirmation of all suggested methods were conducted in accordance with ICH guidelines, producing satisfactory results within the accepted limits. Statistical comparison was achieved between the attained results from suggested methods and those attained from official methods, in which insignificant difference was existed. The suggested methods were successfully employed for identification of the studied drugs as well as determination of their spectral recognition and evaluation of the purity in their combined formulations. The proposed methods followed the principles of green analytical chemistry, where their greenness was evaluated and compared with the official potentiometric and HPLC methods via using four tools, namely, National Environmental Methods Index (NEMI), the Analytical Eco-Scale, the Green Analytical Procedure Index (GAPI) and Analytical greenness metric (AGREE) which affirmed the eco-friendly nature of the proposed methods. Moreover, studying the whiteness features was performed using the recently introduced RGB12 model. The acceptable results along with the sustainability, simplicity, affordability and low-cost of the proposed methods encourages their utilization in the quality control laboratories.
1. Introduction

Hypertension is the prominent cause of cardiovascular disease and globally premature death. The incidence of hypertension is growing globally as a result of ageing of the population, and it is exaggerated upon exposure to risk factors of lifestyle including unhealthy diets and lacking of physical activity. Nevertheless, hypertension treatment was improved and enhanced significantly over the last two decades because of multiple combination therapies (Fuchs and Whelton, 2020; Zhang et al., 2017; Ettehad et al., 2016).

Patients with raised blood pressure have a two-fold increased risk of dying from the coronavirus COVID-19 compared to patients without high blood pressure. It further detected that hypertensive patients who were not on medicines to manage their condition had even increased probability and risk of mortality (Gao et al., 2020).

Candesartan cilexetil (CAN) (Fig. 1a), is chemically designated as 1-cyclohexyloxyxcarbonyloxyethyl 2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]benzimidazole-4-carboxylate (British Pharmacopoeia, 2013). CAN is prodrug where following administration, it undergoes hydrolysis to candesartan through ester hydrolysis (Huang et al., 2013). Candesartan is considered as an angiotensin II receptor antagonist used alone or in combination with other drugs to manage high blood pressure.

Different recent studies have reported that using available angiotensin receptor 1 (AT1R) blockers, such as CAN, can eschew morbidity associated with SARS-CoV-2 virus infection (admittance to the intensive care unit (ICU) and the mechanical ventilation) and mortality (Pinto-Sietsma et al., 2020; Yan et al., 2020; Zhang et al., 2020; Ruilope et al., 2020).

Hydrochlorothiazide (HCT) (Fig. 1b), is chemically designated as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide1,1-dioxide (British Pharmacopoeia, 2013). 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 (Sweetman, 2009).

For effective pharmacological action of the proposed drugs, they are co-formulated in single-pill (Atacand plus® tablet), these double antihypertensive medicines attract the attention of researchers due to their paired pharmacological effects including the drug’s capability to reduce levels of the blood pressure (BP) as well as lowering risk of coronavirus mortality and morbidity of antihypertensive patients. Different analytical methods have been reported for the studied binary mixture of CAN and HCT comprising spectrophotometry (Abdelaleem et al., 2013; Ahmad Charoo et al., 2009; Belal et al., 2013; Daharwal and Singh, 2015; Mohamed et al., 2020; Mukthinuthalapati and Kumar, 2015; Üstündag and Dinç, 2021; Workie et al., 2017; Youssef et al., 2010; ÜSTÜNDAĞ and Erdal, 2021; Erk, 2003a) and chromatography methods (Bharathi et al., 2012; Bonthu et al., 2018; de Diego et al., 2018; Erk, 2003b; Khedr, 2008; Madhavi et al., 2017; Singh et al., 2014; Üstündag and Dinç, 2021; Youssef et al., 2010; Gaurkhede and Chandewar, 2018; Pappula et al., 2019).

Mathematical spectrophotometric methods using built in spectrophotometer’s software are now being utilized more frequently for evaluation of drugs in pharmaceutical formulations (Obaydo et al., 2021; Lotfy and Saleh, 2016; Saleh et al., 2022; Rostom et al., 2021, 2022; Lotfy and Hagazy, 2012; Lotfy and Omran, 2018; Lotfy et al., 2014, 2019, 2020a, 2020b, 2020c, 2021, 2022; Ahmed and Lotfy, 2021, 2022; Samir et al., 2014; Obaydo and Sakur, 2019; Obaydo and Alhaj Sakur, 2019; Al Zakri et al., 2019; Darweish et al., 2022) as well as the recently developed approaches based on absorptivity centering (Lotfy et al., 2020a, 2020c; Lotfy and Omran, 2018; Obaydo et al., 2021) owing to their inherent benefits such as simplicity, accuracy, reduction of expensive reagents consumption and eschewing time consuming or special software utilization.

The concept of White Analytical Chemistry (WAC) is presented as an extension of Green Analytical Chemistry (GAC) (El-Kafrawy et al., 2022; Gatuszka et al., 2013). In addition to green aspects, WAC takes into account other key criteria affecting the quality of the method, analytical (red) and practical (blue). This was challenging for researchers to provide a multi-criteria approach to assess the entire analytical process, including performance, safety- and environmental considerations (Nowak et al., 2020; Nowak and Kościelniak, 2019; Elbordiny et al., 2022). In reference to the RGB color model, according to which mixing of red, green and blue light beams gives the impression of whiteness, a white analytical method shows the coherence and synergy of the analytical, ecological and practical attributes. WAC is closer to the idea of sustainable development due to a more holistic view, as it strives for a compromise that avoids an unconditional increase in greenness at the expense of functionality. The usefulness of the sustainable method is conditioned by the analytical efficiency expressed by validation criteria (accuracy, precision, sensitivity), as well as purely practical and economic considerations, e.g. cost of analysis, speed of analysis, and general simplicity of the method. There is an urgent need to incorporate GAC with the concept of sustainable development in order to achieve the proper balance between the greenness of the method, its usefulness, and the practical and economic aspects. An ecofriendly, ideal, economic- and applicable analytical method is considered white.

Spectrophotometric analytical methods’s utilization for the examination and purity check of pharmaceuticals plays a substantial role in the manufacturing process of these medicines.
role to adhere to the necessary of statutory certification of the drugs and their pharmaceutical products by the industry. Spectralprint recognition is a potential interpretation of the purity index using signals data of the extracted parent spectrum of each drug in the combinations and those of the pure drugs. Accordingly, the development of novel spectrophotometric methods that could be used in quality control and purity assessment of pharmaceutical products is critical for confirming the appliance of the drug products to consumers in terms of both safety and effectiveness (Rostom et al., 2022; El-Hanboushy et al., 2022; Lotfy et al., 2020a).

The co-formulated dosage form of the studied drugs (Atacand plus® tablets) in ratio, 1.28:1.0 for CAN and HCT, respectively showing completely overlapping spectra which hinder the extraction of their parent spectra (zero-order) by the well-established methods based on the extension of one of the analytes such as ratio subtraction, extended ratio subtraction, unified constant subtraction and constant multiplication (Lotfy and Saleh, 2016, 2018). Therefore, there is a need of developing innovative technique devoted to extract the parent spectrum (PS) of the analytes in binary mixtures with completely overlapped spectra with green and white aspects which could be applicable in the quality control laboratories. As a result, the aim of this work is to develop specific, environmentally friendly UV spectrophotometric methods for simultaneous quantification of CAN and HCT in bulk and their pharmaceutical formulations (Atacand Plus® tablets) allowing estimation at their absorbance maxima with optimum accuracy and precision using ethanol as solvent via recently introduced resolution tool namely, factorized spectrum (FS). Applying the purity study using spectralprint recognition via purity index (SR-PI) of the studied drugs as well as the greenness and whiteness evaluation of the developed methods added superior advantages to this work regarding human and environmental safety. A comparative study has been conducted between the results attained by the proposed methods versus those attained by official methods to ascertain the efficiency for applying in quality control laboratories.

2. Theoretical background

2.1. Concept of paired wavelength data processing technique (PWDPT)

This innovative technique used for the analysis of binary mixtures (X&Y) their spectra are showing complete overlapping (with or without isoabsorptive point) via data processing of recorded signals at two chosen paired wavelengths to extract the parent spectrum (PS) (zero order spectrum) of each analyte. Thus, allowing determination of X & Y, separately at their \( \lambda_{\text{max}} \) (using regression equation representing absorbance and versus the correlating concentrations) with maximum accuracy and precision. In addition, the extracted PS affirm the spectral profile of each component of interest. This technique is applied via three complementary steps, signal analysis (statistical manipulation or arithmetic signal difference), resolution tool’s preparation (factorized spectrum) and finally spectrum manipulation. This technique including the following methods:

2.1.1. Absorbance resolution (AR)

**Principle:** Selection of two wavelengths \( \lambda_1 \) and \( \lambda_2 \) is performed where X possesses a variable valuable of absorbance while Y shows equal values. Thus, this absorbance difference in the mixture is related to component X only due to Y is intrinsically equals to zero. The applied resolution steps are summarized as follows:

**a Signals Analysis:** Statistical equation is computed representing the linear relationship of the difference of absorbance substituting \( \Delta A \) at \( \lambda_1 \) and \( \lambda_2 \) versus the recorded absorbance \( A_1 \) at \( \lambda_1 \) of different concentration of pure X.

\[
\Delta A = \text{Slope } A_1 + \text{Intercept}
\]  

**b Resolution tool’s preparation:** Factorized \( D^0 \) spectrum at \( A_1 \) (FS\(_{A1}\)): The scanned \( D^0 \) of pure analyte X is divided by the recorded absorbance at isoabsorptive point using spectrophotometer’s software.

\[
\text{Factorized } D^0 \text{ spectrum of } X = \frac{X}{A_i}
\]

**c Spectral manipulation:** For analysis of mixture X and Y, \( \Delta A \) at \( \lambda_1 \) and \( \lambda_2 \) is calculated and substituted in equation (1) to get the postulated absorbance value \( (A_1) \) corresponding to the X only at \( \lambda_1 \) then multiplied this value by FS\(_{A1}\) (\( \frac{X}{A_i} \)) to get PS of X. Then, PS of Y is obtained by difference between gross spectrum of each mixture, and extracted PS of X via spectrum subtraction method.

2.2. Amplitude resolution (PR)

**Principle:** The innovative method could be applicable for ratio spectra of two analytes X and Y in binary mixture using one of these components as a divisor. The applied resolution steps are summarized as follows:

**a Signals Analysis:** Statistical equation is computed representing the linear relationship of the difference of absorbance substituting \( \Delta P \) at \( \lambda_1 \) and \( \lambda_2 \) versus the recorded amplitude \( P_1 \) at \( \lambda_1 \) of different concentration of pure X.

\[
\Delta P = \text{slope } P_1 + \text{intercept}
\]

**b Resolution tool’s preparation:**
Factorized ratio spectrum of pure X using $P_1$ (FS$_{P1}$): is prepared through using the ratio spectrum of pure component of X using $D^0$ of Y as a divisor. Afterwards, the obtained ratio spectrum is divided by the recorded amplitude at $\lambda_1$ utilizing spectrophotometer’s software.

\[
\text{Factorized ratio spectrum of } X = \frac{X}{P_1} \tag{4}
\]

c **Spectral manipulation:** For analysis of mixture X and Y, $\Delta A$ at $\lambda_1$ and $\lambda_2$ is calculated and substituted in equation (3) to get the postulated amplitude value ($P_1$) corresponding to the X only at $\lambda_1$ then multiplied this value by FS$_{P1}$ ($\frac{X}{P_1}$) to get ratio spectrum of $\frac{X}{Y}$ then multiplied by Y$'$ to get PS of X. Then, PS of Y is obtained by difference between gross spectrum of each mixture, and extracted PS of X via spectrum subtraction method.

### 2.3. Ratio extraction method (RE)

**Principle:** The innovative method could be applicable for ratio spectra of two analytes X and Y in binary mixture using one of these components as a divisor. The drug of interest has optimum amplitude difference while interfered drug is constant so, its amplitude difference showing nil difference. The applied resolution steps are summarized as follows:

a **Signals Analysis:** The amplitude signals of ratio spectrum of X using Y$'$ as a divisor are recorded at two different wavelengths where $Y/Y'$ is constant, so amplitude difference is calculated at the chosen wavelengths.

b **Resolution tool’s preparation:** Factorized ratio spectrum of X using $\Delta P$ (FS$_{\Delta P}$): is prepared through using the ratio spectrum of pure component of X using $D^0$ of Y as a divisor. Afterwards, dividing the obtained ratio spectrum by the $\Delta P$ at $\lambda_1$ and $\lambda_2$.

\[
\text{Factorized ratio spectrum of } X = \frac{\Delta X}{\Delta P} \tag{5}
\]

c **Spectral manipulation:** For analysis of mixture of X and Y, the ratio spectra of each mixture are obtained using Y as a divisor. The amplitude difference at $\lambda_1$ and $\lambda_2$ is calculated then multiplied by the factorized ratio spectrum of X representing $\Delta P$ then multiplied by Y$'$ to extract PS of X. Then, PS of Y is obtained by difference between gross spectrum of each mixture, and extracted PS of X via spectrum subtraction method.

### 3. Experimental

#### 3.1. Apparatus and operational system

The study was conducted by utilizing a Shimadzu (UV-1800 spectrophotometer) affixed to ACER computer and operated by UV-Probe 2.43 system software. Using quartz cells-1.0 cm, accurate recording of the absorption over the range of 200.0 nm–400.0 nm at 0.1 nm as interval was conducted.

#### 3.2. Materials

3.2.1. Authentic materials

Pure CAN sample was kindly supplied by Pharaonia pharma, Borg Al Arab-Alexandaria-Egypt, while HCT was provided by the Egyptian International Pharmaceutical Industries (EIPICO), 10th of Ramadan City-Sharqia Governorate-Egypt. Their purity was found to be 99.58% ± 0.56 for CAN and 99.79% ± 0.65 for HCT, according to their official methods (European Pharmacopoeia, 2016; United States Pharmacopeia and National Formulary, 2019).

3.2.2. Pharmaceutical formulations

Atacand Plus® tablet, manufactured by AstraZeneca, 6th of October-Giza-Egypt, two formulas are available, formula I (B.N.19015) comprising 16.0 mg CAN and 12.5 mg HCT per tablet while formula II (B.N. 200340) comprising 32.0 mg CAN and 25.0 mg HCT per tablet. Purchasing of all the previously mentioned dosage forms was performed from local pharmacies.

3.2.3. Chemicals and solvents

Ethanol of HPLC-grade was kindly procured from Sigma-Aldrich, Darmstadt-Germany.

#### 3.3. Standard solutions

3.3.1. Standard stock solutions

Stock solutions (1000.0 $\mu$g/mL) of all proposed drugs were prepared accurately by dissolving 100.0 mg of each of the proposed drugs, separately in ethanol in 100-mL volumetric flask, afterwards the volume was attuned to the mark through utilizing the same solvent and were kept at the refrigerator.

3.3.2. Working solutions

Further dilutions of the stock solutions were performed to attain a concentration of (100.0 $\mu$g/mL) for the proposed drugs.
3.3.3. Synthetic mixtures
Accurate portions with various ratios were transferred accurately from the working solutions (100.0 µg/mL) for all proposed drugs. The volume was attuned to the mark by ethanol. Scanning of previously prepared mixtures’ spectra was performed at wavelength region (200.0–400.0 nm) and then saved on the computer.

3.4. Procedure
Accurate portions equivalent to 50.0–500.0 µg CAN and 20.0–240.0 µg HCT were transmitted from their correlating working solutions (100.0 µg/mL) to two separate sets of volumetric flasks (10-mL), then utilizing ethanol, the volume was attuned to the mark. Scanning of absorption spectra of conducted standard solutions was carried out in the region from 200 to 400 nm and then saved on the computer.

3.4.1. Spectral characteristic
Using standard working solutions (100.0 µg/mL), CAN (16.0 µg/mL) and HCT (12.5 µg/mL), were prepared and scanned accurately in a wavelength region (200.0–400.0 nm) utilizing ethanol as blank and their D0 were saved on the computer.

3.4.2. Construction of calibration curves
Utilizing the average of three experiments, construction of calibration curves of (CAN) and (HCT) was conducted through plotting their absorbance at their maxima 270.5 nm and 255.0 nm, respectively against their correlative concentrations, afterwards accurate computation of regression equations was performed.

3.5. Preparation of resolution tools
3.5.1. Factorized spectra (FS)
- Factorized D0 spectrum of CAN for (AR) method: Zero order absorption spectra (D0) of pure CAN of concentration within its linearity range was divided by the recorded value of the absorbance at Aiso (257.2 nm) using arithmetic function in spectrophotometer’s software and saved on the computer.
- Factorized ratio spectrum of HCT using CAN as a divisor for (PR) method: The obtained ratio spectrum of pure HCT of concentration within its linearity range utilizing CAN (30.0 µg/mL) as a divisor is divided by the amplitude’s value at Piso (257.2 nm) using arithmetic function in spectrophotometer’s software and saved on the computer.
- Factorized ratio spectrum of HCT using CAN as a divisor for (RE) method: The obtained ratio spectrum of pure HCT of concentration within its linearity range utilizing CAN (30.0 µg/mL) as a divisor is divided the amplitude difference (ΔP274.5–287.0 nm) and saved on the computer.

3.5.2. Data processing
- For (AR) method: Statistical equation representing the absorbance difference of various concentrations of CAN within its linearity range at 257.2 nm and 280.9 nm (ΔA257.2-280.9 nm) against absorbance at 257.2 nm (λiso) was computed.
- For (PR) method: Statistical equation representing amplitude difference of the ratio spectra of various concentrations of HCT within its linearity range at 257.2 nm and 274.5 nm (ΔP257.2–274.5) utilizing standard solution of CAN (30.0 µg/mL) against corresponding amplitude at 257.2 nm (λiso) was computed.

3.6. Analysis of synthetic mixtures
3.6.1. Absorbance resolution method (AR)
Absorbance values at 257.2 nm and 280.9 nm of the scanned D0 absorption spectra of the binary mixture were recorded, followed by calculation of the difference between them. Afterwards, the absorbance at Aiso (257.2 nm) of CAN in each D0 spectrum of the binary mixture was attained by utilizing the previously computed statistical equation representing absorbance difference at (ΔA257.2-280.9 nm) against absorbance at λiso (257.2 nm) for pure CAN. The parent spectrum (D0) of CAN could be obtained by multiplication of the calculated Aiso (257.6 nm) by the corresponding factorized of CAN. Thus, parent D0 spectrum of HCT was restored via subtraction of the attained D0 of CAN from its gross spectra of its corresponding binary mixture. CAN and HCT concentrations of each binary mixture were calculated through substituting in the correlating regression equations at their maxima 255.0 nm and 270.5 nm for CAN and HCT, respectively.

3.6.2. Amplitude resolution method (PR)
Division of D0 of each binary mixture (CAN and HCT) by D0 of standard solution of CAN (30 µg/mL) was performed to obtain the ratio spectra of (HCT/CAN), and then saved in the computer. Amplitudes at 257.2 nm and 274.5 nm of the ratio spectra of each binary mixture were recorded, followed by calculation of the difference between them. Afterwards, the amplitude at Piso (257.2 nm) of HCT in each ratio spectrum of the binary mixture was attained by utilizing the previously computed statistical equation representing amplitude difference at (ΔP257.2–274.5 nm) against amplitude at Piso (257.2 nm) for pure HCT using CAN as a divisor. The parent spectrum (D0) of HCT could be attained by multiplication of the calculated Piso (257.2 nm) by the corresponding factorized ratio spectra of HCT, followed by multiplication by the divisor’s spectrum of CAN (30.0 µg/mL). Thus, parent D0 spectrum of CAN was restored via subtraction of the attained D0 of HCT from its gross spectra of its corresponding binary mixture. CAN and HCT concentrations of each binary mixture were calculated through substituting in the correlating regression equations at their maxima 255.0 nm and 270.5 nm for CAN and HCT, respectively.
3.6.3. Ratio extraction (RE) method

Division of $D^0$ of each binary mixture (CAN and HCT) by $D^0$ of standard solution of CAN (30.0 μg/mL) was performed to obtain the ratio spectra of (HCT/CAN), then saved in the computer. Amplitudes at 274.5 nm and 287.0 nm of the ratio spectrum of each binary mixture were recorded, followed by calculation of the difference between them. The parent $D^0$ of HCT could be attained by multiplication of the calculated amplitude difference ($\Delta P_{274.5-287.0\text{nm}}$) by the corresponding factorized ratio spectra of HCT followed by multiplication by the divisor’s spectrum of CAN (30.0 μg/mL). The parent $D^0$ spectrum of CAN could be obtained after spectrum subtraction of the attained $D^0$ of HCT from its gross spectra of its corresponding binary mixture. CAN and HCT concentrations of each binary mixture were calculated through substituting in the correlating regression equations at their maxima 255.0 nm and 270.5 nm for CAN and HCT, respectively.

3.7. Assessment of pharmaceutical formulations

3.7.1. Atacand plus® tablets (two formulas)

3.7.1.1. Sample preparation. Accurately weighing of ten tablets, followed by grinding were performed. An accurate weight of the mixed sample equivalent to one tablet from each of the two formulas, (formula I comprising 16.0 mg CAN and 12.5 mg HCT per tablet while formula II comprising 32.0 mg CAN and 25.0 mg HCT per tablet) was separately transferred into two beakers, then 50-mL ethanol was added with continuous magnetic stirring for about 10 min. Each solution was separately filtered through a 0.45 μm filter paper into two 100-mL volumetric flasks then an appropriate dilution with ethanol was performed to get solution with final concentration claimed to be 16.0 μg/mL and 12.5 μg/mL of CAN and HCT, respectively.

3.7.1.2. Spectral analysis. Analysis and determination of the proposed drugs in their pharmaceutical formulations were accomplished using the presented methods discussed before in the section of analysis of synthetic mixtures (section 3.6).

3.7.1.3. Calculation of concentrations. Calculation of concentrations of CAN and HCT was performed utilizing their correlating regression equations.

3.7.2. Spectral print recognition via purity index (SR-PI)

The above stated and described procedures were performed for each entire tablet (10 tablets were utilized) to extract the parent spectra of CAN and HCT. Recording the absorbance value at wavelengths pairs 255.0 nm and 305.0 nm for CAN and 270.5 nm and 320.0 nm for HCT for extracted spectrum of pharmaceutical formulations and authentic spectrum of the pure drugs was performed using the proposed methods.

4. Results and discussion

Spectrophotometry is a well-established and invaluable technique in quality control laboratories for routine drug testing around the world. The presence of completely overlapping spectra in drug mixture hinders their direct determination and subsequently
necessitates an efficient mathematical separation procedure otherwise their mechanical separation is mandatory. Additionally, drug mixtures having one or more distinctive spectral characteristics encourage the progress of new spectral resolution techniques which are superior other than traditional ones by their capabilities of reducing manipulation steps. In addition, spectrophotometry has also taken important role towards green analytical chemistry, replacing hazardous solvents with more sustainable ones.

In this work, validation of simple three spectrophotometric methods, namely, absorbance resolution (AR), amplitude resolution (PR) and ratio extraction (RE) was the main purpose of the present work to quantify and analyze the proposed drugs in their pure form and diverse pharmaceutical formulations using PWDPT. The principle of PR and RE methods is based on HIAM strategy (Saleh et al., 2022) where the amplitude relating to the drug of interest is transformed into its corresponding parent spectrum (D^0).

Those methods are able to obtain the parent spectra which are the basic spectrum of each drug and considered as their fingerprint, so they are used to get the concentration of each component at their maxima. Additionally, they are superior owing to their fast quantitative resolution of samples composing of two or more substances without a need for any chemical pretreatment.

To illustrate the power of (PWDPT), the antihypertensive formulations consisting of CAN and HCT in their ratio, 1.28:1.0 for CAN and HCT, respectively were taken as models. By scanning standard solutions of the proposed drugs, it was found that CAN and HCT were completely overlapped which prevented their direct resolution, Fig. 2.

4.1. Experimental optimization

The overlain spectra of CAN and HCT binary mixture suggested that PWDPT was a suitable for their determination. Ethanol was utilized as solvent system, because it was found that it has optimum characteristics regarding studied drugs’ solubility, costs of the production and disposable process, signal sensitivity and finally its score of the environmental, health & safety (EHS). The scanning range was 200.0–400.0 nm, and the scanning speed was medium. During APE 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 of CAN. 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 remained unaffected. Using a divisor concentration of 30.0 µg/mL for CAN gave the best results concerning accuracy, reproducibility and recovery in synthetic mixtures and various pharmaceutical formulations. In AR method, wavelengths (257.2 nm and 280.9 nm) were selected for determination of CAN while wavelengths (257.2 nm and 274.5 nm) and (274.5 nm and 287.0 nm) were selected for determination of HCT in PR and RE methods, respectively. Optimized method parameters for (PWDPT) are shown in Table S1 (in the Supplementary Information).

4.2. Analysis of synthetic mixtures

The developed (PWDPT) for the analysis of CAN and HCT consist of three complementary steps, signals analysis (statistical manipulation or arithmetic signal difference), resolution tool’s preparation (factorized spectrum) and finally spectrum manipulation. This technique including three innovative methods namely, absorbance resolution (AR), amplitude resolution (PR) and ratio extraction (RE) as follows:

4.2.1. Absorbance resolution method (AR)

The essential requirement for AR method is choosing two wavelengths; one of them is isoabsorptive point (λ_iso), where the interfering component shows equal absorbance value while the analyte of interest shows substantial absorbance difference. Accordingly, the difference between those selected two points on the D^0 of a mixture is directly related to the concentration of the analyte of interest, independence of the interfering ones. The applied resolution steps are summarized as follows:

a Signals Analysis: Choosing the suitable wavelengths is the key role concerning simplicity, selectivity. Isoabsorptive point was selected as one of the chosen wavelengths which added a privilege to this method since both factorized spectra of studied drugs could be used as a resolution tool. In this procedure, the absorbance difference at λ_iso (257.2 nm) and 280.9 nm was used for CAN determination where HCT has zero absorbance difference, Fig. 2. Regression equation was computed showing linear relationship among the absorbance difference of pure CAN at (ΔA257.2–280.9nm) against A257.2nm (λ_iso) with the following equation:

\[ ΔA_{257.2–280.9nm} = 0.5665A_{λ_{iso}} (257.2nm) + 0.0084 \quad (R^2 = 1.0000) \]  

b Resolution tool’s preparation: The factorized spectrum of pure CAN was prepared by division the scanned D^0 of CAN by its recorded absorbance at A257.2 nm (λ_iso).

c Spectral manipulation: For analysis of mixture, the absorbance value A_{iso} at λ_iso (257.2 nm) of CAN in the mixture was obtained via substituting in equation (6).

The D^0 of CAN in the mixture was obtained via multiplication of the absorbance value at λ_iso by the corresponding factorized CAN. Subsequently, D^0 of HCT could be successfully resolved by utilizing the method of spectrum subtraction, where the attained D^0 of CAN in each mixture was subtracted from the corresponding mixture’s gross D^0 absorption spectrum.

CAN and HCT concentrations were calculated by utilizing their correlating regression equations which represent a linear relationship among their absorbance values at 255.0 nm for CAN and 270.5 nm for HCT against their correlating concentrations in the range (5.0–50.0 µg/mL) and (2.0–24.0 µg/mL) for CAN and HCT respectively.
4.2.2. Amplitude resolution method (PR)

This novel resolution method is applied at ratio spectra of the studied binary mixture having isosbestic point using interfering component as a divisor. The essential requirement for PR method is choosing two wavelengths; one of them is iso-point ($\lambda_{iso}$); where the interfering analyte shows equal amplitude value (constant) while the analyte of interest shows substantial amplitude difference. Accordingly, the difference between those selected two points on the $D_0$ of a mixture is directly related to the analyte of interest’s concentration, independent of the interfering ones. The applied resolution steps are summarized as follows:

a Signals Analysis: Selection of suitable wavelengths plays a key role concerning selectivity and sensitivity. Isoabsorptive point was selected as one of the chosen wavelengths which added a privilege to this method since the factorized spectra of both drugs could be used as a resolution tool in the data processing steps.

In this procedure, amplitude difference between 257.2 nm ($\lambda_{iso}$) and 274.5 nm ($\lambda_2$) for ratio spectra of HCT using CAN (30.0 $\mu$g/mL) as a divisor where CAN is constant with zero amplitude difference at these selected wavelengths, Fig. 3. Regression equation was computed showing linear relationship among the amplitude difference of HCT at 257.2 nm and 274.5 nm ($\Delta P_{257.2-274.5\text{nm}}$) against $P_{iso}$ (257.2 nm) with the following equation (7).

$$\Delta P_{257.2-274.5\text{nm}} = 2.2224 \times P_{iso\ (257.2\text{nm})} + 0.0089 \quad \left( R^2 = 1.0000 \right) \quad (7)$$

b Resolution tool’s preparation: The factorized ratio spectrum of pure HCT was prepared by dividing the ratio of (HCT/CAN) by its recorded amplitude at 257.2 nm ($\lambda_{iso}$).

c Spectral manipulation: For analysis of mixture, the amplitude of HCT in the mixture at 257.2 nm ($P_{iso\ (257.2\text{nm})}$) could be determined through substitution in equation (7).

Ratio spectrum of HCT/CAN could be obtained by multiplying the obtained $P_{iso\ (257.2\text{nm})}$ of each mixture by previously prepared factorized HCT. Then, by multiplying the ratio spectrum of HCT/CAN by the CAN divisor (30.0 $\mu$g/mL), parent $D_0$ of HCT was obtained. Subsequently, $D_0$ of CAN of each mixture could be successfully resolved by utilizing the spectrum subtraction method, where the attained $D_0$ of HCT in each mixture was subtracted from the corresponding mixture’s gross $D_0$ absorption spectrum.

CAN and HCT concentrations were calculated by utilizing their correlating regression equations which represent a linear relationship among their absorbance values at 255.0 nm for CAN and 270.5 nm for HCT and against their correlating concentrations in the range (5.0–50.0 mg/mL) and (2.0–24.0 mg/mL) for CAN and HCT respectively.

4.2.3. Ratio extraction method (RE)

This novel resolution method is 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 interfering component displays the same amplitudes (constant) and the component of interest displays significant amplitude difference. The applied resolution steps are summarized as follows:

![Fig. 3. Ratio spectra of binary mixture of CAN———) 16.0 $\mu$g/mL and HCT (—) 12.5 $\mu$g/mL using CAN 30.0 $\mu$g/mL as a divisor showing the selected $\lambda_{iso}$ (257.2 nm) and $\lambda_2$ (274.5 nm).](attachment:image.png)
a Signals Analysis: \( D^0 \) of the binary mixture of (CAN+HCT) is divided by \( D^0 \) CAN (30.0 \( \mu g/mL \)) to get ratio spectrum of \( (\frac{CAN}{HCT}\) + constant \( \frac{CAN}{CAN} \)). Upon selecting two wavelengths for HCT ratio spectra and applying subtraction of these two amplitudes \((\frac{HCT}{CAN})_1 \cdot (\frac{HCT}{CAN})_2\), cancellation of the constant will be achieved, and the divisor analyte’s interference will be omitted so this amplitude difference will be directly related to the analyte of interest. The optimum wavelength pair was studied and found to be maximum \( \Delta P \) values via using peak maxima at 274.5 nm and peak trough at 287.0 nm, Fig. 4.

b Resolution tool’s preparation: The factorized ratio spectrum of pure HCT was prepared by dividing the ratio of \( (\frac{HCT}{CAN}) \) by the amplitude difference \( \Delta P \) at 274.5–287.0 nm.

c Spectral manipulation: The ratio spectrum of \( (\frac{HCT}{CAN}) \) in each mixture was obtained by multiplying the value of the amplitude difference \( \Delta P \) at 274.5–287.0 nm by the aforementioned prepared factorized ratio spectra of pure HCT, Fig. 4.

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

CAN and HCT concentrations were calculated by utilizing their correlating regression equations which represent a linear relationship among their absorbance values at 255.0 nm for CAN and 270.5 nm for HCT and against their correlating concentrations in the range (5.0–50.0 \( \mu g/mL \)) and (2.0–24.0 \( \mu g/mL \)) for CAN and HCT respectively.

4.3. Proposed methods’ validation

Diverse parameters of ICH guidelines were evaluated with the aim of guaranteeing the analytical validity of the proposed methods (ICH, 2005).

4.3.1. Linearity and range

Establishing the working linearity range was carried out with adhering to the limits of absorbance of the Beer’s law (Rostom et al., 2021). Satisfactory and acceptable linearity was revealed by good recovery, minor values of the intercept and nearness of the correlation coefficients(r) to unity. Table 1 summarizes the linearity ranges obtained by each of the proposed methods and their regression equations.

4.3.2. Detection and quantitation limits

Limit of detection and limit of quantification concentrations for CAN and HCT were determined based on standard deviation of the response and the slope of the calibration curve as per the ICH guidelines. Calibration curve prepared in linearity study was used for this purpose. The equations \( LOD = 3.3 \cdot \delta / S \) and \( LOQ = 10 \cdot \delta / S \) were utilized where \( \delta \) is the standard deviation of the response and \( S \) is the slope of calibration plot. Results are assembled in Table 1 where they demonstrate sensitivity of the developed spectrophotometric methods.

![Fig. 4. Ratio spectra of binary mixture of CAN(———) 16.0 \( \mu g/mL \) and HCT (—) 12.5 \( \mu g/mL \) using CAN 30.0 \( \mu g/mL \) as a divisor showing the selected \( \lambda_1 \) (274.5 nm) and \( \lambda_2 \) (287.0 nm).](image-url)
4.3.3. Accuracy
The suggested methods’ accuracy was assured by the acceptable percent recoveries attained after analyzing three concentration levels for each studied drug (15.0 $\mu$g/mL, 35.0 $\mu$g/mL and 45.0 $\mu$g/mL) for CAN and (4.0 $\mu$g/mL, 12.0 $\mu$g/mL and 20.0 $\mu$g/mL) for HCT through the developed linearity range for the studied drugs, Table 1.

4.3.4. Precision
Examination of triplicates of three disparate concentrations was conducted by the presented methods on the same day in addition to three consecutive days to assess both repeatability and intermediate precision of those methods utilizing concentrations of (10.0 $\mu$g/mL, 30.0 $\mu$g/mL and 40.0 $\mu$g/mL) and (6.0 $\mu$g/mL, 14.0 $\mu$g/mL and 22.0 $\mu$g/mL) for CAN and HCT, respectively, Table 1.

4.3.5. Specificity
Assuring the capability of the proposed methods to quantify and analyze the studied drugs in mixtures possessing diverse composition ratios was conducted and satisfactory percent recoveries values results were obtained, Table 2.

4.3.6. Robustness
Robustness of the developed spectrophotometric methods was evaluated by calculating RSD% demonstrated through small modifications in the established analytical conditions. Triplicate analysis of three concentrations using ethanol from different commercial suppliers (Sigma-Aldrich, Darmstadt-Germany and Merck Darmstadt-Germany) was done. The method proved to be robust with %RSD values which did not exceed 2.0%, Table 1.

4.3.7. Stability of solutions
Stability of the working solutions in ethanol was tested and it was found that no changes in spectral features were noticed for the stock and calibration solutions of the studied drugs in ethanol for a minimum period of 2 weeks when stored at 4 $^\circ$C.

4.3.8. System suitability
System suitability is done to demonstrate the suitability of the UV spectrophotometric system being used for the analysis. Six

Table 1
Regression parameters and validation sheet for determination of pure CAN and HCT by the proposed methods.

| Drug | Method | CAN (D$^0$ ($\lambda$ max 255.0 nm)) | HCT (D$^0$ ($\lambda$ max 270.5 nm)) |
|------|--------|---------------------------------|---------------------------------|
| Linearity range ($\mu$g/mL) | 5.0–50.0 | 2.0–24.0 |
| Regression equation Parameters | | |
| Slope | 0.0296 | 0.0680 |
| Intercept | 0.0012 | 0.0062 |
| Correlation coefficient (r) | 1.0000 | 0.9999 |
| Detection and quantitation limits | | |
| LOD | 0.341 | 0.294 |
| LOQ | 1.033 | 0.892 |
| Accuracy a (Mean ± %RSE) | 99.79 ± 0.120 | 98.79 ± 0.226 |
| Precision (± %RSD) | | |
| Repeatability b | 0.558 | 0.384 |
| Intermediate precision c | 0.705 | 0.835 |
| Robustness (%RSD) | 0.710 | 0.531 |

a Accuracy was checked using concentrations (15.0 $\mu$g/mL, 35.0 $\mu$g/mL and 45.0 $\mu$g/mL) and (4.0 $\mu$g/mL, 12.0 $\mu$g/mL and 20.0 $\mu$g/mL) for CAN and HCT, respectively.
b Repeatability, percentage relative standard deviation of three different concentrations in triplicate in the same day of (10.0 $\mu$g/mL, 30.0 $\mu$g/mL and 40.0 $\mu$g/mL) and (6.0 $\mu$g/mL, 14.0 $\mu$g/mL and 22.0 $\mu$g/mL) for CAN and HCT, respectively.
c Intermediate precision, percentage relative standard deviation of three different concentrations in triplicate in three consecutive days of (10.0 $\mu$g/mL, 30.0 $\mu$g/mL and 40.0 $\mu$g/mL) and (6.0 $\mu$g/mL, 14.0 $\mu$g/mL and 22.0 $\mu$g/mL) for CAN and HCT, respectively.

Table 2
Determination of the studied drugs in the laboratory prepared mixtures by the proposed methods.

| Recovery % ± SD | CAN: HCT | CAN (D$^0$) | HCT (D$^0$) |
|----------------|---------|-------------|-------------|
| Ratio | AR | SS of D$^0$ HCT via PR | SS of D$^0$ HCT via RE | SS of D$^0$ CAN via AR | PR | RE |
| (1.28:1.0) a | 100.50 | 100.81 | 99.79 | 100.58 | 100.55 | 99.07 |
| (2.56:1.0) | 100.67 | 99.69 | 99.77 | 100.87 | 99.63 | 99.63 |
| (1.02:1.0) | 100.74 | 100.28 | 98.63 | 99.67 | 100.25 | 100.75 |
| (3.01:1.0) | 99.92 | 99.65 | 100.23 | 100.58 | 99.25 | 100.25 |
| (3.02:1.0) | 99.47 | 100.53 | 98.99 | 99.43 | 100.23 | 100.58 |
| Mean ± SD | 100.26 ± 0.55 | 100.19 ± 0.51 | 99.48 ± 0.65 | 100.23 ± 0.63 | 99.98 ± 0.53 | 100.06 ± 0.70 |

a Average of three replicates.
b Ratio as dosage forms.
replicates of standard solution (10.0 μg/mL) of CAN and HCT were prepared from a stock solution in the selected solvent (ethanol) and absorbance was determined at their \( \lambda_{\text{max}} \) (255.0 and 270.5 nm for CAN and HCT, respectively) of each replicate using UV spectrophotometer. Percentage relative standard deviation (%RSD) was calculated for the absorbance and it was found to be 0.498% and 0.614% for CAN and HCT, respectively.

4.4. Application of the proposed methods of pharmaceutical formulations

Determination of the proposed drugs’ concentration in Atacand plus® tablets was carried out utilizing the proposed spectrophotometric methods and all of them showed acceptable percentage recoveries. This permits their utilization for regular analysis of the proposed drugs in their combined formulation, Table 3. The accuracy of pharmaceutical formulations was certified by standard addition technique as revealed in Table 3 using various, known, and definite concentrations of authentic standard CAN and HCT then applying the aforementioned methods was conducted and acceptable results were attained.

4.5. Statistical analysis

For Assuring the attained results of the presented methods for analyzing the studied drugs, statistical analysis based on Student t-test and F-test was carried out for the accurate comparison between the results obtained by developed methods and those obtained by the official methods for CAN (European Pharmacopoeia, 2016) and HCT (United States Pharmacopeia and National Formulary, 2019). It was found that at p-value 0.05, the values of computed Student t and F test are not more than their respective theoretical ones indicated negligible difference between the developed and official methods as revealed in Table S2 (in the Supplementary Information). In addition, the statistical analysis was implemented between the results of developed methods and those of official methods utilizing of one way ANOVA test. The results attained from ANOVA analysis indicated insignificant difference among the developed and official methods as revealed in Table S3 (in the Supplementary Information).

4.6. Spectral print recognition via purity index (SR-PI)

Using advanced spectrophotometric methods based on the extraction of the parent spectrum of each cited drugs which act as spectral print is advantageous due to its capability to assess and evaluate the sample purity. Assessment of purity is greatly critical respecting compounds having a biological activity as impurities’ traces with or without great potency can probably cause undesirable action (Coyle, 1972). This SR-PI 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 the parent extracted spectrum and its scanned spectrum. To assess the SR-PI of the cited drugs, the absorbance ratio at two chosen wavelengths for the identical concentration of the extracted spectrum from the commercial formulations after applying the developed methods and the authentic spectrum of the cited drugs should be calculated (\( \lambda_1/\lambda_2 \) of extracted spectrum/\( \lambda_3/\lambda_4 \) of authentic spectrum) and it should be around 1.000 (0.991–1.000) to ascertain the purity. The two selected wavelengths are 255.0 and 305.0 nm for CAN and 270.5 and 320.0 nm for HCT. SRI was evaluated for CAN and HCT in each individual Atacand plus® tablet (utilizing 10 tablets). The purity profile was calculated via utilizing their extracted D0 spectra of CAN (16.0 μg/mL) and HCT (12.5 μg/mL) from the two formulas of the pharmaceutical dosage forms by the developed methods and their equivalent D0 of pure drugs of the same concentrations found in the analyzed products. The attained values of SR-PI from Atacand plus® tablets were all acceptable and satisfactory verifying their purity, Table 4.

4.7. Greenness profile assessment

The compliance of the presented spectrophotometric methods to the hypotheses of the green analytical chemistry was listed

**Table 3**

Determination of the studied drugs in pharmaceutical formulations by the proposed methods and application of standard addition technique.

| Pharmaceutical dosage form | CAN (D0) | HCT (D0) |
|-----------------------------|----------|----------|
|                             | AR       | SS of D0 HCT via PR | SS of D0 HCT via RE | SS of D0 CAN via AR | PR | RE |
| Pharmaceutical dosage form* (Atacand plus®) | | | | | | |
| Formula I (B.N. 19015) (1.28 CAN:1.0 HCT) (%Found ± SD) | 99.72 ± 0.66 | 100.88 ± 0.44 | 99.90 ± 0.60 | 99.03 ± 0.61 | 99.11 ± 0.60 | 99.03 ± 0.54 |
| Formula II (B.N. 200340) (1.28 CAN:1.0 HCT) (%Found ± SD) | 99.57 ± 0.69 | 100.92 ± 0.49 | 99.40 ± 0.64 | 99.73 ± 0.78 | 100.28 ± 0.43 | 100.26 ± 0.46 |
| Standard Addition* (Atacand plus®) | | | | | | |
| Formula I (1.28 CAN:1.0 HCT) (%Recovery ± SD) | 100.21 ± 0.73 | 100.14 ± 0.48 | 99.23 ± 0.84 | 99.47 ± 0.83 | 100.09 ± 0.43 | 99.59 ± 0.73 |
| Formula II (1.28 CAN:1.0 HCT) (%Recovery ± SD) | 100.84 ± 0.64 | 99.43 ± 0.48 | 99.01 ± 0.92 | 98.98 ± 0.82 | 99.22 ± 0.64 | 99.81 ± 0.58 |

* Average of six experiments.

**Table 3**

Determination of the studied drugs in pharmaceutical formulations by the proposed methods and application of standard addition technique.

| Pharmaceutical dosage form | CAN (D0) | HCT (D0) |
|-----------------------------|----------|----------|
|                             | AR       | SS of D0 HCT via PR | SS of D0 HCT via RE | SS of D0 CAN via AR | PR | RE |
| Pharmaceutical dosage form* (Atacand plus®) | | | | | | |
| Formula I (B.N. 19015) (1.28 CAN:1.0 HCT) (%Found ± SD) | 99.72 ± 0.66 | 100.88 ± 0.44 | 99.90 ± 0.60 | 99.03 ± 0.61 | 99.11 ± 0.60 | 99.03 ± 0.54 |
| Formula II (B.N. 200340) (1.28 CAN:1.0 HCT) (%Found ± SD) | 99.57 ± 0.69 | 100.92 ± 0.49 | 99.40 ± 0.64 | 99.73 ± 0.78 | 100.28 ± 0.43 | 100.26 ± 0.46 |
| Standard Addition* (Atacand plus®) | | | | | | |
| Formula I (1.28 CAN:1.0 HCT) (%Recovery ± SD) | 100.21 ± 0.73 | 100.14 ± 0.48 | 99.23 ± 0.84 | 99.47 ± 0.83 | 100.09 ± 0.43 | 99.59 ± 0.73 |
| Formula II (1.28 CAN:1.0 HCT) (%Recovery ± SD) | 100.84 ± 0.64 | 99.43 ± 0.48 | 99.01 ± 0.92 | 98.98 ± 0.82 | 99.22 ± 0.64 | 99.81 ± 0.58 |

* Average of six experiments.

\( \text{Average of three experiments (pure added equivalent to 4.0, 8.0, 16.0 μg/mL of CAN and 3.125, 6.25, 12.5 μg/mL of HCT).} \)
throughout numerous assessment tools in order to figure a clear picture about the greenness profile of the presented methods. In addition, a greenness comparison between the proposed methods and the official methods for CAN (European Pharmacopoeia, 2016) and HCT (United States Pharmacopeia and National Formulary, 2019) was performed as follows:

4.7.1. National Environmental Methods Index (NEMI)

NEMI is considered from the first used qualitative tools for prompting the greenness of an analytical methodology, in which the design of simple circular diagram was established and divided into four quadrants. Each quarter shows specific criteria, namely; the usage of PBT (persistent, bioaccumulative and toxic) chemicals, consumption of hazardous chemicals, corrosiveness and the amount of waste generated (Keith et al., 2007; Tobiszewski, 2016). The proposed spectrophotometric methods are considered the greenest ones displaying fortunately predominant green color of the four quadrants as ethanol doesn’t exist either in the PBT list or in the TRI (Toxic Release Inventory TRI Chemical List for Reporting, 2019). In addition, the pH of the study samples was non-corrosive (pH is not less than 2 and not higher than 12) and the amount of waste produced was less than 50 g/sample, Table 5. On the other hand, HCT official HPLC method shows 3 out of 4 quadrants are green shaded, since including hazardous solvents

Table 4

The uniformity of dosage unit and purity index for Candesartan (CAN) and Hydrochlorothiazide (HCT) in Atacand Plus® tablets.

| Tablet No. | Formula I | Formula II |
|------------|-----------|------------|
|            | CAN (D⁰)  | HCT (D⁰)   | CAN (D⁰)  | HCT (D⁰)   |
|            | Purity index |          | Purity index |          |
|            | AR | SS of D⁰ | HCT via PR | HCT via RE | SS of D⁰ | CAN via AR | SS of D⁰ | CAN via PR | HCT via RE | SS of D⁰ | CAN via AR | SS of D⁰ | CAN via PR | HCT via RE | SS of D⁰ | CAN via AR | SS of D⁰ | CAN via PR | HCT via RE | SS of D⁰ | CAN via AR |
| Tablet 1   | 0.989 | 0.993 | 0.996 | 0.991 | 0.991 | 0.999 | 0.999 | 0.992 | 0.993 | 0.996 | 0.99  | 0.994 | 0.996 | 0.999 | 0.996 | 0.99  | 0.994 | 0.996 | 0.999 | 0.996 |
| Tablet 2   | 0.995 | 0.99 | 0.999 | 0.996 | 0.998 | 0.994 | 0.997 | 0.994 | 0.997 | 0.996 | 0.998 | 0.997 | 0.994 | 0.996 | 0.996 | 0.998 | 0.997 | 0.996 | 0.999 |
| Tablet 3   | 0.99 | 0.987 | 0.998 | 0.998 | 0.996 | 0.992 | 0.995 | 0.994 | 0.995 | 0.996 | 0.994 | 0.999 | 0.994 | 0.997 | 0.996 | 0.998 | 0.999 | 0.999 |
| Tablet 4   | 0.992 | 0.994 | 0.895 | 0.997 | 0.997 | 0.995 | 0.995 | 0.995 | 0.995 | 0.995 | 0.994 | 0.999 | 0.996 | 0.996 | 0.997 | 0.998 | 0.996 | 0.996 |
| Tablet 5   | 0.99 | 0.991 | 0.988 | 0.992 | 0.993 | 0.995 | 0.995 | 0.994 | 0.995 | 0.995 | 0.996 | 0.999 | 0.996 | 0.999 | 0.997 | 0.997 | 0.996 |
| Tablet 6   | 0.991 | 0.987 | 0.992 | 0.985 | 0.998 | 0.999 | 0.999 | 0.996 | 0.996 | 0.996 | 0.996 | 0.999 | 0.995 | 0.999 | 0.997 | 0.999 | 0.999 | 0.999 |
| Tablet 7   | 0.988 | 0.999 | 0.999 | 0.988 | 0.991 | 0.998 | 0.998 | 0.998 | 0.999 | 0.999 | 0.994 | 0.992 | 0.996 | 0.996 | 0.997 | 0.999 |
| Tablet 8   | 0.989 | 0.986 | 0.995 | 0.999 | 0.993 | 0.994 | 0.995 | 0.994 | 0.995 | 0.995 | 0.995 | 0.998 | 0.997 | 0.996 | 0.998 |
| Tablet 9   | 0.99 | 0.998 | 0.994 | 0.996 | 0.989 | 0.995 | 0.992 | 0.995 | 0.993 | 0.994 | 0.998 | 0.996 | 0.992 |
| Tablet 10  | 0.992 | 0.994 | 0.988 | 0.998 | 0.995 | 0.994 | 0.999 | 0.995 | 0.999 | 0.999 | 0.992 | 0.996 | 0.992 |
| Mean       | 0.993 | 0.996 | 0.996 | 0.990 | 0.997 | 0.998 | 0.996 | 0.990 | 0.995 | 0.995 | 0.996 | 0.993 | 0.996 | 0.996 |
| SD         | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.001 | 0.002 | 0.002 | 0.001 | 0.002 | 0.003 | 0.002 | 0.002 |

Table 5

Greenness assessment of the proposed spectrophotometric methods by Eco-scale, NEMI, GAPI and AGREE tools and whiteness assessment by RGB12 model.

| Parameters | Penalty points (PPs) | NEMI tool | GAPI tool | AGREE tool |
|------------|----------------------|-----------|-----------|------------|
| Eco-scale assessment | | | | |
| 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 | | | |

Whiteness Assessment (RGB12 Model)

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*a* Ethanol is set a signal word ‘danger’ with two pictograms and consumed volume per sample analysis is < 10 mL (sample cuvette).

*b* Score of ‘0’ is given as for spectrophotometric technique; the energy used is ≤ 1.0 kWh per sample.
in the mobile phase. Whereas, CAN official potentiometric method is designated to be the least green one, yielding more than 50 g/sample of waste and working under corrosive pH, Table S4 (in the Supplementary Information).

### 4.7.2. Analytical eco-scale system

Achieving evaluation with more specific, precise and semi-quantitative parameters was obtained upon implementing analytical Eco-scale, in which the influences of numerous method parameters comprising the hazardousness and amount of the used reagents, waste generation, energy consumption, types of waste treatment and any probable occupational risks were allocated as penalty points (PPs) (Gatulska et al., 2012). The calculated penalty points are subtracted from the value of the ideal green process (100), an excellent green analysis scores >75, an acceptable green analysis scores >50, and if the method scores <50, it will be considered as inadequate green analysis. The eco-scale analysis shown in Table 5 supported the NEMI findings, placing the proposed spectrophotometric methods, which received a score of 93, above the other procedures, followed by HCT official HPLC method with a score of 78 and CAN official potentiometric method with the least score of 68, Table S4 (in the Supplementary Information).

### 4.7.3. Green Analytical Procedure Index (GAPI)

GAPI tool is an approach composing of hybrid of both NEMI and Eco-scale. It has been presented to assess the whole analytical procedures’ green character (Plotka-Wasyllka, 2018). This tool utilizes a certain pictogram with five pentagons with color-coded extending from green through yellow to red, indicating low, medium, and high environmental impact of each level. The GAPI tool provides information regarding 15 different parameters which evaluate the environmental effect of the method’s main steps (sample preparation, collection, preservation, transport and storage, general method type, reagent and compounds used and instrumentation). The developed spectrophotometric methods possess the greatest green shaded sections (7) and least red shaded ones (2), Table 5. On the other hand, CAN official potentiometric method shows most sections shaded red (6), Table S4 (in the Supplementary Information).

### 4.7.4. Analytical greenness metric (AGREE)

AGREE tool (Pena-Pereira et al., 2020) is a software which could be downloaded, producing simple colored pictogram comprising twelve sections which cover twelve principles of green analytical chemistry. The final score is a fraction of unity from zero to one that appears in the middle of the AGREE pictogram. The result associated color in the middle is the combination of colors presenting the performance of the 12 AGREE pictogram sections, where each section is colored ranging from deep green to deep red based on its environmental impact. The ideal method has a score of one taking dark green color. Therefore, AGREE metric is considered user-friendly, comprehensive, easy to apply and very fast. The presented AGREE pictogram of the developed methods in Table 5 illustrates the greenness of the methods with an overall score of 0.74 which is better than CAN official potentiometric method (0.41) and HCT official HPLC method (0.52), Table S4 (in the Supplementary Information).

Conclusively, the successfulness of the presented methods lies on their capability to reduce the utilization and consumption of the used solvent and instrumental energy. In addition, the use of ethanol is clearly reflected via the obtained high scores of the analytical eco-scale and AGREE tools with the prevalence of green color in both NEMI and GAPI pictograms as compared to those for the official methods as shown in Table S4 (in the Supplementary Information). Therefore, the developed methods are considered “green methods” which can be safely applied without any vulnerable harm to either the analyst or the environment.

### 4.8. Whiteness tool for assessing analytical chemistry method

Sustainable development should integrate the environment, society, and economy as three main pillars. Available algorithms for global evaluations of analytical methods is provided via recently introduced Red-Green-Blue (RGB) model which is quantitative evaluation tool provides sustainability by calculating the whiteness of the analytical method (Nowak et al., 2020; Nowak and Kościelnia, 2019; Elbordiny et al., 2022). This color model commonly used in electronics, which extends the concept of green chemistry to other primary colors (Red and Blue). Red is assigned to analytical efficiency as expressed by validation criteria such as accuracy, precision, LOD, sensitivity and others), green stands for compliance with GAC principles related to environment safety such as toxicity of the reagents, number and amount of reagents used, and waste generated during the complete process, energy used and the total impact on the environment, while blue represents productivity and practical/economic efficiency including the cost, time, least practical requirements, and operational simplicity. In order to evaluate the method using the RGB 12 algorithm, the red, green and blue tables prepared in the Excel template spreadsheet must be filled out (Nowak and Kościelnia, 2019). Following a computational assessment, the method is assigned a color resulting from the share of individual primary colors, an ideal sustainable, reliable analytical method characterized by a high saturation of all three colors to give white color. The template allows for simultaneous evaluation, 0 being the worst result, and 100 meaning that the method is well fitted for a planned application in respect to particular principle.

The validation results in table (1) confirm that the paired wavelength data processing techniques (PWDPT) have produced values within accepted and appropriate tolerance levels, with the outcome of 97.5% for the red criteria. An advantage of this approach is that biodegradable ethanol solvent with two pictogram was used in small quantity, so these methods among (PWDPT) produced less amount of the waste, with the outcome of 96.3% green criteria. All spectrophotometric methods are considered simple, fast, cost effective, and time consuming, having the blue criteria for the (PWDPT) 98.8%. Collective results showed that the proposed spectrophotometric methods among (PWDPT) technique are sensitive, safe, rapid and costless with an overall score of the whiteness 97.5% as detailed in Table 5.

### 4.9. Comparative study between developed methods and their corresponding reported spectrophotometric methods

The criteria for applying the developed technique (PWDPT) including three innovative spectrophotometric methods to evaluate
binary drugs is the data processing using recorded signals at two paired wavelengths by coupling mathematical analysis of signals (statistical equation or signal difference) and spectrum extraction using resolution tool (factorized spectrum) prepared by built-in spectrophotometric software. These methods, namely, (AR), (PR) and (RE) which successfully have the ability to extract the parent spectra (D^0) of the two studied drugs allowing the determination of their concentrations at their maxima with maximum accuracy and precision using one divisor in case of PR and RE with minimum manipulation steps. PR method has advantage over AR since the slope at the two chosen wavelengths exhibits a higher value with more accurate determination of CAN and HCT, therefore minimizing the error and enhancing sensitivity. RE method is advantageous over both AR and PR methods since it has no limitation in the choice of two wavelengths used in signals analysis with no need for the presence of extension region or iso-point, accordingly it could be applied on all mixtures containing different drugs concentrations with satisfactory results. This unique data processing technique offers advantages over the other reported spectrophotometric methods (Abdelaleem et al., 2013; Ahmad Charoo et al., 2009; Belal et al., 2013; Daharwal and Singh, 2015; Mohamed et al., 2020; Mukthinuthalapati and Kumar, 2015; Üstündag and Dinç, 2021; Workie et al., 2017; Youssef et al., 2010; ÜSTÜNDAG and Erdal, 2021; Erk, 2003a) where it encouraged the extraction of the parent spectra of each drug in the mixture acting as spectral print and confirming their purities.

5. Conclusion

Three simple, sustainable, environment-friendly and selective spectrophotometric methods among PWDPT namely, AR and PR and RE are applied for the analysis of CAN and HCT in their formulations where CAN belongs to ARBs that have an important role for curing patients with modest or severe hypertension symptoms along with their significance minimization of risks related to the serious clinical evolution of COVID-19 for antihypertensive patients suffering from corona virus. Concerning the presented method, RE method showed optimum selectivity with higher sensitivity without any limitation which gave it privilege over AR and PR methods. Such spectrophotometric techniques are implemented in preference to traditional chromatographic techniques owing to their ability to down size energy, time and solvent consumption. Preventing the usage of hazardous solvents was successfully performed via utilizing ethanol which is considered the most appropriate UV organic solvent in addition to its green features. The developed methods were effectively applied to assess the analytes’ purity profiles in their tablet formulation. Additionally, the greenness and whiteness evaluation of the developed methods for studied mixture confirmed the safety, environmental sustainability, better time and cost efficiency. The unique simplicity, accuracy and consistency of the developed methods as well as their superior greenness and whiteness profile and capability of testing purity, encouraged their applicability and suitability in quality control laboratories.

Credit author statement

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Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scp.2022.100806.

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