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Spectrophotometric Estimation of Carvedilol via Schiff's base Reaction with 4-Hydroxybenzaldehyde

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Abstract

A simple, sensitive, precise, and accurate spectrophotometric method was suggested for the estimation of carvedilol (CARVL) as pure, and in its pharmaceutical formulation (tablet): the method is based on Schiff's base reaction included condensation of CARVL with 4-hydroxybenzaldehyde(4-HBED) in the presence of concentrated sulphuric acid. All parameters that affected the formation of the product have been studied and the Optimal condition were selected. The colored Schiff base product gave maximum absorption at 533 nm. The linearity of the proposed method was obeyed Beer's law in the concentrations range of 0.5 to 5 µg/ ml, with a molar absorptivity of 8.463 ×104 l. mol-1 cm-1. The suggested method was applied to the determination of CARVL in Tablet formulations with accepted results of recovery of CARVL without any interfering with the common excipients.

Keywords: Carvedilo Schiff's base reaction, 4-hydroxybenzaldehyde, tablet formulations, spectrophotometry

1. Introduction

The most important use of Carvedilol is in treating high blood pressure, heart failure and in some cases when the heart is not pumping healthy [1].

Carvedilol is almost white or sometimes is crystalline powder, practically insoluble in any dilute acids or water but it is slightly soluble in an alcohol medium. (2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]propan-2-ol is the well-known chemical name and the chemical formula is $C_{24}H_{26}N_2O_4$ with molecular weight = 406.5 g/mol. It has a chemical structure as in figure 1[2].

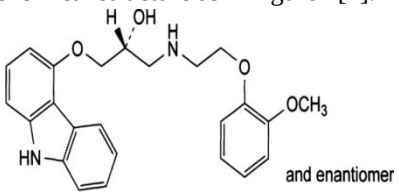

Figure 1. The chemical structure of carvedilol.

Various methods were applied for the estimation of CARVL. The most accurate methods include high performance liquid chromatography [3-8], HPLC/MS [9],UHPLC [10],LC/MS/MS [11] nanosensor probe [12], electrochemical sensor [13], voltammetric determination [14], spectrofluorimetry in presence of atenolol [15] and SPE- spectrofluorimetric [16]. There is no uncertainty that some of the above methods especially chromatographic and electro methods are sensitive and more selective, but expensive instruments are needed. The spectrophotom-etric methods are very simple, easy incorporation, inexpensive devices, and the possibility of having the spectrophotometer in the simplest laboratory. Through the literature survey, it was exposed various analytical procedures were applied for spectrophotometric determination of CARVL [17-22]. The present method included condensation reaction to form Schiff's base complex by the reaction of CARVL with 4-HBED.

2. Experimental part

2.1. Apparatus used

All measurements were made by using a Jasco V-630 UV / VIS dual-beam spectrophotometer, using quartz cells with a light path of 1 cm, and a BET ENGINEERING sensor scale was used to measure weight.

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2.2. *Chemicals used and reagents solutions*

The chemicals and analytical reagents used with a high degree of purity.

2.2.1. *Carvedilol solution (50µg/ml)*

This solution preparation include dissolving 0.0050 g of pure carvedilol (Bio-Pharm company, and it is as a gift from the Nineveh Pharmaceutical Company) , in 20 ml of absolute ethanol, and after the completion of the dissolution, the volume is completed to 100 ml with the same solvent in a 100 ml volumetric flask.

2.2.2. *4- Hydroxybenzaldehyde reagent solution, (0.1 W/V%)*

This solution was prepared by dissolving 0.1 g of 4-hydroxybenzaldehyde (Fluka company) with an amount of absolute ethanol, then the volume was completed to 100 ml in a volumetric flask with absolute ethanol.

2.2.3. *Pharmaceutical preparation solution*

2.2.3.1. Carve TAD tablet, 12.5 mg, 50µg/ml

The solution of the pharmaceutical preparation(Carve TAD tablet, TAD Pharma / Gmb Germany, each tablet contains 12.5 mg of carvedilol and the solution was prepared with a weight of 10 tablets, crushed and mixed well and weighed that equivalent of 0.0050 g of pure was dissolved in 20 ml of absolute ethanol, and shaked well and completed the volume to 100 ml with the same solvent, and then filtered it with No.1 filter paper.

2.2.3.2. Tablet (ALPHABETA. 6.25 mg) solution, 50µg/ml

The tablets are produced by the Swiss company (Hoffmann-La Roche). Each tablet contains 6.25 mg of carvedilol, using the same previous method of preparation 50µg/ml of Carve TAD tablet.

3. Appropriate working method and calibration curve

After experimentally fixing the optimal conditions for the determination of CARVL, a standard curve was prepared for the working method as follows: Increasing volumes (0.1-1.0) ml of a solution of 50 µg/ ml of carvedilol were added to a series of volumetric flasks of 10 ml, then 2 ml of the reagent solution 4-HBED (0.1%) was added, then 0.5 ml of concentrated sulphuric acid was added. The flasks were left for 20 minutes at room temperature and the volumes were completed to the mark with absolute ethanol. After that, the absorbance of the samples was measured against the blank solution at the wavelength of 533 nm. Figure 2 represents the straight standard curve that conforms to Beer's law in the range of concentrations 0.5 to 5µg/ 10 ml, and there is a negative deviation at concentrations higher than 5 micrograms / 10 ml and it was found that the value of the molar absorptivity of the resulting product is equal to 8.463×10^4 l. mol. $^{-1}$. cm^{-1} and Sandell's index was 0.0048 µg. cm^{-2}.

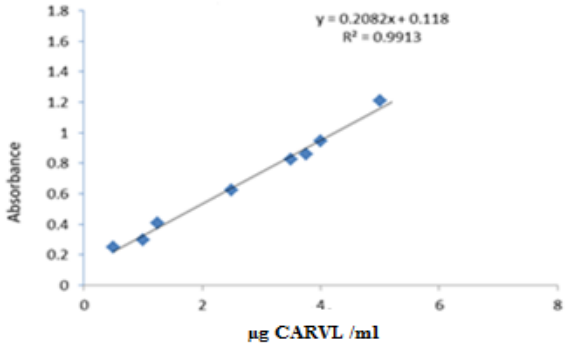


Figure2. Standard curve for determination of carvedilol using condensation reaction with 4-HBED**.**

4. Results and discussion

50 µg of CARVL in a final volume of 10 ml was used for subsequent experiments and the absorbance intensity of the solutions was measured against their blank solutions.

4.1. Principle of the method

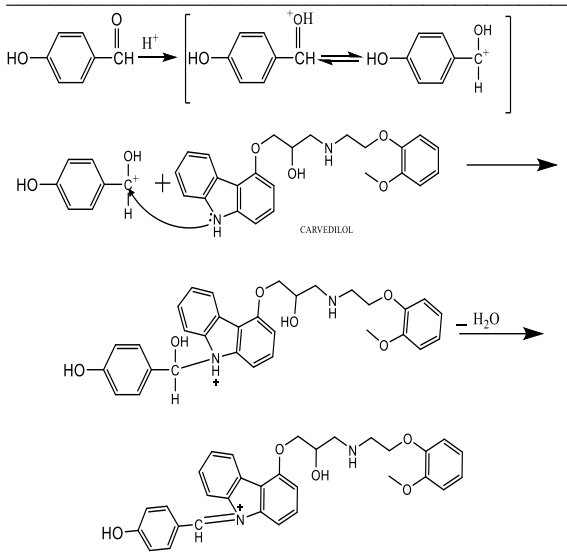
It was observed that an amount of CARVL solution reacted with 4-HBED in an acidic medium to produce a colored solution that gives the highest absorption intensity at the wavelength of 533 nm, The reason for the appearance of the red color is due to the formation of Schiff's bases product through the condensation of CARVL with the carbonyl group in the reagent (4-HBED), as shown in proposed mechanism of the reaction below:

The limit of detection LOD and limit of quantitation(LOQ) were calculated by applying the following mathematical relationships:

$LOD = 3\sigma / S$

$LOQ = 10\sigma / S$

Where σ = the standard deviation of the absorbance of the blank solution at the maximum wavelength and S is the slopes is the slope of the straight line[24].



4.2. Study the optimal conditions for a reaction

In order to obtain a color product with sufficient stability and high absorption intensity, the effect of various conditions on the optical properties of product resulting from the reaction of CARVL with 4-HBED in a final volume of 10 ml was studied.

4.2.1. Aldehyde selection

Several chemical reagents (aldehydes) with the concentration of 0.1% and a volume of 1 ml have been used which can be used as a reagent in the condensation reaction with 1 ml of a 50 µg solution of carvedilol (see Table 1).

Table 1. Selection of the type of aldehyde reagent.

Reagents (0.1%)	A	λ_{max} (nm)	ε :l.mole^{-1}.cm^{-1}
Vanillin	0.376	565	6.1133×10^4
Chlorobenza-ldehyde	0.297	398	4.8288×10^4
Methoxyben-zaldehyde	0.294	420	4.7800×10^4
4- Hydroxyben-zaldehyde	0.401	533	6.5197×10^4

From the results of Table (1), it is found that 4-HBED reagent gave the highest intensity of absorption of the colored product and the peak of the color contrast was good 203 nm as the blank solution gave very little absorption at the maximum wavelength of the measurement (533 nm). Also, the method does not require heat or a long waiting time compared to using vanillin and 2-hydroxynaph-thaldehyde reagents.

4.2.2. Study the effect of the type and quantity of acid used

The effect of various types and different amounts of acids have been studied, concentrating on the intensity of absorption of the resulting product at the maximum wavelength of 533 nm(Table 2).

Table 2. The optimal type of acid and amount used.

Acid used	Absorbance/ml acid			
	0.1	0.3	0.5	0.6
HCl	0.106	0.196	0.321	0.321
HNO_3	0.093	0.184	0.205	0.203
H_2SO_4	0.184	0.486	0.638	0.501
CH_3COOH	0.053	0.068	0.083	0.081
H_3PO_4	0.081	0.130	0.162	0.167

The results in Table 2 indicated that sulphuric acid and with a volume of 0.5 ml were the optimal type and amount of acid.

4.2.3. The effect of the amount of reagent(4-HBED)

To find out the appropriate amount of reagent for the reaction, different quantities of the reagent 4-HBED and for different concentrations of (1.25-5) µg of Carvedilol and the results are shown in Table 3.

Table 3. The optimal amount of reagent.

4-HBED (ml)	Absorbance/μg of ml			
	1.25	2.5	3.75	5
1	0.142	0.261	0.361	0.389
1.٥	0.201	0.294	0.398	0.582
2.0	0.401	0.623	0.856	1.226
3.0	0.344	0.539	0.701	1.025

The results in Table 3 show that a volume of 2 ml of 4-HBED solution with a concentration of (0.1%) gave the highest value of the resulting absorption intensity and the highest value of the determination coefficient(0.9832). Therefore, a volume of 2 ml of the reagent was adopted in the subsequent experiments.

4.2.4. *The effect of time on absorbance.*

To study the stability of the color of the Schiff base product from our reaction by measuring the intensity of absorption of the formed product against its blank solution at different time periods. The stability of the product is followed by solutions containing two different quantities of CARVL, and the results are shown in Table 4.

Table 4: The stability of the Schiff base colored product.

Time, minute	A/µg of CARVL Present in 1 ml	
	2.5	**3.75**
2	0.584	0.837
5	0.591	0.839
10	0.602	0.852
15	0.613	0.855
20	0.620	0.854
25	0.621	0.855
30	0.623	0.857
35	0.625	0.860
40	0.626	0.863
45	0.625	0.866
50	0.627	0.865
55	0.629	0.866
60	0.630	0.866
70	0.631	0.869
100	0.632	0.868
120	0.631	0.869

The results shown in Table 4 indicate that only two minutes after completing the additions are enough to give a complete reaction, and the absorbance remains stable for at least two hours.

4.2.5. Effect of solvents

The effect of several of organic solvents on the spectrum of the resulting product was studied by diluting the reaction solutions with these solvents instead of absolute ethanol. After that, the absorption of the product formed in these solutions was measured and the results are shown in Figure 3 and Table5.

From the results illustrated in table 5, it was found that chloroform solvent and absolute ethanol give the highest absorption intensity of the colored product formed. The absolute ethanol was chosen as a solvent in the subsequent experiments.

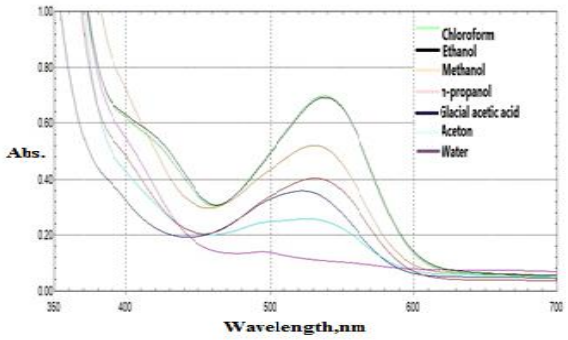


Figure 3. Effect of some organic solvents on the spectrum of the colored product compared with D.W.

Table5: The Effects of various solvents on the spectrum of the resulting colored product.

Solvent	λ_{max} (nm)	A	**Ɛ:l.mol^{-1}. cm^{-1}**
Ethanol	533	0.685	1.1137×10^5
n-propanol	528	0.404	6.5685×10^4
Glacial acetic acid	525	0.347	5.6418×10^4
Chloroform	533	0.687	1.1169×10^5
Water	490	0.111	1.8047×10^4
Methanol	533	0.519	8.4383×10^4
Acetone	530	0.256	4.1622×10^4

5. The absorption spectrum

It was observed when a 50 mg of carvedilol solution is treated with the quantity of reagent 4-HBED at a concentration (0.1%) and under optimal conditions and completing the volume with absolute ethanol, a red-colored product is formed that gives the highest absorption intensity at the wavelength of 533 nm compared to the blank solution that gives nil absorbance (0.091) at the wavelength of measurement as in Figure 4.

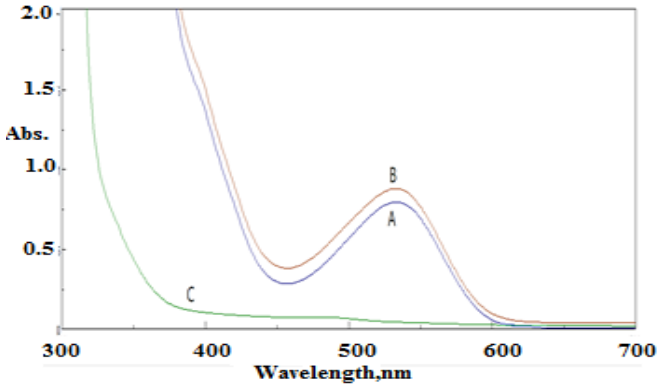


Figure 4. The absorption spectrum of 3.5 µg / ml of carvedilol measured (A) versus the blank solution, (B) versus absolute ethanol, (C) the blank solution versus absolute ethanol.

6. The nature of the colored product

The ratio of the condensation reaction of CARVL with an aldehyde (Schiff base reaction) was estimated by applying the continuous variations method (Job's method) [24]. Figure 5 proves that the condensation ratio is 1:1.

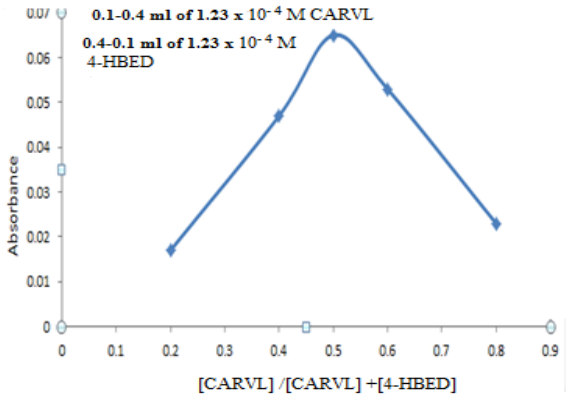


Figure 5. Continuous variations curve (Job's method) for the product formed by condensation of CARVL with 4-HBED.

From the above figure, the coupling ratio of carvedilol with the reagent 4-hydroxybenzaldehyde is 1: 1, and therefore the proposed structure for the colored product formed is as follows(figure 6):

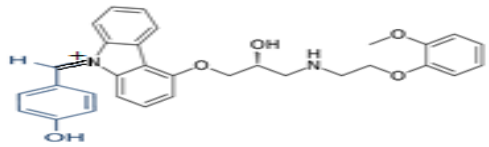


Figure 6. The suggested chemical structure of condensate product (CARVL-4-HBED].

7. Application part

The suggested method was applied in assays carvedilol in its pharmaceutical tablets, by taking different concentrations 1.25, 2.5 and 3.75 µg of the two tablets solutions prepared previously and after completing the additions according to the optimal conditions, the volumes were completed with absolute ethanol in a 10 ml volumetric flasks. Absorbance was measured at the optimum wavelength of 533 nm and the recovery ratio was calculated as shown in Table 6.

The results in Tables 6 and 7 confirm the success of the suggested method for estimating carvedilol in two types of tablets as its pharmaceutical preparation, and the method is accurate and precise based on the RE% and RSD% values sequentially.

Table 6: The results of application part

Drug	µg CARL present/ ml	µg CARL measured/ ml	Recovery* %
Carve TDA 12.5 mg/tablet TDA pharma Gmb, Germany	1.25	1.27	102.2
	2.5	2.47	99.1
	3.75	3.60	96.2
ALPHABETA 6.25 mg/tablet, Hoffmann-La-Roche	1.25	1.21	97.4
	2.5	2.48	99.3
	3.75	3.60	96.1

*Average of five determinations.

Also, the relative error% and relative standard deviation % have been calculated for three concentrations of CARVL in the two tablets in the application part (Table 7).

Table 7: The results of RE% and RSD%

Drug	µg CARVL present/ml	RE%	RSD%
Carve TDA 12.5 mg/tablet TDA pharma Gmb, Germany	1.25	2.162	0.609
	2.5	-0.80	0.553
	3.75	-1.30	0.614
ALPHABETA 6.25 mg/tablet, Hoffmann-La Roche	1.25	-3.20	1.609
	2.5	-0.24	0.388
	3.75	-3.84	2.140

8. Application using the standard addition method

The standard addition method was used to prove that the suggested method can be used in assaying carvedilol with accepted results and there is no effect of additives present with in tablets under an investigation by taking two series of 10 ml volumetric flasks and transfer in the first series of flasks a fixed volume of 0.3 ml of drug solution ((Carve 12.5 mg /tab.), and add to the different volumes of the standard solution of carvedilol 0 -0.5 ml of 50 µg, and transfer to the second series the same volumes of the standard solution of CARVL to a fixed volume of the drug solution (0.5 ml) and the volumes were completed to the marks and measure the absorbance of the solutions against their blank solutions at the wavelength of 533 nm. The curves of two serious as in Figure 7.

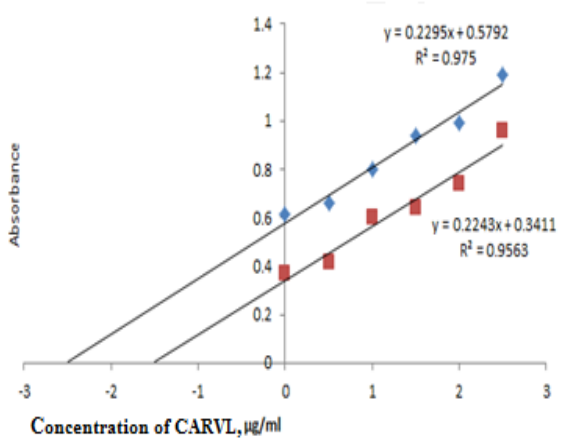


Figure 7. Standard additives curve for determination of CARVL in a medicinal preparation (Carve 12.5 mg / tablet.

Table 8 contain the results extracted from figure 7.

Table 8: The results of standard addition method

Drug	µg./ml taken	µg./ml measured	Recovery%
Carve TDA 12.5 mg/tablet TDA pharma Gmb, Germany	1.5	1.5207	101.38
	2.5	2.5237	100.94

The results in the table 8 show the success of the method in estimating CARVL in pharmaceutical preparation(Carve TAD tablet*)* without any effect of additives**.**

9. Estimation of t-test

The method comparison was based on the choice of t-test at the 95% confidence level for 4 degrees of freedom to find out the degree of congruence between the present method and the standard method approved in the British Pharmacopoeia[25] for the determination of CARVL and to determine the validity of the present method in application to a pharmaceutical preparation. The results in table 9 have been showing that the calculated value for t-exp. is less than the tabular value.

10. Methods comparison

Some of the analytical variables of the suggested method were compared with the variables for a spectrophotometric method for estimating carvedilol and the results .Are listed in the table10.

The results shown in Table show that the suggested method is characterized by excellent sensitivity and its applicability in estimating carvedilol in pharmaceutical preparation at room temperature.

Table 9: The comparison of the proposed method for the determination of CARVL with the standard method**.**

Drug	Recovery % Present method	British Pharma copeia method	t. exp*
Carve TDA 12.5 mg/tablet TDA pharma Gmb, Germany	99.14	100.02	0.152
ALPHABETA 6.25 mg/tablet, Hoffmann-La-Roche	98.8	99.42	0.117

Average of five determinations

Table 10: Comparison of the proposed method with another spectrophotometric method.

Parameter	Present method	Literature method[19]
Temperature (℃)	R.T.	60
Development time (minutes)	20	15 (At water bath)
λ_{max}	533	601
Reagent	4-Hydroxy benzaldehyde	p-Dimethy lamino benzaldehyde
Beer's law range (µg. ml^{-1})	0.5-2.5	50-250
Molar absorptivity (l. mol^{-1}. cm^{-1})	8.463×10^4	0.92×10^3
RSD (%)	Not more than 2.140	1.28
Application	Tablets	-------

11. Conclusions

The proposed method has several characteristics that distinguish it from other methods, including speed, accuracy, and sensitivity. The method depends on the condensation of carvedilol with the reagent 4-hydroxybenzaldehyde in an acidic medium to form a Schiff base, the method has been successfully applied to determine carvedilol in pharmaceutical preparations.

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