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Spectrophotometric Determination of Carvedilol via Oxidation and Bleaching Colour of Methyl Red.

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Abstract

A selective spectrophotometric method for the determination of carvedilol (CAR) by oxidation and bleaching reactions in pure and pharmaceutical forms has been described. The method is based on the oxidation of CAR by known excess of ceric (IV) in acidic medium, exceed amount of the last was used in bleaching methyl red dye (MRD) color. The absorption of residual MRD at 520nm is directly proportional to the increase in carvedilol concentration. Beer's law was obeyed over the concentrations from 0.5 to 50 μg ml^{-1} The molar absorptivity was 1.11381 × 10^4 $l.mol^{-1}.cm^{-1}$. Sandell's indexes was 0.03610124 $\mu g.cm^{-2}$. The proposed method was successfully applied for determination of CAR in tablets.

Keywords: Carvedilol, Methyl red, Oxidation, bleaching dye;

1. Introduction

Carvedilol (CAR) belongs to the agents combining beta-adrenergic antagonists, which are well-known and distinct pharmaceutical compounds that are used in hypertension, high blood pressure treatment [1].As beta antagonists are divided into three groups, carvedilol and possendolol are considered from the third group that have an auxiliary property for vasodilation, it is a formal, non-selective compound [2], [3] antagonist of beta-adrenergic receptors (S-enantiomer-) and alpha-adrenergic receptors [2]in both R (+) and S (-) enantiomers - with equal potency [4]. Beta antagonists work by affecting the response to certain nerve impulses in certain parts of the body and as a result they reduce the heart's need for blood and oxygen by reducing the workload, and also help the heart beat more regularly. Carvedilol used in the treatment of high blood pressure and congestive heart failure by blocking the beta-adrenaline receptors for the adrenaline hormone, it reduces the heart rate and the force of contraction and thus reduces the work of the heart and low pressure, while alpha-adrenergic antagonists prevent the hormone noradrenaline from contracting the smooth muscles of the walls of arteries and veins, which helps to relax the arteries and veins and expand blood vessels, leading to easy blood flow and pressure drops [5]. It is also used to treat mild to moderate and severe chronic heart failure and left ventricular dysfunction after myocardial infarction in clinically stable patients, and carvedilol has a significant antioxidant effect by stopping oxygen from generating free radicals [3]. Carvedilol is in the form of a white powder that melts at a degree of 114-115 ° C. It is characterized by being a weak base that is practically insoluble in water [7] and dilutes acids. As for its solubility in 96% ethanol, it is slightly soluble in absolute methanol [8]. As for the molecular formula of carvedilol $C_{26}H_{24}N_2O_4$, it has the following structural formula and scientific name [9]:(2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2(2-methoxyphenoxy) ethyl] amino] propan-2-ol.

A UV spectroscopic method [8], Fluorometric method [10,11] were followed to determined CAR, these methods were based on the interaction of the charge transfer of CAR with the iodine receptor in acetonitrile medium, or on the formation of the two-ion complexes between CAR with acid dyes, sulfaphthaline, bromothymol blue and bromocresol green in chloroform medium [12].

Oxidation of the drug compound with ferric ammonium sulfate, followed by a complex formation of Fe (II) ion with potassium hexacyanoferrate or oxidation of diphenyl hydrazine followed by coupling with CAR has been used for determination of CAR[13].

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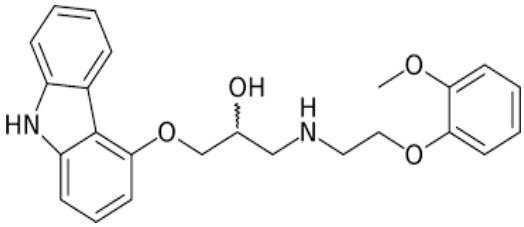

M.wt = 406.5 g/mol

Figure 1: The chemical structure of Carvedilol

Chromatographic methods based on reverse phase HPLC were also followed to determine CAR [14-16]. Other electrochemical method based on the use of polymer and colored carbon nanotubes for the determination of CAR was published [18].

Some methods require advanced techniques that may not be available in all laboratories and within the reach of all researchers, other methods require organic medium to enhance the dissolution of the prepared complexes as acetonitrile or chloroform, or use toxic reagent as potassium hexacyanoferrate and diphenyl hydrazine. The aim of the research is to estimate the drug CAR in simple procedure, smooth and available technique, using safe and friendly environment dye methyl red which is used in histopathology to detect the acidic nature of tissue and presence of organisms of acidic cell walls nature.

2.Experimental

Apparatus:

All spectroscopy measurements were performed with a Shimadzu 1800UV- Visible Japan dual-beam spectrophotometer. Quartz cells with a 1 cm light path were used and all weights were performed using a four decimal place sensor balance of the type Electric balance and the use of the water bath of the type Elektro.mag.

Chemicals

All chemicals and reagents were used with a high degree of purity.

CAR solution (100 µg.ml^{-1})

0.0100 g of the pure CAR powder has been dissolved in 5 ml of methanol with stirring and diluted up to the mark with absolute methanol in 100 ml volumetric flask.

Prepare methyl red dye (25 µg.ml^{-1})

The solution was prepared by dissolving 0.0025g of dye in 100 ml of absolute ethanol, completing the volume to the mark in a 100 ml volumetric flask with ethanol and placing the solution in a dark flask[19].

Ceric (IV) sulphate solution (300 µg.ml^{-1})

Prepare at a concentration of 300 µg.ml-1 by dissolving 0.0300 g of the medicinal compound in its pure form with 1.0M sulphuric acid and complete the volume to 100 ml volumetric flask using the same solvent.

Sulphuric acid solution (5M)

The solution was prepared by diluting 27.2 ml of concentrated acid (sp.gr. 1.84, 98%) to 100 ml with distilled water.

Intervention Solutions (5000 µg.ml^{-1})

Intervention solutions were prepared at a concentration of 5000 µg.ml-1 by dissolving 0.5000 grams of them in 100 ml of distilled water.

Solutions of surfactants

SDS and CTAB solutions are prepared at a concentration of 1 x 10^{-3} while a Triton (X-100) solution of 2% (w/v) is prepared by dissolving each in 100 ml of distilled water.

Pharmaceutical solutions, (100 µg.ml^{-1})

CAR pharmaceutical solutions (Carvedilol 25 mg / tablet and Carvedilol Hexel 12.5 mg / tablet) solutions.

Ten pharmaceutical tablets (Hexel Carvedilol tablets 12.5 mg / tablet and Carvedilol 25 mg / tablet) were weighed separately from the various original companies, were pestle and mixed well, taken as equivalent to 0.01 g and dissolved in 75 ml of absolute methanol while moving and completing the volume up to 75 ml of methanol, followed by filtration and washing the filter paper several times to get rid of suspended matter. The volume was completed in a 100 ml volumetric flask with absolute methanol and the solution was obtained at a concentration of 100 µg.ml-1 for each pharmaceutical product separately, then it was diluted by taking volumes of the aforementioned solutions to prepare three different concentrations (10, 20, 30) with the same solvent[20].

3.Results and discussion

3.1- Preliminary study

Subsequent experiments were conducted in a 10 ml volumetric flask using 2 ml at a concentration of 100 µg.ml^{-1} of the solution of the drug compound CAR and measuring the absorption of MRD (25 µg.ml^{-1}) at the wavelength of 520 nm versus the blank solution[21].

3.2- Study the optimal reaction conditions for oxidation and bleaching colour of methyl red dye MRD.

Prepare a calibration curve of the dye to find the largest amount of MRD dye within the linear range of the curve. Increased volumes (0.1-1) ml of dye at a concentration of 25 µg.ml^{-1} were added in 10 ml volumetric flask in the acidic medium add 0.5 ml of 5 M (H_2SO_4), then complete the volume to the limit of the mark with absolute ethanol It was found that the dye gives a maximum absorption at 520 nm, and that the linear range of the dye is (0.25-2.5 µg.ml^{-1} (Figure 2). Therefore, the concentration of 2.5 µg.ml^{-1}

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was used in the subsequent studies as it is the highest value for the limits of Beer's law**.**

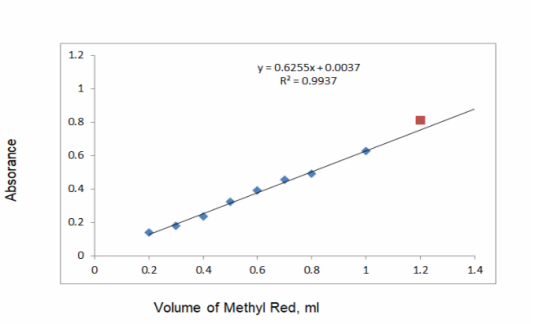

Figure 2: The Standard curve of MRD

The best MRD volume of 1 ml was chosen in the subsequent experiments**.**

3.3- Effect of oxidant.

The effect of a number of oxidizing agents was studied to determine the extent of their effect on the absorption of the color of the dye and it's bleaching by using 2 ml of 300 µg oxidizing agents separately in the presence of 2 ml of 100 µg.ml^{-1} of CAR and 0.5 ml of 5 M H_2SO_4. The results in Table (1) showed that Ce (IV) is a better oxidizing agent for CAR oxidation and MRD bleaching.

Table 1 : Choosing the best oxidizing agent

Oxidizing agent	Absorbance
N-bromosuccinimide	0.035
Ceric (IV) sulphate	0.430
Ferric Chloride	0.042

Ceric (IV) was adopted as an oxidizing agent in subsequent experiments.

3.4- Studying the selection of the best amount of oxidizing agent[23]

Effect of adding increasing amounts of Ce (IV) oxidizing agent to a series of 10 ml volumetric flask containing 1 ml of 25 µg. ml^{-1} MRD and 0.5 ml of 5 M H_2SO_4 with shaking of the solutions, standing for five minutes to oxidize the dye and bleach its color then dilute them with distilled water and measure the absorbance at 520 nm, The optimum amount for bleaching the dye is 1 ml of Ce (IV) oxidizing agent, As shown in the figure 3.

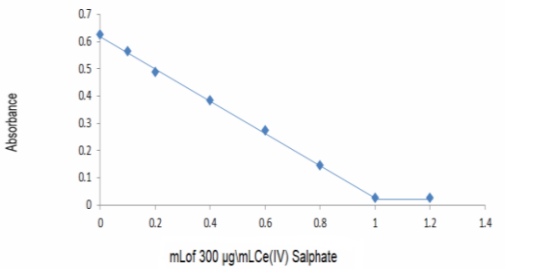

Figure 3: The amount of oxidizing agent needed to bleach the dye

The best volume of the of Ce (IV) was 1 ml and this volume was adopted in subsequent experiments.

3.5- Study the selection of the best acid[22]

A study was conducted to choose the effect of the best type of different strong and weak acids. The results shown in Table (2) showed that the best acid is sulphuric acid, using 0.5 ml at a concentration of 5 M.

Table 2 : Select the acid

Type of acid (5M)	HNO_3	H_2SO_4	HCl	CH_3COOH
Absorbance	0.423	0.462	0.427	0.341

3.6- Selection of the best concentration and amount for sulphuric acid

The effect of different concentrations of H_2SO_4 from (1-7) M to interact with CAR at a concentration of 20 µg and 1 ml of 300 µg.ml-1 Ceric (IV) of was studied. Next, the solutions were shaken and left for five minutes, then 1 ml of MRD was added at a concentration of 25 µg.ml-1, and then leave it for five minutes to complete oxidation of MRD and bleach its color, then dilute it with distilled water in a 10 ml volumetric flask, and the highest absorption was measured against the reagent blank at the wavelength of 520 nm. The 5 M solution, which showed the highest absorption, as recorded in Table (3) and 5 M was chosen for the best acid concentration. Table (4) shows the best volume is 1.5 ml.

Table 3 : Choosing the best concentration of acid

Molarity H_2SO_4	1	2	3	4	5	6	7
Absorbance	0.392	0.417	0.429	0.434	0.463	0.429	0.390

Table 4 : Choosing the best volume of acid

Ml of H_2SO_4 (5M)	0.5	1	1.5	2.0	2.5
Absorbance	0.462	0.488	0.511	0.506	0.499

The results showed that the use of 1.5 ml of 5M H_2SO_4 gave the best absorption for the determination of CAR in MRD oxidation and bleaching, and this amount and concentration were relied on in subsequent experiments.

3.7- Study of oxidation time

The time effect of the reaction is studied, to oxidize CAR with a measured amount of Ce (IV) in an acidic medium and left for different periods. This is followed by adding 1 ml of MRD leaving it for different periods of time, and then diluting it with distilled water to the mark. Then measure the

absorbance of MRD at a wavelength of 520 nm, and the results are shown in the table (5).

Table 5 : Effect of oxidation time and bleach colour

Standing time(min) after adding Ce (IV) Sulphate	Standing time(min) after addition MRD							
	3	5	7	9	10	12	15	20
3	0.489	0.498	0.495	0.493	0.488	0.480	0.478	0.472
5	0.511	0.515	0.510	0.502	0.490	0.465	0.432	0.425
7	0.484	0.492	0.487	0.482	0.477	0.471	0.469	0.465
9	0.391	0.394	0.390	0.389	0.387	0.382	0.378	0.374
10	0.330	0.387	0.384	0.381	0.375	0.371	0.372	0.369

Table (7) shows that the best oxidation time is 5 minutes, oxidation of the medicinal compound, 5 minutes for oxidation and bleaching of the MRD, then dilution with distilled water, and it is to be depending in subsequent studies.

3.8 - Order of addition effect

The effect of different surface-active substances (positive, negative, neutral) on dye absorption and color contrast was studied. The results listed in table (6) showed that the addition of surfactants led to a decrease in the absorption of the dye, therefore, they were excluded from use in subsequent experiments.

Table 6 : Effect Surface active substances

Surfactant	Absorbance/ ml of surfactant Solution		
	0.5	1	2
SDS $1x10^{-3}$ M	0.414	0.424	0.409
CTAB $1x10^{-3}$ M	0.465	0.495	0.446
Triton (X-100) 2%	0.450	0.430	0.421

3.9- Study the sequence of additions

A study was conducted on the effect of the addition sequence on the absorption of the remaining dye. The results presented in Table (7) showed that the sequence (II) (D = Carvedilol, R=Methyl Red, O = Oxidizing agent, A=Acid) adopted in the current work method gave the highest absorption of the (still) existing dye, and this sequence was relied on in the subsequent study.

Table 7 : Effect of Addition Sequence

Reaction components	Order number	Absorbance
D+R+A+O	(I)	0.499
D+A+O+R	)II(	0.515
D+O+A+R	(III)	0.510
O+R+D+A	(IV)	0.045
R+O+A+D	(V)	0.065

3.10- Study the effect of time on the stability

The stability of the unreacted MRD was studied by measuring the absorption of the remaining dye MRD against the blank solution at different times of time at the wavelength of 520 nm and by following the optimal reaction conditions for the current working method, the volume was diluted with distilled water in a 10 ml volumetric flask, and the results are shown in Table (8). The colored remaining dye was stable for 40 minutes at temperature room (22 °C).

Table 8 : The effect of time on the stability of unreacted MRD

Time (min.)	0	5	10	15	20	25	30
Absorbance of 20 µg ml^{-1} Carvedilol	0.515	0.515	0.516	0.515	0.515	0.515	0.514
	35	40	50	60(min.)	70	90	120
	0.514	0.506	0.487	0.479	0.474	0.470	0.403

3.11- Absorption spectrum

The absorption spectrum of the dye compound resulting from the reaction of CAR with MRD in the presence of cerium (IV), was performed in the acid medium, and the final absorption spectrum diagram showed the highest absorption density at the wavelength of 520 nm (Figure 4) illustrate that.

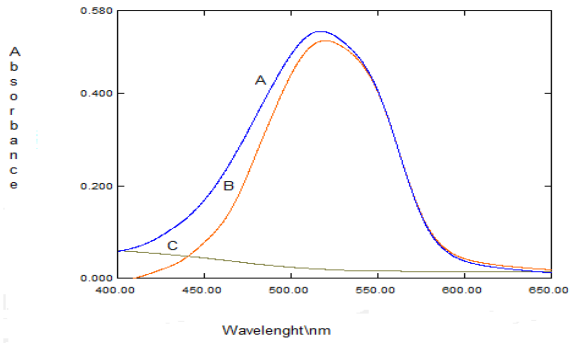

Figure 4: Absorption spectrum of 20 µg CAR solution (A) versus a blank solution, (B) versus a distilled water, and (C) a blank solution versus a distilled water.

3.12- Recommended procedure and calibration

According to the optimal conditions of the proposed method, a series of 10 ml volumetric flasks containing increased microgram concentrations were added and followed Beer's law limits within the linear range (0.5-50) µg.ml^{-1} of CAR solution and 1.5 ml of 5 M H_2SO_4, in the presence of 1 ml of 300 µg.ml-1 of

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the oxidizing agent cerium (IV), which was left for 5 minutes. Followed by the addition of 1 ml of 25 µg.ml-1 MRD, Molar absorptivity is 1.1138 x 10^4 l. mol $^{-1}$. cm $^{-1}$ and Sandell's sensitivity value was 0.036495 µg.cm-^{2}. While the value of detection limit was 0.0604625 µg.ml^{-1} and the limit of quantification was 0.66632 µg.ml^{-1}.

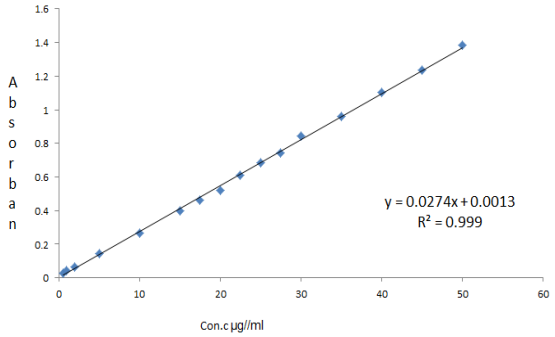

Figure 5 : Standard curve for estimation of CAR

3.13-Analytical and statistical values of the proposed method

Table (9) Shows the analytical values reached from the standard curve, the molar absorptivity coefficient and the sensitivity of the Sandell's index. The table also includes the values of the detection limit (LOD) and the quantification limit (LOQ) that were calculated by making ten replications of the form of the form solution and measuring its absorption against distilled water at the wavelength of 520 nm by applying LOD and LOQ equation.

LOD = $(3x\sigma_b)/S$

LOQ = $(10 \times \sigma_b)/S$

σ_b =Standard deviation

S= Slope of the standard curve

Table 9 : Statistical values for estimating carvedilol

Parameter	Value
Linearity range (µg\ml)	0.5-50
Slop	0.0274
Intercept	0.0013
Molar Absorptivity (l.mol^{-1}.cm^{-1})	1.11381×10^4
Sand ell's index (µg .cm^{-2})	0.036495
Correlation coefficient R^2	0.999
LOD (µg.ml^{-1})	0.1632941
LOQ (µg.ml^{-1})	0.5443137

3.14- Accuracy and precision of the proposed method

The accuracy and precision of the current method were examined by calculating the recovery ratio and the relative standard deviation value, using five replicates of three different concentrations of CAR solution. The results obtained in Table (10) indicate good accuracy and compatibility of the proposed method.

Table 10 : Accuracy and precision of the proposed method

Drug	Amount of Drug taken µg/ ml	Amount of Drug Found µg/ ml	Recovery%*	Average Recovery %	Relative error %*	Relative Standard deviation RSD%*
Carvedilol	10	9.9	99	99.73	1	1.049
	20	19.984	99.92		-0.08	0.443
	30	30.085	100.283		0.283	0.491

*Average of five determinations.

3.15- Interferences studies

Varying quantities of interfering substances were added from (100-500) µg to 10 µg of CAR following the recommended procedure.

It was found that the additives do not interfere with the proposed method as results shown in Table (11).

Table 11 : The effect of Interferences

Foreign Compound	Recovery% of 10 µg /ml CAR per µg Foreign Compound		
	100	250	500
Starch	101.54	100. 57	98.65
Glucose	98.84	98.45	97.30
Lactose	100.38	100.19	99.61
Sucrose	103.46	103.85	102.11

3.16- Application

The application was carried out to test the efficiency and success of the proposed method by applying it to pharmaceutical preparations of CAR in a tablet at three different concentrations (10, 20, 30) µg. ml^{-1}, by following the approved working method. The results obtained shown in Table (12) demonstrate the successful application of the developed method to pharmaceutical forms for the determination of CAR with high accuracy and with good precision.

Table 12 : Application of the proposed method to pharmaceutical preparations of Carvedilol

Pharmaceutical Preparations	µg Taken	µg Found	Recovery (%)	RE%* (%)	RSD%* (%)*	Average Recovery (%)
Carvedilol tablet /25 mg carvedilol (Pharma .international Co Amman - Jorden)	10	9.573	95.731	-4.269	0.942	98.73
	20	20.061	100.305	0.305	0.687	
	30	30.049	100.163	0.163	0.175	
Carvedilol exal tablet/12.5 mg Carvedilol (Salutas Phram GubH–Barleben)	10	9.6254	96.254	-3.746	0.991	98.16
	20	19.811	99.055	-0.945	0.425	
	30	29.753	99.177	-0.823	0.344	

* Average of five determinations.

3.17- Evaluation of analytical data[24]

The standard addition method was applied using the approved method for estimating CAR to prove the efficiency and success of the application of the approved method for estimation and it was free from interference of additives and excipients. Figure

(6) for CAR and the data listed in Table (13) show the success of the standard addition method and were in good agreement with the proposed method. To estimate CAR within the acceptable range of ± 5% error indicating the selectivity of the present method and satisfactorily.

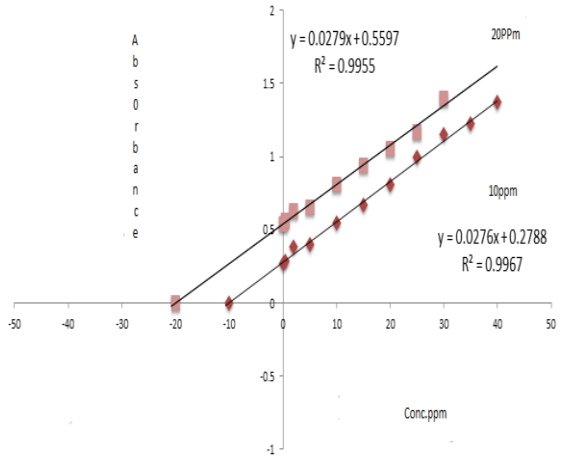

Figure 6: Standard Addition Method

Table 13 : Determination of CAR in pharmaceutical tablets by the standard addition

Pharmaceutical preparation	Amount Present µg.ml^{-1} Drug	Amount Fount µg.ml^{-1} Drug	(%)Recovery*
Carvedilol tablet /25 mg carvedilol Pharma international Amman - Jorden.Co	10	10.101	101.01
	20	20.06	100.3

3.18- Comparison with previously published work[25]

The present method has been compared with other published papers for determination of carvedilol. Table 14 show that the proposed method take place at room temperature, need not to adjustment of pH, safe compared with the other methods which use toxic reagent and it is not expensive by consume ethanol for dissolution only, moreover, it is sensitive, involve wide range of linearity.

Table 14 : Comparison of the present method with other spectrophotometric methods

Parameter	Present method	Published Method (17)	Published Method (18)
Procedure	Bleaching reaction	Schiff`s base reaction	Schiff`s base reaction
Temperature (˚C)	R.T	60	R.T
Reagent	Methyl Red	p-Dimethyl amino benzaldehyde	4-Hydroxy benzaldehyde
Linearity range (µg\ml)	0.5-50	50-250	0.5-2.5
Maximum wavelength (nm)	520	601	533
Molar Absorptivity (l.mol^{-1}.cm^{-1})	1.11381 × 10^4	0.92 × 10^3	8.4638 × 10^4
Sand ell's index (µg .cm^{-2})	0.036495	0.44181	0.0048025
Medium	aqueous unbuffered	Buffer pH 4	Absolute ethanol
Application	Tablet	Tablet	Tablet

4. Conclusions

The proposed method showed the success of the present method in according to the analytical results and the approved method is selective, accurate and precise with high recovery percentage 99.73%.

5. Acknowledgement

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