Journal of Kufa for Chemical Sciences
ISSN(P) :2077-2351 ISSN(E): 3006-3280
Journal Homepage:
https://journal.uokufa.edu.iq/index.php/jkcs/index

Article

Spectrophotometric Determination of Carvedilol Via Oxidative Coupling Reaction Using Leichmanns Dye

Ahlam A. Shehab

Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq
*ahlam99@uomosul.edu.iq

ABSTRACT

A sensitive spectrophotometric method has been developed for the determination of carvedilol in both pure form and pharmaceutical prerarations. This method based on the oxidative coupling reaction of carvedilol (CAR) with the Leichmann dye in the presence of ceric (IV) sulphate as the oxidizing agent in an acidic medium forming a colored product. The established color has a maximum absorbance of 655 nm, The molar absorptivity was (1.012 × 104 L/ mol . cm) for concentrations obeyed Beer's law in the range (0.5 - 34) µg. mL $^{-1}$. The method was of high accuracy and precision, as the recovery rate was 97.96%, the relative standard deviation rate was less than 1.5% and the correlationcoefficient more than 0.99 which indicates that the developed method is a good. The CAR product was formed in the ratio of 1:1 (CAR: leichmann dye). The optimal conditions for the reaction were studied and the method was successfully applied to determine CAR in the pharmaceutical preparations.

Keywords: Spectrophotometry, Oxidative Coupling, Carvedilol, Leichmann dye.

Introduction

Carvedilol (CAR) is a type of non-selective beta-adrenergic antagonist (beta-adrenergic antagonist) and it is a third generation drug that has vasodilating properties. It stimulates blood vessels due to its antagonism to alpha-1-adrenergic receptors and reduces systolic and diastolic blood pressure by reducing peripheral

resistance. It also has an important effect in expanding blood vessels [1,2], and Figure 1 shows the chemical structure of CAR [3].

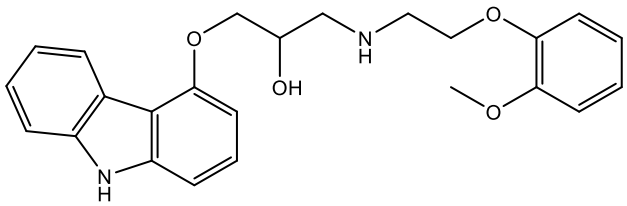


Figure1: Carvedilol ($C_{24}H_{26}N_2O_4$)

(2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol.M.wt = 406.5 g/mol

Is in the form of a white crystalline powder that dissolves in absolute methanol and slightly soluble in ethanol (96%) and insoluble in water and dilutes acids.
The most important side effects of carvedilol are decreased heart rate, decreased blood pressure, pain and tightness in the chest, sensitivity to the drug, headache, and uncontrollable low blood sugar in diabetics. There are other common symptoms such as fatigue, general weakness, and dizziness[4,5].
Various analytical methods have been described to estimate the drug compound under study. Below is a review of some of these methods:
Sensitive spectroscopic methods for the determination of carvedilol in pharmaceutical preparations have been published, based either on its ion pairs complex formation with the dye Aerochrome Black T in KCl-HCl buffer solution and extracting it to the chloroform layer [6] or oxidizing it using cerium sulfate (IV)-methyl orange reaction systems in a medium sulfuric acid [7] or by oxidative coupling method with potassium dichromate and phenothiazine in hydrochloric acid [8] or by condensation with Para-hydroxybenzaldehyde reagent in concentrated sulfuric acid [9]. Or it forms a charge transfer complex with the reagent DDQ in ethanol in the presence of a surfactant Tween-60 [10] or the relative spectrum in the ultraviolet region [11]. It can be directly estimated by fluorometric spectroscopy in methanol, and the excitation was at 285 nm and measurement at 335 nm [12] or indirect estimation based on the principle of carvedilol quenching of eosin dye fluorescence at 545 nm wavelength excitation of 301.5 nm in the acetate baffering medium [13]. Cyclovoltammetry [14] and RP-HPLC coupled with a UV detector and photodiode system were also applied [15-17] for the analysis of carvedilol in pharmaceuticals and biological models.
Due to the importance of estimating the CAR drug, a sensitive spectroscopic method was developed in this research for spectrophotometric determination of

CAR by using an acidic medium between the ceric (IV) sulphate and Leishmann dye.

The objective of this study is developed a selective and sensitive UV-Visible spectrophotometric method for determination of carvedilol.

Experimental Part

Apparatus and Software: Recording of the absorption spectra in the spectral range of 500-700nm was performed by Shimadzu UV-1800 Double-beam spectrophotometry. Glass cells with a width of 1 cm are used. Weighing by using a sensitive scale (ADAM) and heating by using A water bath was carried (mag.elektro).

Chemical Solutions: All the chemicals used were of a high degree of purity.

A 100.0 $mg.L^{-1}$ standard stock solution of ***Carvedilol*** was prepared in absolute methanol. Accurately, about 0.01 mg of CAR was weighed and transferred to 100 mL volumetric flask, methanol added to dissolve the drug then completed the volume to the mark with methanol. The solution was diluted appropriately to get the working concentrations. Moreover, prepare a ***Leichmanns dye*** solution (50 $mg.L^{-1}$) by dissolving 0.005 g in 5 mL methanol with constant heating and stirring, then transfer to a 100 mL volumetric flask and complete the volume with methanol to the mark, solution of Ceric (IV) Sulphate (300 $mg.L^{-1}$), Sulfuric acid (5 M) were prepared for experiments.

Initial tests: When adding 1.0 mL of Leishman dye at a concentration of (50 $mg.L^{-1}$) to 1.0 mL of CAR (100 $mg.L^{-1}$), and 1.0 mL of oxidizing agent ceric (IV) sulphate and 1.0 mL of sulfuric acid, complete the volume with distilled water to 10 mL and leave the solution for 5 minutes at room temperature (22°C), and the spectrum of the resulting solution was measured and found to be the maximum absorption of the medicinal compound is at the wavelength of 655 nm.

Results and Discussions

Study the optimal reaction conditions: Various factors affecting the oxidative coupling reaction of the dye were studied, with a final volume (10) mL . The solution was prepared using (2) mL with a concentration of (100) $mg.L^{-1}$ of CAR to obtain high stability and maximum absorption intensity of the remaining dye at the wavelength (655) nm.

Study type and quantity of oxidizing agent: The effect of different types of oxidizing agents was studied to find out the best oxidizing agent for dye oxidation, by taking 2 mL of a solution of CAR (100 $mg.L^{-1}$) in a volumetric flask capacity (10mL) and adding to it (1.0 mL) of sulfuric acid and fixed volumes of oxidizing agents (2 mL , concentrating 300 $mg.L^{-1}$) the solutions were left for 5 minutes, then Leishman dye was added in an amount of 2mL (50 $mg.L^{-1}$) and wait 5 minutes, followed by dilution with distilled water to the extent of the mark, the

measurement is at 655 nm, and the results shown in (figure 1) indicate that the ceric (IV) sulphate is the best oxidizing agent as it gave the maximum absorption and was chosen in the subsequent experiments.

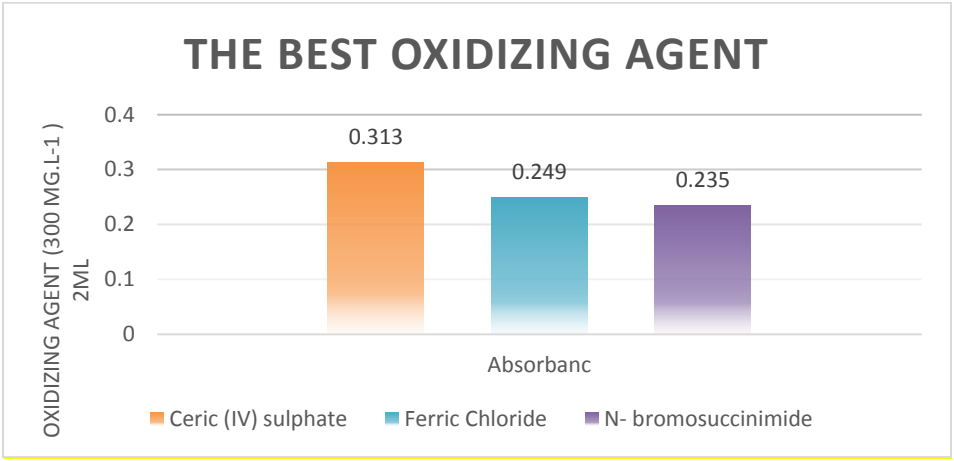


FIGURE 1. Study the best oxidizing agent

The effect of quantities of (0.5 – 3) mL at a concentration of 300 $mg.L^{-1}$ of the oxidizing agent, ceric (IV) sulphate, increased the oxidation of the dye in 10 mL volumetric flask containing a fixed amount of 2mL of Leichmann's dye and 1 mL of 5 M of concentrated sulfuric acid. (figure 2) illustrates the best volume of oxidizing agent is (1.5 mL).

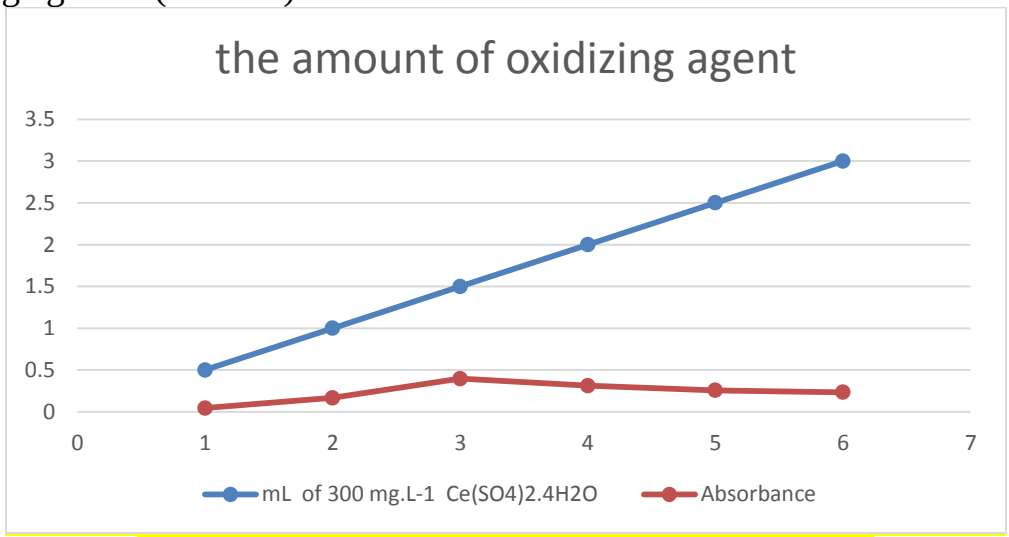


FIGURE 2. Study the amount of oxidizing agent

Study the type of acid: In order to choose the right acid to get the highest sensitivity, we prepared solutions containing equal concentrations of CAR (2 mL) and added 1.5 mL of oxidizing agent and 2mL of dye (50mg.L-1) and waiting for

5 minutes, then diluting the solutions with distilled water to the mark and measuring the absorption at 655 nm against the form solution, figure (3) shows that the best acid is sulfuric acid, as it led to the highest absorption at 655 nm, and its use was relied on in subsequent studies.

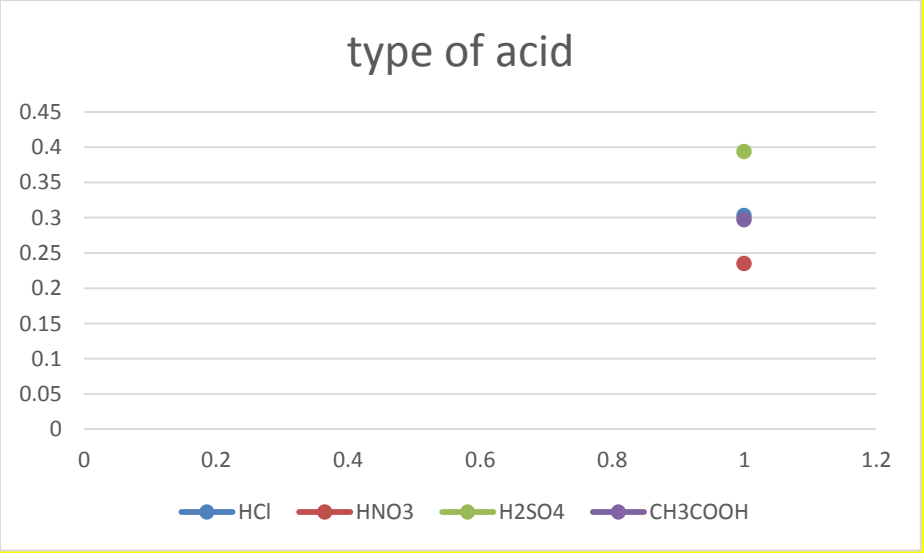


FIGURE 3. Study the type of acid

Study the concentration and amount of acid H_2SO_4: Different concentrations of sulfuric acid from (1 - 7 M), which affect the absorption of the dye, were studied in 10 mL volumetric flasks containing a fixed amount of the drug under study 2mL at (100 $mg.L^{-1}$), after which the optimum amount of oxidizing agent was added to 1.5 mL (300 $mg.L^{-1}$) and the solutions were left for a period of time after 5 minutes, Leishman dye was added to the volume of 2mL (50 $mg.L^{-1}$) with shaking, the solutions were left for 5 minutes, then diluted with distilled water to the point of the mark,(figure 4) shows that the best concentration of sulfuric acid when using (5M) gave the highest and most stable absorption intensity of the staining product of the dye at the wavelength of 655nm, the (5M) concentration of sulfuric acid was relied on in the subsequent experiments, while (figure 5) indicates that the best amount of sulfuric acid was (1 mL), as it gave the highest absorption of the remaining Leishmann dye, so this amount was used in the subsequent experiments.

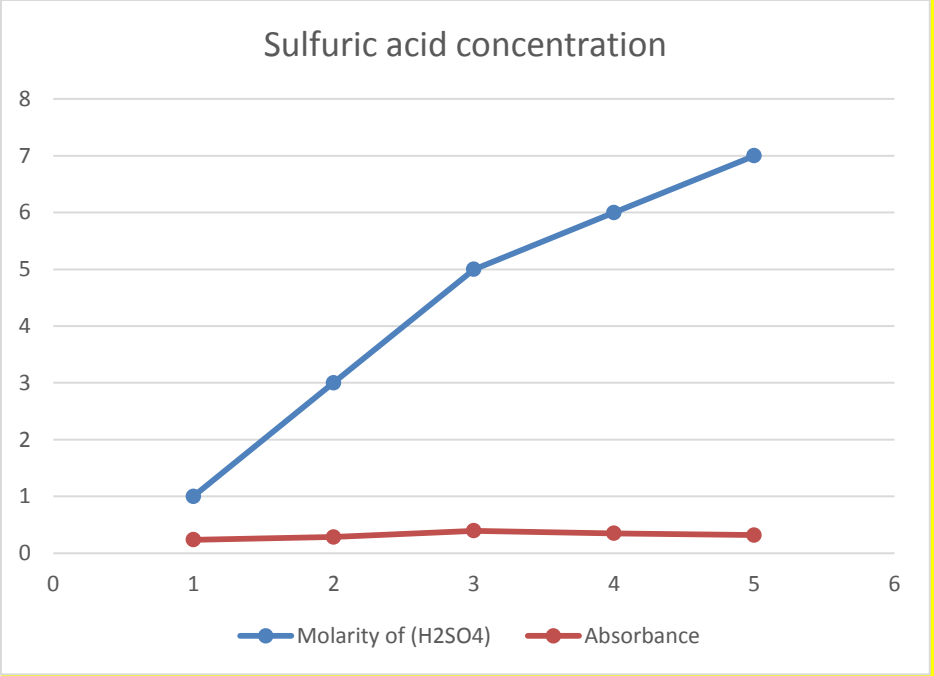


FIGURE 4. Study of Sulfuric acid concentration

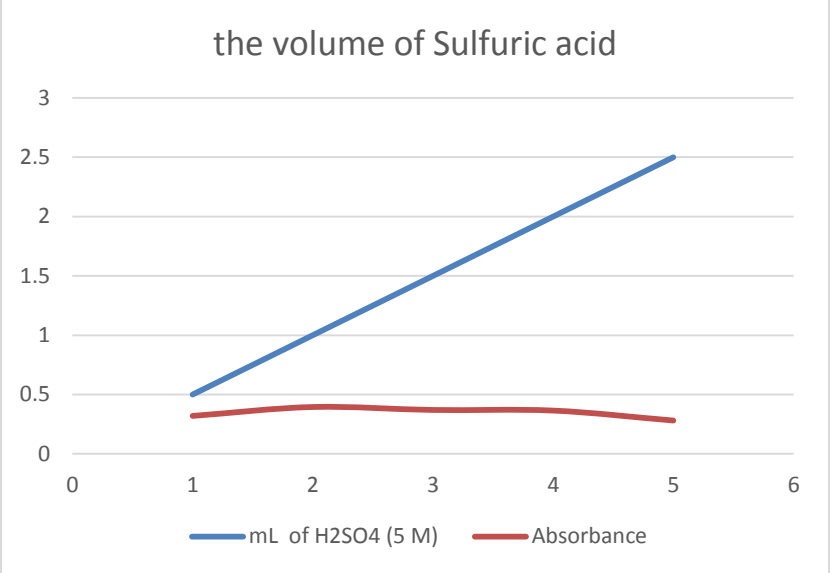


FIGURE 5. Study the volume of Sulfuric acid

The effect of (1.0 mL) of sodium hydroxide at a concentration of (5 M) on the absorption was also studied. It was found that adding it causes a decrease in the intensity of absorption, so adding the base was excluded.

Study the amount of Leichmann dye: For the purpose of selecting the best amount of dye that is subject to the limits of Beer's law, Different volumes (0.5 - 4) mL of dye at a concentration of 50mg.L^{-1} were used in 10 mL volumetric flask containing (2 mL) of the drug, 1 mL of sulfuric acid (5 M) and 1.5 mL of the

oxidizing agent were added to it, and the solutions were left with shaking for 5 min., it was diluted with distilled water to the point of the mark and the absorption was measured at the wavelength of 655 nm. From the results obtained, it was found that using (2.0 mL)of dye gave the best volume that follows Beer's law, as shown in Figure 6.

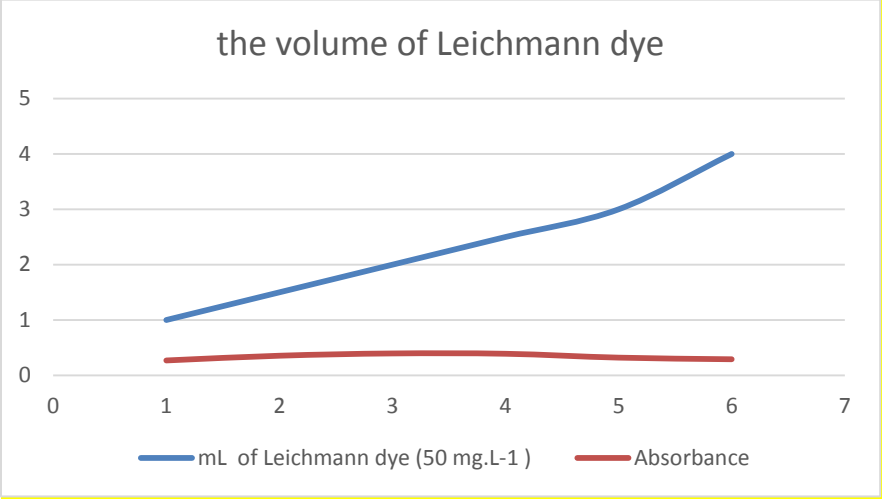


FIGURE 6. Study the volume of Leichmann dye

Study of CAR oxidation time: The oxidation time of CAR was studied at different times following the present method of work using 2 mL of CAR, after which 2 mL of the dye solution (50 $mg.L^{-1}$) was added. The absorption of the product formed by the dye was measured at wavelength of 655nm.From the results recorded in a figure (7), it was found that the best time is (7) minutes for oxidation, and it was relied upon in subsequent experiments.

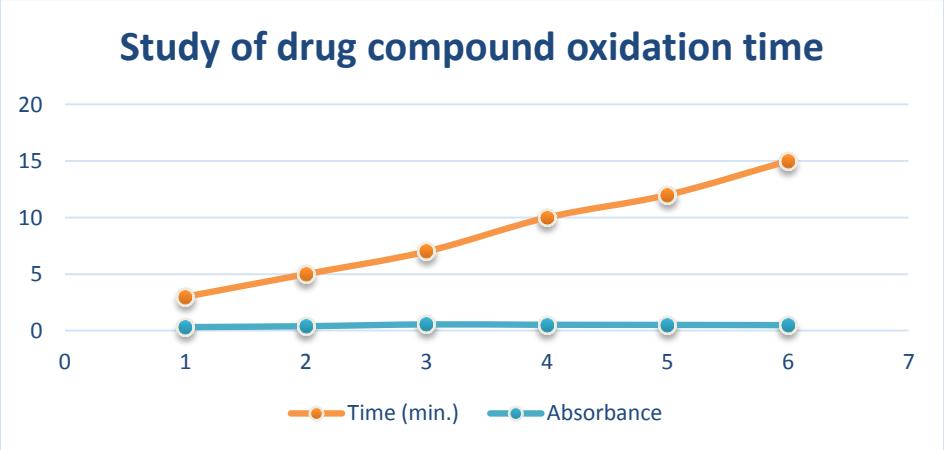


FIGURE 7. Study of drug compound oxidation time

Study of surfactant substances: Different types of surfactants (positive, negative and neutral) were studied to determine the extent of their effect on the absorption and stability of the dye. Increasing amounts of surfactants (0.5 - 2.0) mL each were added separately to a group of 10 mL volumetric flask containing the compound.

The drug under study (10 $mg.L^{-1}$) and the optimal amount in the current working method of sulfuric acid, cerium (IV) ion and Leichmann dye, and the absorption was measured at the wavelength of 655 nm, as shown in the following figure 8:

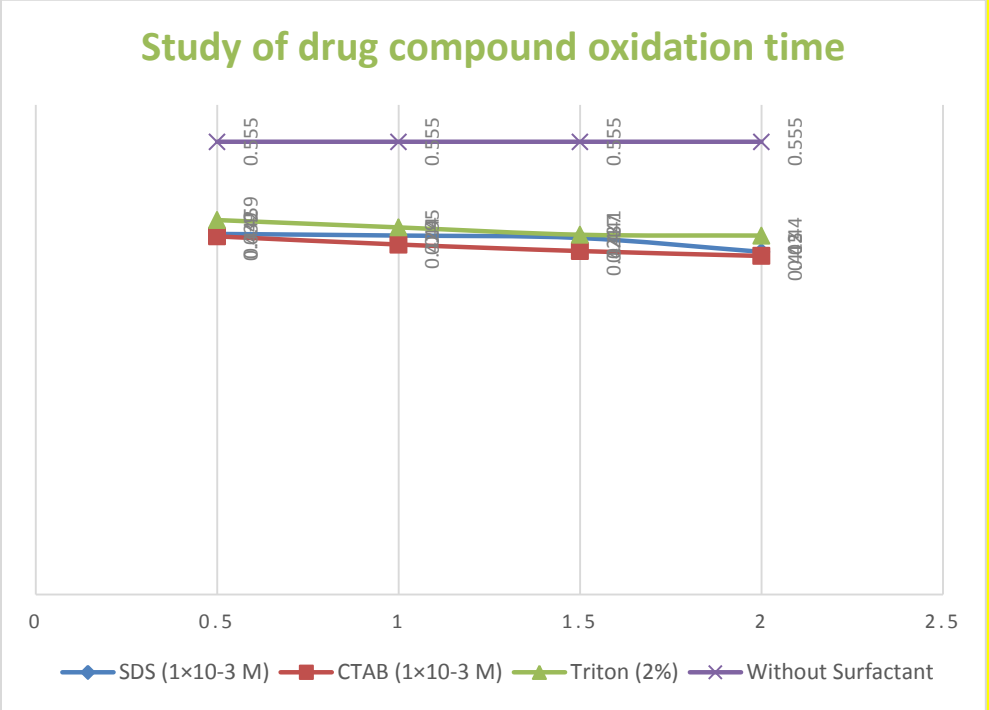


FIGURE 8. Study of drug compound oxidation time

The results obtained indicate that the use of surfactants mentioned in the above table leads to a decrease in the absorption and their inefficiency in increasing the sensitivity of the approved method of work. Therefore, its use in subsequent trials was excluded.

The effect of the addition sequence: The sequence of addition of the reactants affecting the absorption was studied, as several laboratory experiments were conducted. the results listed in table (9) indicate using the order (I), which is the sequence used in the method and the appropriate method for determination, and any change in the order of addition of the reactants has a negative impact on the determine. it was approved in the subsequent study.

Table 1. Effect of the addition sequence on the absorbance of the Leichmann dye

Order Number	Order of addition	Absorbance
I	S + A + O + R	0.556
II	S + O + A + R	0.285
III	R + A + O + S	0.371
IV	R + S + A + O	0.385

S= CAR , R= Reagent (Leichmann dye) , O= Oxidizing agent , A= Acid

Study of time and its effect on absorbance and stability: The stability of the stained product was studied using the aforementioned work method and it was studied at different time periods at temperature (22°C) with a wavelength of 655 nm. Fig. 9 illustrates that the dye formed stabilizes after 5 minutes, followed by dilution with distilled water, and remains stable for 60 minutes.

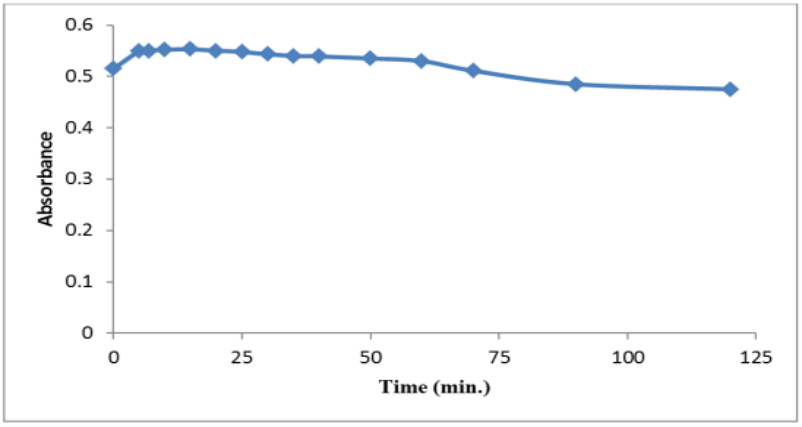


FIGURE 9: The effect of time

Absorption spectrum: After fixing the optimum conditions for CAR, spectroscopy the final absorption of it as it gave the highest absorption intensity of 655 nm, as shown in Fig. 10.

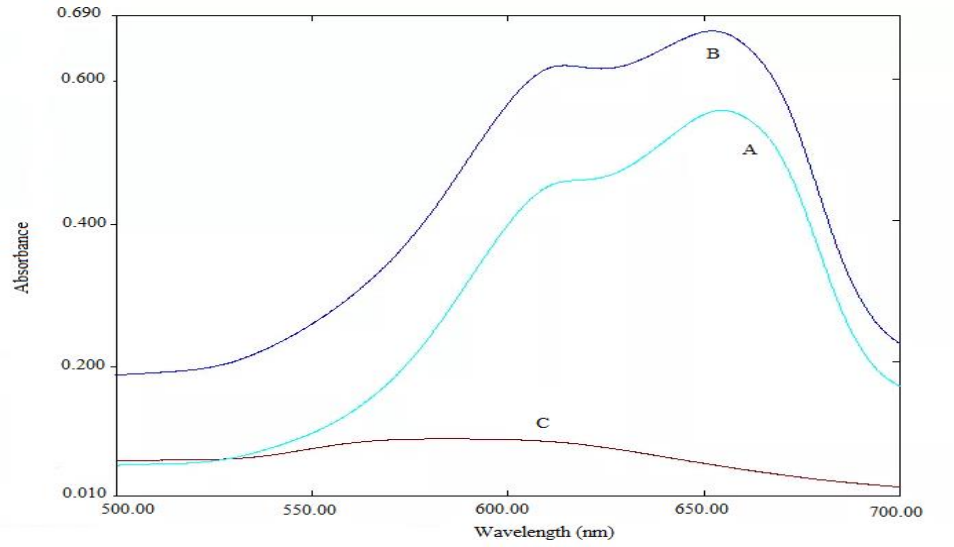


FIGURE 10. Absorption spectrum of CAR 2 mL (100 $mg.L^{-1}$)

A: vs. blanck solution B: vs. distilled water C: blanck's solution vs. distilled water

Working method and standard curve: The standard curve could be prepared by following the current working method and as follows:

To a set of flasks (capacity 10 mL), increasing quantities of CAR at a concentration of 100 $mg.L^{-1}$ are added to cover the microgram concentrations listed in table 10, then, 1 mL of sulfuric acid (5 M) and 1.5 mL of oxidizing agent (300 $mg.L^{-1}$) were added to the group. The solutions of the reactants were shaken and left for 7 Minutes followed by adding 2 mL of dye (50 $mg.L^{-1}$) with shaking and leaving the solutions for 5 minutes after being diluted with distilled water to the point of the mark, followed by measuring the absorbance at wavelength 655 nm against the blank solution. Fig.11 shows the standard curve of CAR that the adopted method follows the boundaries of beer's law within the linear range of concentrations (0.5–34) $mg.L^{-1}$ and that there is a negative deviation after the upper determined limits of Beer-Lambert's law, the value of the correlation coefficient (0.999) which indicates that the curve has excellent linear characteristics.

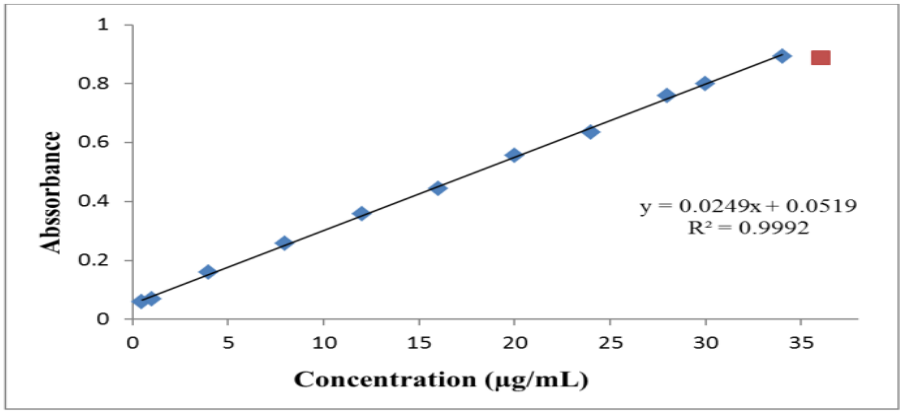


FIGURE 11. Standard curve for CAR

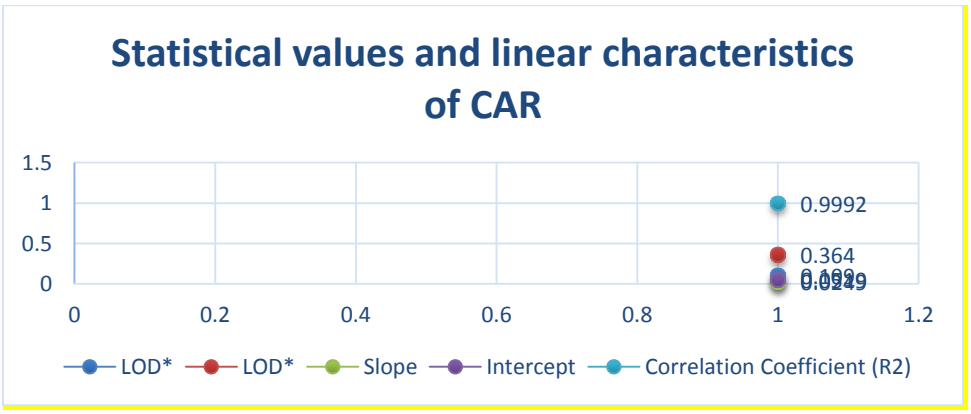


FIGURE 12. Statistical values and linear characteristics of CAR

*Average of ten determination.

The accuracy and precision of the determination of CAR were studied by applying the aforementioned method of action by calculating the relative standard deviation (RSD%) and the recovery rate. the results obtained in table 11 indicate good accuracy and compatibility with the proposed method for different concentrations (10, 20, 30) µg/mL for determining CAR.

RSD% = S/$\bar{X}$ ×100 , $\bar{X}$ = Σxi/N , Recovery% =O – T/ T × 100+ 100 when S: standard deviation , $\bar{X}$ = Arithmetic mean , N= numbed of reads

O= measured value , T = true value

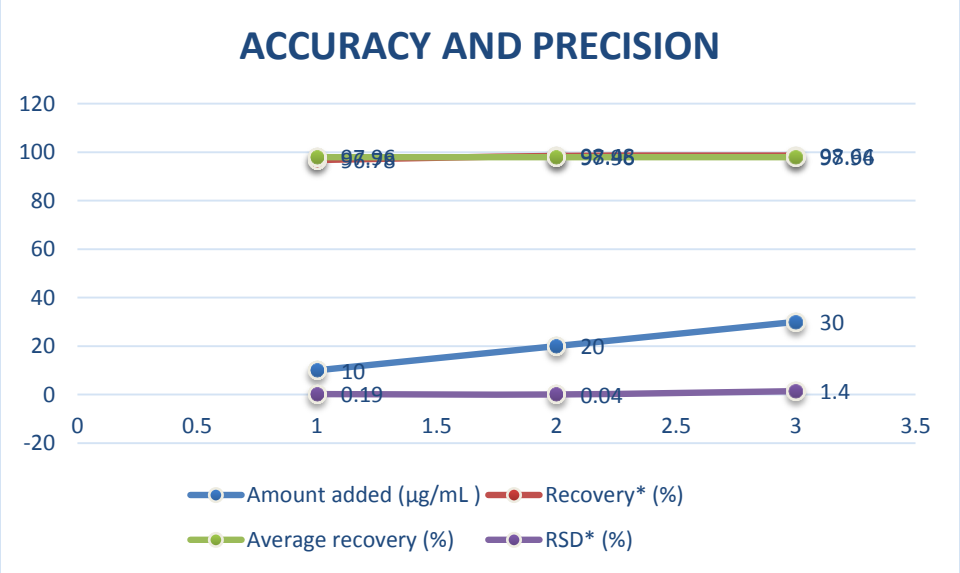


FIGURE 13. accuracy and precision

*Average of five determination.

The effect of the complex nature: The "Continuous Change Method"[18] Job's Method to find out the compositional ratio of the product of Leichmann's dye with CAR using dilute solutions of the dye and the studied compound with a concentration of (1 ×10^{-4} M). The results obtained in Fig. (5) confirm that the result is composed of a ratio of (1:1).

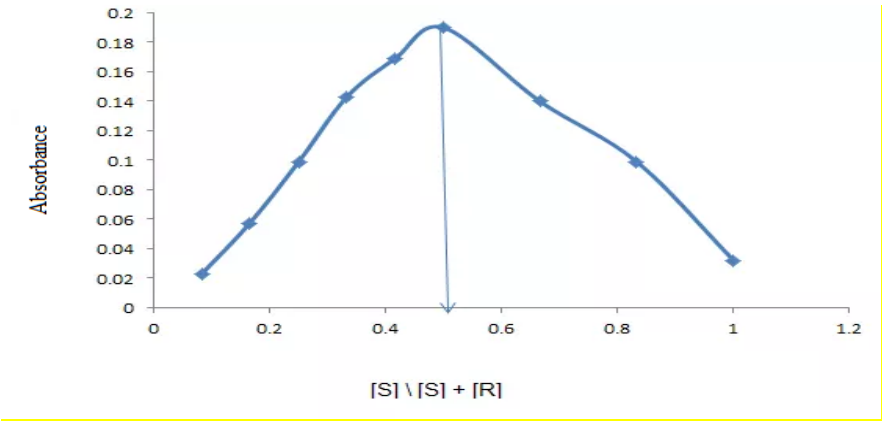


FIGURE 14. Continuous Changes for volumes.

Pharmaceutical solutions for CAR (Carvidol 25 mg / tablet and Cavedilol HEXAL 12,5 mg / tablet)-

The solutions were prepared with the same concentration of the medicinal compound (100 mg.L^{-1}), as 10 tablets were carefully weighed from the two dosage forms, after which they were crushed and mixed well from each medicinal compound separately, then a weight equivalent to 0.01 g was taken from each medicinal tablet and dissolved in 75 mL of methanol, followed by a process. Filter and wash the filtrate several times with methanol, then complete the volume to 100 mL , using the same solvent to the mark.The solutions were prepared at various concentrations (10, 20, 30 mg.L^{-1}) by dilution with methanol as a solvent.

The application: The application was performed on pharmaceutical preparations for the determination of CAR in the form of tablets of different origins by preparing three various concentrations (10, 20, 30 mg.L^{-1}) and following the same method of action described above, and as shown in the following table:

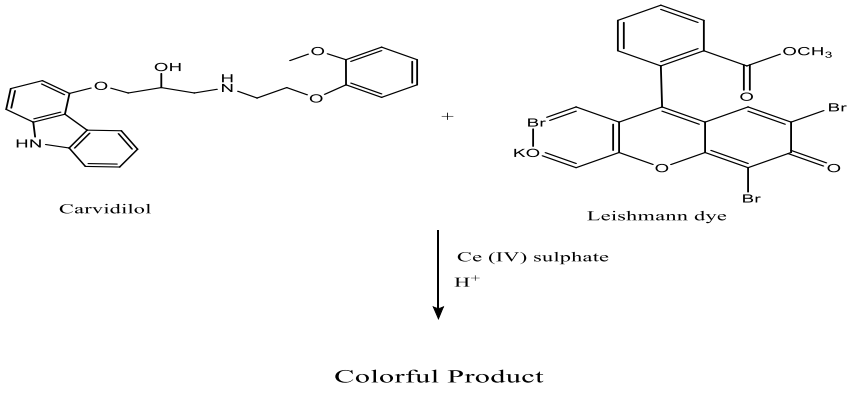
Table 2. Determination of the drug compound in the pharmaceutical preparations

Pharmaceutical preparation	Certified value	Amount present (µg. mL $^{-1}$)	Drug content found*(mg)	Recovery* (%)	Average recovery (%)
Carvidilol tablet (pharma intemational Co. Amman – Jorden)	25 mg	10	24.42	97.68	98.77
		20	24.64	98.56	
		30	25.02	100.08	
Carvidilol Hexal tablet Carvidilol (Salutas Pharm GubH – Barleben)	12.5 mg	10	12.32	98.56	99.17
		20	12.91	103.28	
		30	11.96	95.68	

*Average of five determinations

The results obtained in the above table demonstrate the successful application of the developed method on pharmacological forms to quantify CAR with high accuracy and are well precision.

The Suggested Chemical Reaction



Conclusion

A simple and accurate analytical method was developed aiming to determine the CAR using oxidation and then coupling with the dye in the presence of the oxidizing agent and in acidic medium to form a color product that showed the highest absorption density at 655 nm. The method follows Beer's law within the range (0.5 – 34 $mg.L^{-1}$), and the proposed method is selective, has good accuracy and compatibility, with a recovery rate of (97.96)%, while the value of the relative standard deviation is less than (1.5)% and the value of molar absorbance is (1.012×10^4) l / mol .cm.

Acknowledgments: We, the authors, extend our thanks and praise to Mosul University employees for their support and our encouragement.

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