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Research Article

Simple Methods for the Spectrophotometric Determination of Carvedilol

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Two simple spectrophotometric methods are described for the determination of carvedilol (CAR). Method A is the condensation reaction of CAR with *p*-dimethylaminobenzaldehyde (PDAB), and the reaction mixture exhibits maximum absorbance at 601 nm. Method B is based on the charge transfer complex formation of CAR with *p*-chloranil; the color developed is measured at 662 nm. The calibration graphs are found to be linear over 50.00–250.00 and 20.00–100.0 μg mL^{-1} with molar absorptivity values of 0.92×10^3 and 0.257×10^4 L mol^{-1}cm^{-1} for CAR-PDAB and CAR-*p*-chloranil, respectively. Statistical comparisons of the results are performed with regard to accuracy and precision using Student's *t*-test and *F*-test at 95% confidence level. The methods are successfully employed for the determination of CAR in pharmaceutical preparations, and the results agree favorably with the reference and proposed methods.

1. Introduction

Carvedilol (1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol) belongs to a group of medicines called beta-adrenergic blocking agents, that are indicated for the treatment of hypertension, angina pectoris, and heart failure. Beta-blockers work by affecting the response to some nerve impulses in certain parts of the body. As a result, they decrease the heart's need for blood and oxygen by reducing its workload. They also help the heart to beat more regularly. CAR is used for treating high blood pressure and congestive heart failure. By blocking the receptors, CAR reduces the heart's rate and force of contraction and thereby reduces the work of the heart. CAR also blocks adrenergic receptors on arteries and causes the arteries to relax and the blood pressure to fall. The drop in blood pressure further reduces the work of the heart since it is easier to pump blood against a lower pressure. The FDA first approved CAR in 1995 [1]. Many methods have been used for the determination of carvedilol such as HPLC [2–7], capillary electrophoresis [8], fluorometry [9], and differential pulse voltammetry [10]. Recently a second polymorph of carvedilol has also been reported [11]. The two spectrophotometric methods [12, 13] reported earlier for the determination of CAR are in the UV region, and it is well known that UV-spectrophotometry is not a selective method, and therefore excipients can interfere with the method. The other three methods [14, 15] involve reaction of CAR with reagents like bromocresol green, ninhydrin, acetaldehyde, and nitroprusside. A comparison of the performance characteristics of the reported methods and proposed methods is given in Table 1.

Our aim is to develop a simple, sensitive, accurate, and cost-effective method for the spectrophotometric determination of CAR. The present investigation reports three simple and accurate methods for the determination of CAR in pure form and in pharmaceutical formulations. Method A involves the condensation of CAR with PDAB. Method B is based on the charge transfer complex formation. It is known that electron acceptors and electron donors can interact in solution to form intensively colored charge transfer complexes. Studies showed that amines are excellent electron donors and can interact with the electron acceptor. Hence, in the second method, tetrachlorobenzoquinone (*p*-chloranil)

Table 1: Comparison table for reported methods and proposed methods.

Reagents	Range (μg mL^{-1})	λ_{max} (nm)	Molar absorptivity (Lmol^{-1} cm^{-1})	Reference
Methanol	4.00–36.00	285	1.54×10^4	[12]
Bromocresol green	5.00–25.00	415	1.8×10^4	[14]
Ninhydrin	0.20–1.20	402	2.571×10^4	[15]
Acetaldehyde + SNP	0.60–2.00	558	1.617×10^4	[15]
Proposed methods				
p-Chloranil	20.00–100.00	662	0.257×10^4	—
PDAB	50.00–250.00	601	0.92×10^3	—

SNP: sodium nitroprusside; PDAB: *p*-dimethylaminobenzaldehyde.

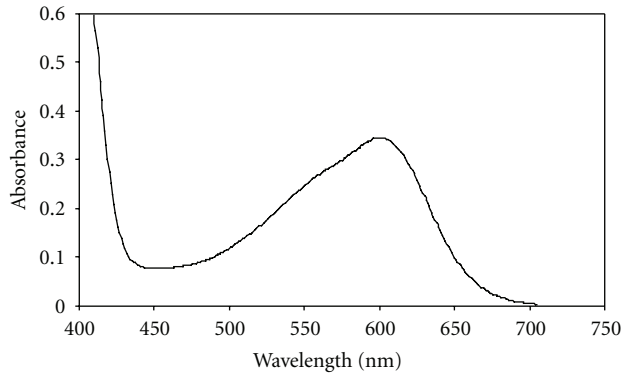


Figure 1: Absorption spectrum of carvedilol-*p*-dimethylaminobenzaldehyde system.

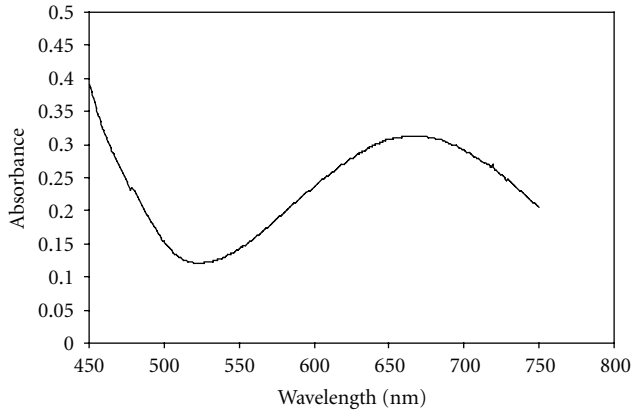


Figure 2: Absorption spectrum of *p*-chloranil system.

is used as electron acceptor from the aliphatic amine group of the drug.

2. Experimental

2.1. Instrument. A UV-2550 PC (Shimadzu, Japan) visible spectrophotometer with matched 1 cm quartz cells was used for all measurements.

2.2. Reagents and Chemicals. All reagents and chemicals used were of analytical grade. All the reagents were prepared with distilled water. The solution of *p*-chloranil (0.5%) was prepared using acetone. PDAB (1%) was prepared in 6 M HCl.

2.3. Standard Solutions of CAR. A 1000 μg mL^{-1} CAR solution was prepared in absolute alcohol/acetone and diluted to get the required working concentration.

2.4. Determination of CAR

2.4.1. Method A. Different aliquots of CAR containing 50.00–250.00 μg mL^{-1} were transferred into a series of 10 mL standard flasks using a microburette. Then, 2 mL of 3% PDAB and 1 mL buffer of pH 4 were added, and the contents were heated for 15 min at 60°C in a water bath. The contents were then cooled to room temperature, and the volume was made up to 10 mL with absolute alcohol. Increase in the absorbance was measured at 601 nm (Figure 1).

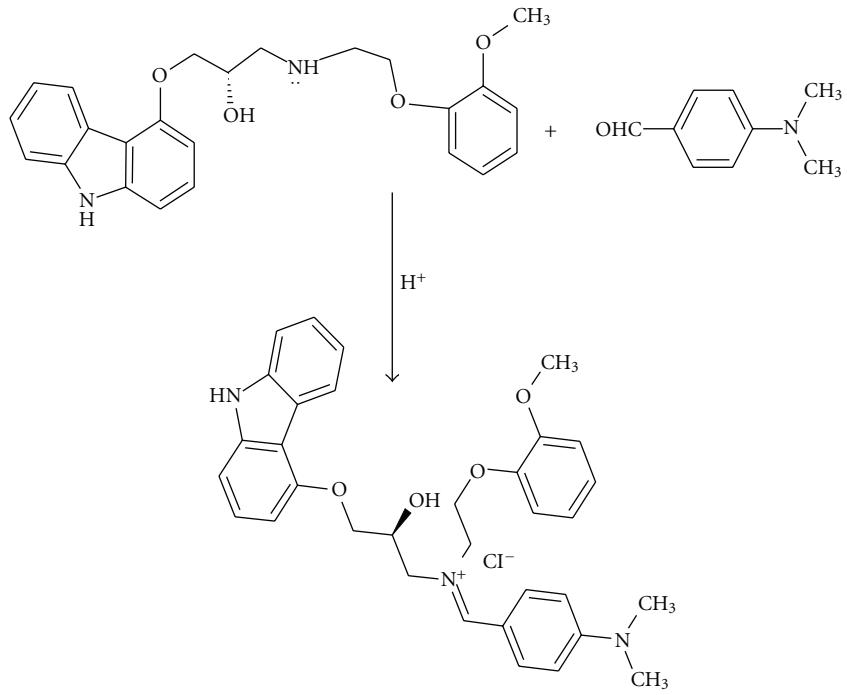
2.4.2. Method B. Aliquots containing 20.00–100.00 μg mL^{-1} of CAR were transferred into a series of 5 mL calibrated flasks using a microburette. To this 1 mL of 0.5% *p*-chloranil was added and kept for 1 hour, and the solution was made up to the mark with acetone. Absorbance of each solution was measured at 662 nm (Figure 2).

2.4.3. Assay of Formulations. Two tablets of Carloc (25 mg) and four tablets each of Carca (3.125 mg) and Carvil (3.125 mg) were taken and ground into a fine powder and dissolved in suitable solvent, filtered into different 100 mL standard flasks, the solutions were made up to the mark with respective solvents. Suitable aliquots were next subjected to analysis by using the procedures described above.

3. Results and Discussions

3.1. Absorption Spectra and Optimization of Reagent Concentrations

3.1.1. Method A. The method is based on the condensation of CAR with PDAB to form a blue colored product which is shown in Scheme 1. The absorbance of the colored product measured is a quantitative measure of the concentration of



Scheme 1: Reaction of CAR with PDAB.

CAR. Constant absorbance values were obtained with 2 mL of 1% PDAB.

Absorbance of each solution was measured at 601 nm. The reaction was carried out in acidic medium. Preliminary experiments were carried out to fix the initial concentration of HCl. It was found that 6 M HCl gave maximum color intensity. An acidic buffer of pH 4 was used in the present investigation. Different volumes (0.2 mL to 2.0 mL) of the buffer were tested for the color stability. One mL of the buffer of pH 4 was found to be ideal.

3.1.2. Method B. The charge transfer complex forming reactions are based on that π-acceptors react with the basic nitrogenous compounds as n-donors to form charge transfer complexes or radical anions according to the polarity of the solvent used. Scheme 2 represents the reaction of CAR with *p*-chloranil.

Preliminary experiments were performed to fix the initial concentration of *p*-chloranil; it was found that 1 mL of 0.5% *p*-chloranil shows a good increase in the absorbance. The polarity of the solvent used in the reaction between p-acceptors and n-donors can influence the formation of charge transfer complexes, and preliminary tests were performed to find out the most favorable solvent for the formation of the colored product. The solvents studied were ethanol, absolute alcohol, and acetone. There was a very small change in the color of the solution in presence of ethanol and absolute alcohol, whereas acetone gave a bluish green colored product which could be measured at 662 nm.

3.2. Analytical Data. Adherence to Beer's law was studied by measuring the absorbance values of solutions varying in drug concentration. The analytical parameters such as molar absorptivity, Beer's law limits and Sandell's sensitivity values were calculated. A linear correlation was found between absorbance and concentration ranges given in Table 2. The table also has values for correlation coefficient, intercept, and slope. The LOD and LOQ were calculated according to ICH guidelines as LOD $= 3.3 \times \sigma/S$ and LOQ $= 10 \times \sigma/S$, where σ is standard deviation of y-intercept of regression lines (standard deviation of response) and S is slope of calibration curve [16]. Sensitivity of the proposed methods is determined by calculating Sandell's sensitivity (μg/cm^2/0.001 Abs unit), which can be defined as smallest weight of substance that can be detected in column of unit cross-section. The calibration graphs are described by the equation $Y = a + bX$ (Y = absorbance, a = intercept, b = slope, X = concentration in μg mL^{-1}) obtained by the method of least squares.

3.3. Accuracy and Precision. The accuracy of the methods established by analyzing the pure drugs at different levels within working limits and the precision ascertained by calculating the relative standard deviation of replicate determinations on the same solution containing the drugs at different levels are presented in Table 3. The relative error and relative standard deviation indicate the high accuracy and precision for the methods. In order to check the validity of the proposed methods, carvedilol is determined in some commercial formulations. From the results it is clear that there is close agreement between the results obtained by the proposed methods and the label claim.

3.4. Interference Study. In the pharmaceutical analysis, it is important to test the selectivity towards the excipients and fillers added to the pharmaceutical preparations. Species that

Table 2: Analytical parameters.

	Method A	Method B
λ_{max} (nm)	601	662
Beer's law limit (μg mL^{-1})	50.00–250.00	20.00–100.00
Molar absorptivity (Lmol^{-1} cm^{-1})	0.92×10^3	0.257×10^4
Sandell's sensitivity (μg cm^{-2})	0.050	0.158
Limit of detection** (μg mL^{-1})	1.650	0.6626
Limit of quantification** (μg mL^{-1})	5.0000	2.0080
Regression equation*	$Y = a + bX$	$Y = a + bX$
Slope (b)	0.0020	0.0049
Intercept (a)	0.0124	0.0203
Correlation coefficient (r)	0.9988	0.9988

* Y is the absorbance and X the concentration in μg mL^{-1}.
**Calculated according to ICH guidelines.

Table 3: Evaluation of accuracy and precision.

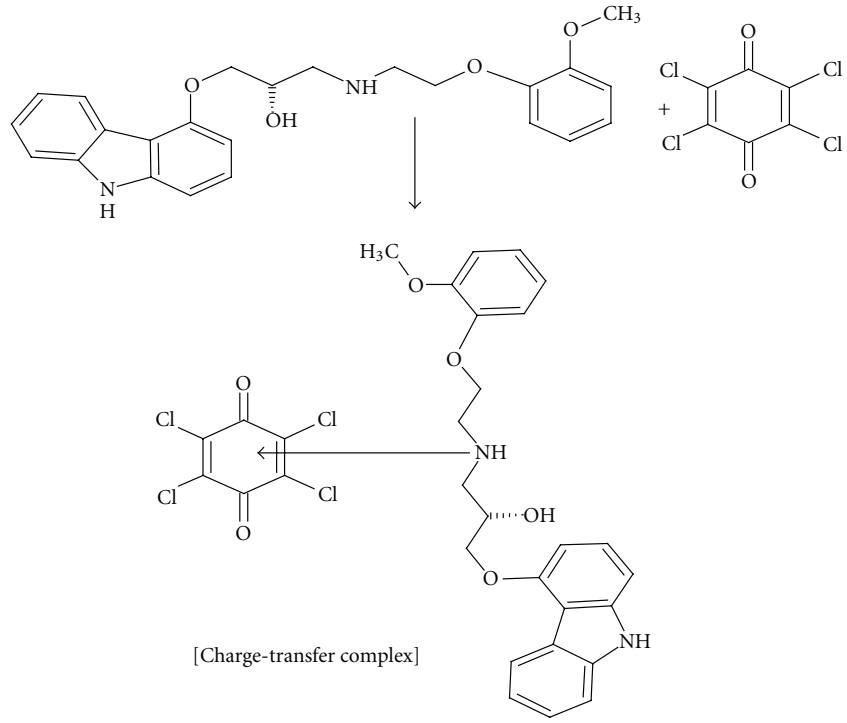
Method A				
Amount taken (μg mL^{-1})	*Amount found (μg mL^{-1})	RE (%)	SD (μg mL^{-1})	RSD (%)
100.00	100.40	0.40	0.01	1.39
150.00	149.70	0.66	0.02	1.49
200.00	200.20	0.10	0.02	0.98
Method B				
Amount taken (μg mL^{-1})	**Amount found (μg mL^{-1})	RE (%)	SD (μg mL^{-1})	RSD (%)
20.00	19.89	0.55	0.18	0.92
40.00	40.12	0.30	0.21	0.54
80.00	79.62	0.48	0.64	0.81
100.00	99.35	0.65	0.59	0.60

* Mean value of four determinations.
**Mean value of three determinations.
RE: relative error; SD: standard deviations; RSD: relative standard deviation.

Table 4: Results of assay of formulations.

Brand name	Carca[a]	Carvil[b]	Carloc[c]
Labeled amount (mg)	3.125	3.125	25.0
Reference value [15]			
Percent of label claim ± SD	99.8 ± 0.20	99.92 ± 0.17	98.99 ± 0.18
(1) PDAB			
(i) Amount found	3.115	3.110	24.97
(ii) Percent of label claim ± SD	99.7 ± 0.17	99.54 ± 0.26	99.89 ± 0.19
(iii) t-test	1.73	1.71	1.12
(iv) F-test	1.38	2.33	1.11
(2) p-Chloranil			
(i) Amount found	3.104	3.134	24.80
(ii) Percent of label claim ± SD	99.35 ± 0.15	100.3 ± 0.15	99.2 ± 0.13
(iii) t-test	1.73	1.55	2.37
(iv) F-test	1.77	1.28	1.91

* Mean of four determinations.
[a]Intas Pharma, [b]Zydus (C&D), [c]Cipla Ltd.
Values of t at 95% confidence level = 3.182.
Values of F at 95% confidence level = 6.39.



Scheme 2: Charge transfer reaction of CAR with *p*-Chloranil.

can occur in the real samples together with the drug were investigated. To investigate the effect of tablet fillers on the measurements involved in the methods, standard addition method was carried out. It was observed that starch, glucose, and talc did not interfere in the measurements.

3.5. Applications. The proposed methods had been successfully applied to the determination of carvedilol in four different branded tablets. The content of the tablet formulations is calculated by applying suitable dilution factor. The results for three pharmaceutical dosage forms are compared statistically with those of the tabulated value at 95% confidence level. The calculated Student's *t*-test does not exceed the tabulated value, indicating that there is no significant difference between the proposed methods and the tabulated value in respect to accuracy and precision. The results are listed in Table 4.

4. Conclusions

Simple spectrophotometric methods for the determination of carvedilol have been developed and validated according to ICH guidelines. The proposed methods are accurate and precise as indicated by good recoveries of the drugs and low RSD values. All the analytical reagents are inexpensive, have excellent shelf life, and are available in any analytical laboratory. The methods could be used to monitor the content uniformity of tablets. The proposed methods could be applied for routine analysis and in quality control laboratories for quantitative determination of the cited drugs both in the pure and dosage forms.

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