## RESEARCH





A unique revolutionary eco-friendly spectrophotometric technique for solving the spectral overlap in the determination of carvedilol and ivabradine in their binary combination: stability study

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## Abstract

**Background** Carivalan<sup>®</sup> dosage form containing carvedilol and ivabradine is widely indicated for patients with stable angina. Three precise, eco-friendly and reproducible spectrophotometric techniques were created for resolving the researched drugs in their mixtures using zero and/or ratio spectra. Technique I is a factorized dual wavelength coupled with spectrum subtraction (FDW-SS), technique II is a ratio difference, and technique III is a constant center coupled with spectrum subtraction (CC-SS). Moreover, CAR and IVA were simultaneously determined in the existence of their oxidative degradation products exploiting the newly developed induced tripartite amplitude difference coupled with ratio subtraction (ITAD-RS) technique.

**Results** The calibration curves for CAR and IVA showed linearity within 3.0–30.0 µg/ml, each. Techniques' precision, accuracy, and linearity ranges were resolved and validated in harmony with ICH guidelines. Additionally, the specificity was examined by examining created combinations of the proposed drugs with LOD of 0.258 and 0.290 for CAR, while for IVA 0.272 and 0.204.

**Conclusion** These techniques were used to determine the presence of the provided drugs in Carivalan<sup>®</sup> tablets. There is statistical comparison between the found results of the offered spectrophotometric techniques and the previously reported ones with no discernible variance in the acquired results.

**Keywords** Carvedilol, Ivabradine, Ratio difference, Constant center, Dual wavelength, Spectrum subtraction, Induced tripartite amplitude difference, Factorized spectrum, Greenness assessment

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## Background

Carvedilol (Fig. 1a) is chemically [1-(9H-carbazol-4-yloxy)-3-[[2-(2methoxyphenoxy) ethyl] amino] propan-2-01]. It is a non-selective beta-blocker which works by reducing heart beats and facilitating smoother blood circulation. Additionally, it widens some blood arteries, acting similarly to an alpha blocker, which in turns helps in lowering the blood pressure [1, 2].

Ivabradine (Fig. 1b) is chemically (3-[3-[[(7S)-3,4-dimethoxy-7-bicyclo[4.2.0]octa-1,3,5-trienyl]



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Fig. 1 Chemical structures of a carvedilol and b ivabradine

methyl-methylamino]propyl]-7,8-dimethoxy-2,5-dihydro-1H-3-benzazepin-4-one). It is a drug that lowers heart rate by precisely and selectively blocking the cardiac pacemaker current;  $(I_f)$  and hence regulates the heart rate via direct sinus node inhibition without affecting myocardial contractility or blood pressure [3, 4].

Carivalan® containing CAR and IVA has received a widespread indication for treating the symptoms of stable angina, where few analytical HPTLC [5] and HPLC [6, 7] techniques for analyzing CAR and IVA have been stated. In the meantime, their simultaneous determination was achieved spectrophotometrically by only one reported research [8], while no spectrophotometric techniques have been previously conveyed for the declared drugs' resolving in the occurrence of their degradation products.

It is well known that the chromatographic analytical technique, which is distinguished by its superior accuracy and reproducibility in the process of samples' separation and analysis in parts per million (PPM) level as well as its speed and automation [9], is one of the most well-liked and frequently used techniques in the field of drug analysis. However, and particularly in light of the current conditions faced by the majority of the world's nations, especially developing nations, many analysts and researchers now turn to the usage of less expensive analytical techniques while keeping accuracy and precision.

The UV spectrophotometric approach is at the forefront of the methodologies that achieve most researchers' objectives because of its ease of use, low implementation cost besides widespread accessibility in laboratories for quality control [10].

This work focuses on the creating and validation of three green UV spectrophotometric techniques for the selective resolution of CAR and IVA in their pure forms and Carivalan<sup>®</sup> tablets by utilizing zero and/or ratio spectra; FDW-SS, RD [11–13] and CC-SS techniques [13–15]. Furthermore, induced tripartite amplitude difference (ITAD), a newly developed spectrophotometric technique, was able to solve the issue of resolving and analyzing CAR and IVA in their quaternary mixture with their oxidative degradates and succeeded in determining them without even requiring any preliminary separation steps. Green assessment of the offered, official and the reported techniques was achieved by using Analytical Eco-Scale [16], National Environmental Methods Index (NEMI) [17], Green Analytical Procedure Index (GAPI) [18] and Analytical Greenness Metric (AGREE) [19]. The proposed techniques' suitability for the intended usage has been validated in accordance with ICH criteria [20].

## Factorized dual wavelength coupled with spectrum subtraction technique (FDW-SS)

This technique depicts coupling between dual wavelength [21–24] and spectrum subtraction [24–26] for analyzing CAR and IVA in their mixture, where one drug X shows a noteworthy absorbance difference at two selected wavelengths which is directly proportional to its concentrations. While the other interfering drug *Y* shows equal absorbance reading with an insignificant absorbance difference at the same selected wavelengths ( $\Delta A = \text{zero}$ ), thus its effect is negligible. Recovering the parent  $D^0$  of X is accomplished by firstly preparing X's factorized  $\Delta A$  spectrum using computer software via dividing  $(D^0)$  X at any concentration within Beer's law by the  $\Delta A$  between the chosen wavelengths;  $\frac{X(D^{\circ})}{\Delta A}$ 

Secondly,  $\Delta A$  is recorded in the laboratory mixtures between the chosen wavelengths then multiplied by X's factorized  $\Delta A$  spectrum.

$$\Delta A. \frac{X(D^{\circ})}{\Delta A} = \text{Recovered}(D^{\circ}) of X$$
(1)

While  $D^0$  of the interfering drug *Y* is obtained via spectrum subtraction [25–27] technique;  $D^0(X+Y) - D^0(X)$ .

# Induced tripartite amplitude difference coupled with ratio subtraction technique (ITAD-RS)

An advanced technique that can be used on a ratio spectrum of quaternary mixtures made up of  $\left(\frac{[X+Y+Z+W]}{W'}\right)$  at selected wavelengths at which component X has a  $\Delta P$  noteworthy value and the amplitude readings of *Y* are not equal ( $\Delta P \neq 0$ ) at the same chosen wavelengths.

Meanwhile, component *Z* exhibits an equal amplitude value with a zero difference ( $\Delta P$ =0); thus, the effect of *Z* is canceled. It is a moderation of both induced dual-amplitude difference [27] and dual-amplitude difference [28] techniques.

The effect of *Y* was canceled via calculating the equality factor [13, 29] of *Y* by getting average Py1/Py2 at the suggested wavelengths (FY). Component *Y* will be canceled by calculating the difference. As a result, *P* (FyPm1–Pm2) is only connected to *X*.

A factorized induced ratio spectrum of X ;  $\frac{X/W'}{\Delta P.Fy}$  is set via division of the ratio spectrum of X by the calculated induced amplitude difference.

For laboratory mixtures, the induced amplitude difference values ( $\Delta P$ . Fy) at the suggested wavelengths are noted and multiplied by X's factorized induced ratio spectrum to acquire X's ratio spectrum.

$$\Delta P.Fy.\frac{\frac{X}{W}'}{\Delta P.Fy} = \frac{X}{W'}$$

Multiplying the recovered ratio spectrum of *X* by the divisor, *W*, will attain the parent  $D^0$  of X;  $\frac{X}{W'} \times W' = X(D0)$ 

Finally, subtracting the regained  $D^0$  of X from the quaternary mixture's  $D^0$  spectrum and applying the same previously mentioned steps, the other components in the mixtures will be determined.

Exploiting the factorized induced ratio spectrum and the divisor's spectrum multiplication in this newly developed method allows the mentioned component's determination at its  $\lambda_{max}$  through minimal signal output analysis of data. In addition, random error is highly minimized since the preparation of factorized spectrum is founded on the response only in contrast to normalized spectrum which is based on concentration.

### Methods

## Instrumentation

Shimadzu double-beam spectrophotometer (UV, Japan), Sonicator (Model 3510, UK), Analytical Balance, Italy, Digital Stirring Hot Plate, China, Pure laboratory flex to obtain distilled water Silica gel F254 ( $20 \times 20$  cm) pre-coated TLC plates (Sigma-Aldrich, Germany). The degradation tracing and plate visualization were carried out using a UV lamp with a wavelength of 254.0 nm.

#### **Reagents and chemicals**

Pure samples of CAR and IVA were kindly obtained from Global Napi, 6th of October, Egypt. The pureness was certified to be  $99.88 \pm 0.95$  and  $99.60 \pm 1.06$ , respectively, depending on their official [1] and reported [8] techniques.

The market-available Carivalan<sup>®</sup> tablet dosage form, made in France by Les Laboratories Servier Industrie, was purchased. According to the product label, Carivalan <sup>®</sup> batch number (29,044) contains 12.5 mg of CAR and 5.0 mg of IVA. 30% H<sub>2</sub>O<sub>2</sub> (ADWIC, Egypt).

#### **Standard solutions**

To prepare stock solutions of carvedilol and ivabradine (100.0  $\mu$ g/ml), in volumetric flasks, 100 ml, 10 mg of each of CAR and IVA was dissolved separately in distilled water.

In two rounded flasks, 10 mg of each of CAR and IVA was refluxed with 10 ml of 30%  $H_2O_2$  for 8 h at 80 °C. After refluxing, hydrogen peroxide was evaporated using hot plate at 50 °C, transfer oxidative degraded product to volumetric flask, 100 ml, which is completed with distilled water to prepare standard solutions of 100.0 µg/ml for each.

### Laboratory mixtures

Exact amounts of the stated drugs were conveyed into different volumetric flasks, 10 ml, with varied ratios and topped off with distilled water to produce variable sets as follows:

Set A: Binary mixture containing CAR and IVA.

Set B: Quaternary mixture containing CAR and IVA along with their oxidative degradation products.

### Procedures

#### Stress stability studies

Studied drugs' stress studies were conducted as per ICH guidelines [30] by applying different stress acidic, basic, photolytic, thermal and oxidative surroundings. Different molar concentrations of HCl and NaOH were tried out where, in each trial, 10 ml of each of the investigated medications was combined individually with either HCl or NaOH. Reflux was applied at 100 °C for 3 h.

To conduct oxidative trials, 10 mg of each medication was refluxed individually in 10 ml of 10, 20 and 30%  $\rm H_2O_2$  for 8 h at 80 °C.

Drugs' photostability was tested on their solid powdered form by the exposing them to UV light, 254.0 nm for 10 h.

Finally, drugs' thermal stress testing was performed in glass ampoules by heating them in a thermostatic oven at  $10 \degree$ C boosts (50–100 °C) for 10 h.

### Spectral characteristics

 $D^0$  spectra of each examined drugs were measured at 200.0–400.0 nm.

### Construction of calibration graphs

To prepare calibration standards across concentrations of  $3.0-30.0 \ \mu\text{g/ml}$  for CAR and IVA, from each standard stock solution (100.0 \ \mu\g/ml), an accurately transferred amounts were conveyed to separate series of volumetric flasks, 10 ml, and finalized with distilled water. The D<sup>0</sup> spectra acquired after scanning solutions between 200.0 and 400.0 nm were recorded. The calibration graph for each suggested technique was performed via the mean of three experiments, as shown below:

# Factorized dual wavelength coupled with spectrum subtraction technique (FDW-SS)

The regression equations were created by plotting the stored D<sup>0</sup> maximum absorbance of CAR and IVA at 285.6 and 287.0 nm, respectively, against the associated concentrations. A factorized  $\Delta A$  spectrum for IVA was constructed using spectrophotometric software by division of D<sup>0</sup> of a certain concentration of IVA by ( $\Delta A$ ) at 285.2 nm and 255.0 nm.

### Ratio difference technique

The ratio spectra were produced via division of the scanning spectra of the CAR and IVA solutions by the absorption spectra of the IVA (15.0  $\mu$ g/ml) and CAR (15.0  $\mu$ g/ml) standard solutions, respectively. CAR's and IVA's concentrations were plotted against their respective amplitude differences at 252.6 and 275.0 nm and 294.0 and 320.0 nm, respectively.

# Constant center coupled with spectrum subtraction technique (CC-SS)

The regression equations have been calculated by setting two calibration curves. The first one is between the maximum absorbance of  $D^0$  of CAR and IVA at 285.6 nm and 287.0 nm and their related concentrations, respectively. The second one is between the  $\Delta A$  at 294.0 nm and 320.0 nm versus amplitudes at 294.0 nm for IVA after generating a ratio spectrum by dividing of the stored absorption spectra of IVA by the absorption spectrum of CAR; 15.0 µg/ml.

## Induced tripartite amplitude difference coupled with ratio subtraction technique (ITAD-RS)

The regression equations were computed by plotting the maximum absorbance of the stored  $D^0$  of CAR and IVA at 285.6 nm and 287.0 nm versus their corresponding concentrations, respectively.

The equality factor of IVA ratio spectra was calculated by using amplitude ratios' average at 296.0 nm  $(P_{296.0})$  and 242.7 nm  $(P_{242.7})$  using CAR's oxidative degradate spectrum as a divisor.

Via dividing the ratio spectrum of a certain concentration of CAR using CAR's oxidative degradate's spectrum as a divisor by  $\Delta P$  at 242.7 nm and 296.0 nm after multiplying the former by *F*, (*FP*<sub>242.7</sub>–*P*<sub>296.0</sub>), factorized induced ratio spectrum for CAR was created.

Through the division of the ratio spectrum of IVA using CAR's oxidative degradate's spectrum as a divisor by the value of the  $\Delta P$  at 242.7 nm and 296.0 nm, factorized ratio spectrum for IVA was created.

### Laboratory mixtures' analysis

Different mixtures in different sets, set A (binary mixture of CAR and IVA) and set B (quaternary mixture of CAR and IVA with their oxidative degradates), were analyzed by carrying out the following manipulation actions for each method:

# Analysis of set A containing CAR and IVA in their binary mixtures

# Factorized dual wavelength coupled with spectrum subtraction technique (FDW-SS)

The D<sup>0</sup> of IVA was obtained by noting  $\Delta A$  at 285.2 nm and 255.0 nm and multiplying by IVA's factorized  $\Delta A$  spectrum in the prepared laboratory mixtures of CAR and IVA. CAR's D<sup>0</sup> spectrum was resulted from the subtraction of IVA's D<sup>0</sup> spectrum from the spectra of laboratory mixture.

## Ratio difference technique

The  $D^0$  of binary laboratory mixture in set A was divided separately by the absorption spectrum of standard IVA' and CAR' (15.0 µg/ml, each). The amplitudes of the obtained ratio spectra were noted at 252.6 and 275.0 nm for CAR and 294.0 and 320.0 nm for IVA. The concentrations of CAR and IVA were resolved from their related regression equations.

# Constant center coupled with spectrum subtraction technique (CC-SS):

Via employing the formerly manipulated ratio spectra in RD method, the following steps were employed: Firstly, IVA's ratio spectrum in each mixture was recorded at 294.0 nm and 320.0 nm using CAR's  $D^0$  as a divisor. Secondly, the postulated amplitude at 294.0 was acquired from the correspondent related regression equation followed by subtracting the postulated amplitude from the recorded one to obtain a constant value; CAR/CAR'. Finally, via multiplying the constant value by the spectra of 15.0 µg/ml standard CAR', the parent  $D^0$  of CAR was obtained in which its concentration might be determined using the matching regression equation built at its maxima.

As a result, the  $D^0$  of IVA could be calculated via subtraction of the acquired spectra of CAR from the spectra of its related mixtures' spectra. The concentration of IVA was estimated using the relevant regression equation.

## Analysis of set B containing CAR and IVA with their oxidative degradates in their quaternary mixtures Induced tripartite amplitude difference coupled with ratio subtraction technique (ITAD-RS)

CAR/CAR's degradate ratio spectrum in mixtures of set B was acquired after multiplication of CAR's factorized induced ratio spectrum by  $(FP_{242.7} - P_{296.0})$  of each mixture's ratio spectrum. After multiplication of the former ratio spectrum, CAR/CAR's degradate by the  $D^0$  spectrum of the CAR oxidative degradates, CAR's  $D^0$  was calculated.

The ratio spectrum comprising, IVA+IVA's degradate+CAR's degradate/CAR's degradate was acquired by subtraction of the obtained ratio spectrum; CAR/CAR's degradate from that of quaternary mixture.

For IVA, its ratio spectrum in the combination can be acquired by multiplication of  $\Delta P$  at 242.7 nm and 296.0 nm by the formerly set IVA's factorized ratio spectrum. IVA's parent D<sup>0</sup> was acquired after multiplication of the acquired ratio spectrum by the spectrum of the CAR oxidative degradate.

CAR and IVA concentrations were figured using their respective regression equations generated at their maxima.

### Application to pharmaceutical formulation

Five Carivalan<sup>®</sup> tablets were crushed and blended together. Amounts equivalent to 25.0 mg CAR and 10.0 mg IVA were precisely moved to beaker, 100 ml, dissolved in 30 ml distilled water, sonicated and filtered into a volumetric flask, 100 ml, then accomplished with distilled water.

Further dilutions were proceeded to produce concentrations of 10.0  $\mu$ g/ml for CAR and 4.0  $\mu$ g/ml for IVA. The proposed techniques were used for the analysis of the examined Carivalan<sup>®</sup> following the described

measures under laboratory-prepared mixtures analysis to compute their concentrations utilizing the related regression equation for each proposed drug.

### Results

As a result of the current global conditions that most countries are going through either economically or practically, the main movement has become toward using analytical techniques at the lowest costs and in the easiest and most accurate ways at all once.

This was the main reason behind the resort to the use of spectrophotometric technique in drugs' analysis, as it proved accuracy, reliability and simplicity among the rest of the other analytical techniques as well as its availability in most quality control laboratories.

Unfortunately, the presence of extremely overlapped spectra made several challenges to use direct absorbance measurements in zero-order spectra, which led to numerous obstacles during spectrophotometric analysis, particularly for multicomponent mixtures.

Therefore, the primary objective is to concentrate on creating highly advanced eco-friendly spectrophotometric techniques that enable both the researcher and the analyst to explore complex mixtures and to overcome any challenges that may arise.

Carvedilol and ivabradine were chosen as a model for demonstrating the successful application of new spectrophotometric techniques for their analysis, either in their binary or in their quaternary mixture with their oxidative degradation products without the need for pre-separation procedures.

Complete CAR's and IVA's oxidative degradation was observed. Quite the reverse, they conveyed relative acidic, basic, photolytic and thermal stability. Samples were frequently taken out to track the drugs' degradation; for the drugs in question, total oxidation was verified by the disappearance of the TLC spot using TLC aluminum plates.

CAR and IVA along with their oxidative degradation products were UV scanned and found to be highly overlapped in the UV region 200.0–340.0 nm, Fig. 2. Direct measurement of the studied drugs was not possible. Thus, two sets were constructed, one for determining CAR and IVA in their binary mixture (set A) exploiting zero or ratio spectra through the application of FDW-SS, RD and CC-SS methods [15], while the other set (set B) was created for the proposed drugs' determination in the presence of their oxidative degradates exploiting the innovative induced tripartite amplitude difference together with ratio subtraction (ITAD-RS) spectrophotometric technique.



Fig. 2 Zero-order spectra of carvedilol, ivabradine and their oxidative degradation products (10.0 µg/ml, each)

### Set A: CAR and IVA in their binary mixture:

An overlapped  $(D^0)$  spectrum was observed for CAR and IVA. Using suitable recommended techniques, simultaneous determination of the considered drugs was performed.

# Factorized dual wavelength coupled with spectrum subtraction technique (FDW-SS)

This suggested technique was used to analyze CAR and IVA in their binary mixture via picking two wavelengths (285.2 nm and 255.0 nm) where the  $\Delta A$  is proportional to IVA concentration versus a zero  $\Delta A$  for CAR at the selected wavelengths, as shown in Fig. 3a.

Figure 3b shows how the original (D<sup>0</sup>) of IVA was acquired from each combination after noting ( $\Delta A$ ) value at 285.2 nm and 255.0 nm and multiplying this value by IVA's factorized  $\Delta A$  spectrum.

Subtraction of IVA's  $(D^0)$  spectrum from  $(D^0)$  spectrum of the mixture yielded CAR's parent  $(D^0)$  spectrum, Fig. **3.c**.

Via relieving the recorded absorbance values at the maxima of each of CAR and IVA in their corresponding regression equations, each drug's concentration was calculated, Table 1.

#### Ratio difference technique

The paramount principle of this technique is that  $D^0$  of the mixture is divided by  $D^0$  of the interfering element obtaining a ratio spectrum. At this moment, the studied drug has a marked ( $\Delta P$ ) at two wavelengths opposing a canceled contribution of the interfering component for being a constant all over the curve. Thus, the interfering component's determination is possible.

This proposed technique was utilized to determine CAR and IVA simultaneously in binary set A mixtures. Dividing the mixture's absorption spectra by the absorption spectrum of IVA' (15.0  $\mu$ g/ml) was performed to achieve a ratio spectrum. CAR in the mixture was determined using the amplitudes at 252.6 and 275.0 nm, Fig. 4.

Similarly, for the estimation of IVA in the prepared mixture utilizing CAR' (15.0  $\mu$ g/ml) as a divisor, the amplitudes at 294.0 and 320.0 nm were used, Fig. 5.

The estimated  $\Delta P$  of the ratio spectra at 252.6 and 275.0 nm and 294.0 and 320.0 nm, respectively, was shown to have a linear relationship with the corresponding concentrations of CAR and IVA, respectively, Table 1.

RD is advantageous over FDW method which requires the specific choice of two wavelengths for the studied drug opposing an equal absorbance value for the



3b. D<sup>0</sup> of 10.0 μg/ml of IVA showing  $\lambda_{max}$  at 287.0 nm Fig. 3 Spectral scheme of resolution of 10.0 μg/ml of each CAR (red line) and IVA (black line) using DW-SS in set A

| Table 1 Assa | y parameters and | results of determination | of pure samples | s of CAR and IVA by | the proposed methods |
|--------------|------------------|--------------------------|-----------------|---------------------|----------------------|
|--------------|------------------|--------------------------|-----------------|---------------------|----------------------|

|                             | CAR              |                   |       | IVA     |            |                   |       |         |
|-----------------------------|------------------|-------------------|-------|---------|------------|-------------------|-------|---------|
|                             | RD               | FDW-SS            | CC-SS | ITAD-RS | RD         | FDW-SS            | CC-SS | ITAD-RS |
| Linearity (µg/ml)           | 3.0-30.0         |                   |       |         | 3.0-30.0   |                   |       |         |
| Slope                       | 0.6057           | 0.0408            |       |         | 0.43       | 0.0379            |       |         |
| Intercept                   | 0.0044           | 0.0017            |       |         | 0.025      | 0.0173            |       |         |
| Correlation coefficient (r) | 0.9998           | 0.9998            |       |         | 0.9999     | 0.9999            |       |         |
| Accuracy(Mean±SD)           | $99.38 \pm 0.92$ | $98.45 \pm 0.851$ |       |         | 99.47±1.41 | $99.15 \pm 0.874$ |       |         |
| *RSD% <sup>a</sup>          | 0.791            | 1.244             |       |         | 0.823      | 1.202             |       |         |
| **RSD% <sup>b</sup>         | 0.839            | 1.357             |       |         | 1.229      | 1.323             |       |         |
| LOD                         | 0.258            | 0.290             |       |         | 0.272      | 0.204             |       |         |
| LOQ                         | 0.783            | 0.878             |       |         | 0.823      | 0.618             |       |         |

\*RSD%<sup>a</sup>, \*\*RSD%<sup>b</sup>: the intra-day AND inter-day, respectively, (*n* = 3) relative standard deviation of concentrations (6.0, 20.0, 25.0 µg/ml for CAR and 6.0, 18.0, 25.0 µg/ml for IVA)



Fig. 4 Ratio spectra of 10.0  $\mu$ g/ml of CAR (red line), IVA (black line) and their binary mixture (red dotted line), separately in distilled water, using spectrum of IVA (15.0  $\mu$ g/ml) as a devisor showing the two selected wavelengths (252.6 and 275.0 nm)



Fig. 5 Ratio spectra of 10.0  $\mu$ g/ml of IVA (black line), CAR (red line) and their binary mixture (black dotted line), separately in distilled water, using spectrum of CAR (15.0  $\mu$ g/ml) as a devisor showing the two selected wavelengths (294.0 and 320.0 nm)

other interfering component, while in the RD approach, the wavelength selection is considerably more flexible because the interfering element is a straight line for being a constant.

# Constant center coupled with spectrum subtraction technique (CC-SS)

This proposed technique is regarded as a complement to the previously applied RD method, where a ratio spectrum for the binary mixture in set A was obtained with a marked amplitude difference at 294.0 and 320.0 for IVA using CAR' as a divisor. Meanwhile, the practical ratio amplitude of each mixture was noted at 294.0 nm  $\{(IVA/CAR') + (CAR/CAR')\}$ . The supposed ratio amplitude value of (IVA/CAR') was obtained by putting the recorded ratio amplitude in the equation expressing the linear relationship between the  $\Delta P$  at 294.0 and 320.0 nm with the associated ratio amplitudes at 294.0 nm.

$$P_1 - P_2(\Delta P) = 0.8801 P_1 - 0.0862 (r = 0.9999)$$

, where *P*1, *P*2 are the ratio amplitudes of the ratio spectra of different concentrations of IVA (3.0–30.0  $\mu$ g/ml) by utilizing CAR; 15.0  $\mu$ g/ml as a divisor at 294.0 and 320.0 nm.

The constant value (C.V.);CAR/CAR' was figured as the deviation among the measured amplitudes of the mixture's ratio spectra at 294.0 nm and the postulated amplitudes obtained from the established regression equation.;  $[P_{\text{recorded}}] - [P_{\text{postulated}}]$ .

The recovered CAR's  $D^0$  in the mixture, Fig. 3c, was obtained by multiplication of the laboratory mixture's obtained constant (CAR/CAR') by the CAR's divisor.

For IVA, its recovered  $D^0$ , Fig. 3b, can be calculated by subtraction of the acquired spectra of CAR from the corresponding spectra of their laboratory-generated mixtures.

By substituting the recorded absorbance values at the maxima of each of CAR and IVA in their corresponding regression equations, each drug's concentration was calculated, Table 1

## Set B: CAR and IVA in their quaternary mixture with their oxidative degradates:

The determination of CAR and IVA existed with their degradates was not that possible by the commonly used spectrophotometric techniques. Thus, a novel technique, namely, induced tripartite amplitude difference was devised to assign the targeted drugs by overcoming their completely overlapped spectra.

# Induced tripartite amplitude difference coupled with ratio subtraction technique (ITAD-RS)

On the ratio spectrum of CAR, two wavelengths, 242.7 nm and 296.0 nm, were chosen using its oxidative degradate as a divisor.

The ratio spectrum of IVA degradate demonstrates equal amplitude values at these specified wavelengths. The ratio spectrum of IVA, on the other hand, revealed un-equalized values. As a result, an equality factor (*F*) of 2.497  $[P_{296.0}/P_{242.7}]$  was calculated by obtaining the average of  $\Delta P$  of diverse concentrations of IVA's ratio spectra using CAR degradate's spectrum as a divisor at (296.0 nm and 242.7 nm). This factor prospered to match the amplitudes of IVA at the proposed wavelengths, Fig. 6a.

After calculating the  $\Delta P$  at 242.7 nm and 296.0 nm along with the equality factor for each set B quaternary mixture, multiplication of the previous value with the previously prepared CAR's factorized induced ratio spectrum resulted in an additional spectrum reflecting the CAR/CAR's degradate ratio spectrum in the mixture, Fig. 6b. By multiplication of the generated ratio spectrum



Fig. 6 Spectra scheme of resolution of 10.0 µg/ml of each of CAR (red line), IVA (black line), CAR's degradate (red dotted line) and IVA's degradate (black dotted line) using ITAD-RS in set B

by CAR oxidative degradate's  $D^0$  spectrum, CAR's  $D^0$  was successfully obtained, Fig. 6c.

Subtracting the acquired CAR's ratio spectrum from that of the quaternary mixture resulted in a ratio spectrum comprising IVA+IVA's degradate+CAR's degradate/CAR's degradate. The ratio spectrum of IVA/ CAR's degradate in the combination was obtained by recording  $\Delta P$  at 242.7 nm and 296.0 nm and then multiplying by IVA's factorized ratio spectrum, Fig. 6d. The parent  $D^0$  of IVA was revealed afterward by multiplication of the acquired ratio spectrum by the spectrum of CAR oxidative degradate, Figure 6e

CAR and IVA concentrations were obtained using the respective regression equations generated at their maxima, Table 1.

#### Privileges proposed by the developed methods

From the achieved results, it was found that determination of the cited drugs through grabbing the beneficial impact of using spectral factorization gave the proposed methods, FDW-SS and the newly developed ITAD-RS a huge privilege. This was attributed to the great ability of factorized spectrum to recover the parent profile of each drug and their determination at each corresponding maxima. Additionally, manipulation using factorized spectra allows the resolution of the analytes via minimal arithmetic operation and spectrophotometer software's manipulation steps. Moreover, CC-SS method occupied a high capability in analyzing the cited drugs through obtaining their D<sup>0</sup> spectra in addition to highest accuracy in their quantification at their  $\lambda_{max}$ . Finally, and through using a spectral divisor, RD method was able to cancel the interfering component in the form of constant, which is a straight line across the curve; as a result, there would not be any crucial measurements at any particular wavelength, and the difference between any two wavelengths will always equal to zero.

## Discussion

## Method validation

The suggested spectrophotometric techniques were validated in accordance with the ICH guidelines [20]. Accurate linear results were obtained by analyzing 6 concentrations ranging from 3.0 to 30.0 µg/ml of each cited drug conferring to obedience to Beer's law and their concentration in the pharmaceutical dosage form, Table 1. Accuracy of the results was also checked through analyzing different CAR's and IVA's blind samples where their concentrations were obtained from the corresponding regression equations for the percentage recoveries to be calculated, Table 1. Moreover, three concentration levels were prepared and analyzed in triplicates within and between days for each of CAR and IVA to assess the precision of the employed methods obtaining percentage relative standard deviation (RSD %) not exceeding 2%, Table 1.

Table 2 displays the outcomes of laboratory-prepared mixtures: sets A and B having various ratios of the drugs with or without their oxidative degradates, verifying the specificity of the specified techniques when results were within the calibration range and acceptable. The suggested techniques were additionally successful in distinguishing the drugs contained in Carivalan<sup>®</sup> tablets. The

Table 2 Determination of CAR and IVA in their laboratory-prepared mixtures by the proposed methods

| CAR and IVA | AR and IVA in absence of their degradation products (set A) |     |                   |                   |                  |                   |                  |                   |  |
|-------------|---|-----|-------------------|-------------------|------------------|-------------------|------------------|-------------------|--|
| CAR: IVA    | Concentration (µg/ml)                                       |     | Recovery%         |                   |                  |                   |                  |                   |  |
|             |   |     | CAR               |                   | IVA              |                   |                  |                   |  |
|             | CAR   | IVA | RD                | FDW-SS            | CC-SS            | RD                | FDW-SS           | CC-SS             |  |
| 1:2.5       | 6   | 15  | 101.67            | 101.84            | 98.65            | 100.20            | 98.10            | 100.92            |  |
| 1:3         | 8   | 24  | 101.00            | 101.20            | 98.19            | 100.75            | 98.14            | 101.00            |  |
| 1:1         | 10  | 10  | 101.00            | 99.58             | 98.41            | 101.60            | 98.60            | 101.77            |  |
| 2.5:1*      | 15  | 6   | 100.27            | 98.74             | 98.77            | 98.33             | 98.81            | 101.89            |  |
| 3:1         | 24  | 8   | 100.13            | 99.30             | 98.09            | 101.88            | 98.85            | 100.83            |  |
| Mean±SD     |   |     | $100.81 \pm 0.63$ | $100.13 \pm 1.32$ | $99.42 \pm 0.29$ | $100.55 \pm 1.41$ | $98.50 \pm 0.36$ | $101.28 \pm 0.51$ |  |

CAR and IVA in presence of their oxidative degradation products (set B)

| % Degradation | CAR: IVA: CAR':IVA' | Recovery%        |             |
|---------------|---------------------|------------------|-------------|
| CAR':IVA'     |                     | CAR              | IVA         |
|               |                     | ITAD-RS          | ITAD-RS     |
| 10            | 6:15:5.4:13.5       | 99.80            | 98.80       |
| 30            | 8:24:5.6:16.8       | 98.44            | 101.46      |
| 50            | 10:10:5:5           | 99.34            | 101.20      |
| 70            | 15:6:4.5:1.8        | 98.09            | 99.67       |
| 90            | 24:8:2.4:0.8        | 98.27            | 100.13      |
| Mean±SD       |                     | $98.78 \pm 0.74$ | 100.25±1.00 |

\*Ratio found in pharmaceutical dosage form

| Sample            | CAR (mean%±SD)    |                  |                   |             |
|-------------------|-------------------|------------------|-------------------|-------------|
| Method            | RD                | FDW-SS           | CC-SS             | ITAD-RS     |
| Carivalan®        | 90.40±0.69        | 97.95±0.99       | 97.75±0.51        | 101.63±1.56 |
| Batch No.29044    | $99.83 \pm 0.40$  | 99.83±1.19       | 99.48±0.81        | 98.93±1.37  |
| Standard addition |                   |                  |                   |             |
| Sample            | IVA (mean±SD %)   |                  |                   |             |
| Method            | RD                | FDW-SS           | CC-SS             | ITAD-RS     |
| Carivalan®        | 98.17±0.58        | 98.75±1.14       | 100.07±1.98       | 97.87±0.38  |
| Batch No.29044    |                   |                  |                   |             |
| Standard addition | $100.50 \pm 1.39$ | $98.75 \pm 0.43$ | $100.71 \pm 0.26$ | 99.25±1.15  |

Table 3 Determination of CAR & IVA in tablet dosage form by the proposed methods and application of standard addition technique

Table 4 Statistical analysis of the proposed, official and the reported methods of CAR and IVA in their pure forms

| Parameter                          | CAR           |               |       |         |                      |  |  |
|------------------------------------|---------------|---------------|-------|---------|----------------------|--|--|
|                                    | RD            | FDW-SS        | CC-SS | ITAD-RS | Official method*     |  |  |
| Mean                               | 99.38         | 98.45         |       |         | 99.88                |  |  |
| SD                                 | 0.92          | 0.85          |       |         | 0.95                 |  |  |
| Ν                                  | 5             | 5             |       |         | 4                    |  |  |
| Variance                           | 0.85          | 0.72          |       |         | 0.9                  |  |  |
| Student's <i>t</i> test*** (2.365) | 0.796         | 2.352         |       |         |                      |  |  |
| F***                               | 1.059 (9.120) | 1.250 (6.590) |       |         |                      |  |  |
| Parameter                          | IVA           |               |       |         |                      |  |  |
|                                    | RD            | FDW-SS        | CC-SS | ITAD-RS | Reported<br>method** |  |  |
| Mean                               | 99.47         | 99.15         |       |         | 99.6                 |  |  |
| SD                                 | 1.41          | 0.87          |       |         | 1.06                 |  |  |
| Ν                                  | 5             | 5             |       |         | 5                    |  |  |
| Variance                           | 1.99          | 0.76          |       |         | 1.12                 |  |  |
| Student's <i>t</i> test*** (2.306) | 0.165         | 0.734         |       |         |                      |  |  |
| F*** (6.390)                       | 1.777         | 1.474         |       |         |                      |  |  |
|                                    |               |               |       |         |                      |  |  |

\*BP determination of carvedilol by official potentiometric method [1]

\*\*Derivative ratio spectrophotometric method [8] for determination of ivabradine

\*\*\*The values in the parenthesis are the corresponding theoretical values of t and F at (p = 0.05). No significant difference by using one-way ANOVA with F equals 0.637 ( $F_{crit}$  = 3.239) and p equals (0.602)

standard addition approach was used to further evaluate the suggested methods' validity, Table 3.

### Statistical analysis

Tables 4 shows a statistical comparison of the found results acquired for the identified drugs using the proposed techniques, official [1] and the reported spectro-photometric technique [8] in pure forms. The computed t and F values were fewer than the theoretical ones, indicating no statistically significant variance in obtained results between the suggested and reported approaches.

#### Greenness profile assessment

The goal of the green analytical chemistry is to boost the environmental friendliness without compromising the performance of analytical methods by recommending the reduction of hazardous substances' usage. Applying various assessment techniques to compare the proposed methods' levels of greenness allowed for the method's greenness to be evaluated.

Analytical Eco-Scale [31] is a semi-quantitative approach that operates on the method's penalty points (PP) minus 100. National Environmental Methods Index (NEMI) [32] is a circular NEMI pictogram, consisting of four quadrants coded with green if they meet the requirements. Green Analytical Procedure Index (GAPI) [32] is a color-coding representation. The output representation will provide insight into the method's greenness and the list of chemicals that must be verified in this GAPI in accordance with the National Fire Protection Act (NFPA) by applying the necessary data in the 11 quadrants of the GAPI. Analytical Greenness Metric (AGREE) [32, 33] is a software-based quantitative method. The necessary information was entered

into the software to obtain the pictogram. As a result, greenness assessment of the proposed techniques, official [1], and the reported spectrophotometric technique [8] was performed for obtaining a more fruitful and purposeful comparison. The obtained results showed that the proposed spectrophotometric technique is greener than the official and the reported methods as shown in Table 5. Regarding Analytical Eco-Scale, the developed method shows a 97 green Ecoscore in comparison with both official and the reported methods where they possess an Eco-score of 79 and 93 for CAR and IVA, respectively.

Additionally, the developed method's greenness assessment profile by applying national environmental method index tool (NEMI) obtained four full green quadrants as the used solvent; water is completely safe regarding persistence, bioaccumulation and corrosiveness, while only 1 and 2 green quadrants for both the official and reported methods of CAR and IVA, respectively, were obtained.

Moreover, method's complete framework greenness profile was acquired using green analytical procedure index tool (GAPI) where ten green and five yellow zones with a complete absence of any red zones were possessed in comparison with official and reported methods.

Finally, a score value of 0.84 was obtained which is greener than that obtained by official, 0.73, and reported method, 0.76, after applying the analytical greenness metric tool.

Briefly, the usage of distilled water and minimizing instrumental's energy consumption in the proposed methods was reflected by gaining high analytical

**Table 5** Greenness assessment of the proposed, official and the reported methods according to Eco-Scale, NEMI, GAPI and AGREE tools

| Tool                 | Proposed   | Official [1]                      | Reported [8]   |
|----------------------|--|-----------------------------------|--|
| Analytical Eco-scale |  |                                   |  |
| Water                | 0  | _                                 | _  |
| Acetic acid          | _  | 8                                 | 4  |
| Perchloric acid      | _  | 8                                 | _  |
| Energy Consumption   | 0  | 0                                 | 0  |
| Occupational Hazard  | 0  | 0                                 | 0  |
| Waste                | 3  | 5                                 | 3  |
| Total Penalty Point  | 3  | 13                                | 7  |
| Eco-score            | 97   | 79                                | 93   |
| NEMI                 | $\bigcirc$   |                                   | $\bigcirc$   |
| GAPI Tool            |  |                                   |  |
| AGREE Tool           | 00.84<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0<br>0 | 11 12 1 2<br>10 0.73 4<br>8 7 6 5 | 11<br>10<br>0.76<br>4<br>7<br>5<br>5<br>7<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5<br>5 |

greenness scores besides having full green NEMI pictogram and a complete absence of any red zones in GAPI pictogram.

## Conclusion

UV spectrophotometric techniques have several advantages over other analytical techniques, including their simplicity, low solvent usage and low cost. The suggested spectrophotometric techniques demonstrated great sensitivity and accuracy for determining CAR and IVA in dosage form, laboratory-generated combinations, and in presence of their oxidative degradates with no interference and excellent outcomes. Additionally, the greenness profile was argued green by applying various green assessment tools as analytical eco-scale, national environmental method index, green analytical procedure index and analytical greenness metrics. Therefore, the proposed method is capable of routine analysis of the cited drugs in quality control laboratories.

#### Abbreviations

| CAR     | Carvedilol   |
|---------|--|
| IVA     | Ivabradine   |
| FDW-SS  | Factorized dual wavelength coupled with spectrum subtraction       |
| RD      | Ratio difference technique   |
| CC-SS   | Constant center coupled with spectrum subtraction technique        |
| ITAD-RS | Induced tripartite amplitude difference coupled with ratio subtrac |
|         | tion technique   |

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#### Author contributions

HAEF helped in conceptualization, methodology, software data curation, software, validation, writing—original draft preparation, writing—reviewing and editing. DAA was involved in conceptualization, methodology, software data curation, software, validation, writing—reviewing and editing. NVF, AMB and MFAEG contributed to validation, supervision, writing—reviewing and editing.

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The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

Ethics approval and consent to participate

Not concerned.

#### **Consent for publication**

We certify this manuscript has not been published elsewhere and is not submitted to another journal. All authors have approved the manuscript and agreed to submit it to Future Journal of Pharmaceutical Sciences.

#### **Competing interests**

The authors declare that they have no competing interests.

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