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Research Article

VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CARVEDILOL IN TABLETS

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ABSTRACT
Three simple, sensitive, precise and economical UV- spectrophotometric methods have been developed for the determination of Carvedilol in tablet formulation. Method A is simple UV spectrophotometric method and is based on determination of carvedilol in 0.1 N HCl at 241.2 nm. Linearity was obtained in the concentration range of 1 – 12 µg/ml. Method B is first order derivative spectrophotometric method and involved estimation of carvedilol in 0.1 N HCl usingt the first- order derivative technique at 251 nm as maxima and 290.8 nm as minima. Calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. Linearity was obtained in the concentration range of 2- 20 µg/ml. Method C is area under curve (AUC) method. The method involved calculation of integrated value of absorbance with respect to the wavelength between two selected wavelength 246 nm and 228.6 nm, respectively. Linearity was obtained in the concentration range of 2- 20 µg/ml. These methods were successfully applied to pharmaceutical formulations because no interferences from tablet excipients were found. The suitability of these methods for the quantitative determination of cavedilol was proved by validation. The proposed methods were found to be simple, sensitive, accurate, precise, rapid and economical for the routine quality control application in pharmaceutical formulations.
KEYWORDS: Carvedilol, UV spectrophotometric method, first order derivative spectrophotometric method, Area under Curve (AUC) method, tablet formulation

INTRODUCTION

Carvedilol (CAR) is non selective β-adrenergic blocking agent with α1-blocking activity[1]. Carvedilol is chemically (±)-[3-(9*H*- carbazol -4- yloxy)-2-hydroxypropyl] [2-(2-methoxyphenoxy) ethyl] amine[2]. The literature survey reveals HPLC[3-6], HPTLC[7], gas chromatography – electrospray tamdom mass spectroscopy method[8] and capillary electrophoresis[9] methods for the determination of carvedilol in pharmaceutical formulation as well as in biological fluids. The present manuscript describes three simple, single - step, sensitive, validated and economic spectrophotometric methods for the determination of carvedilol in pharmaceutical dosage form.

MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India)

Reagents and Materials

Carvedilol bulk powder was kindly gifted by Ipca Lab. Ltd. Mumbai, India. The pharmaceutical formulation was procured from the local market.Millipore's distilled water (Millipore, USA), Concentrated Hydrochloric Acid (AR Grade, S. D. Fine Chemicals Lts., Mumbai, India) and Whatman filter paper no. 41 (Whatman International Ltd., England) were used in the study.

Preparation of 0.1 N HCl solution

The solution was prepared by diluting 8.5 ml of concentrated hydrochloric acid with distilled water to produce 1000 ml.

Preparation of standard stock solution and working standard solution

An accurately weighed quantity of CAR (20 mg) wastranseferred to a 100 ml volumetric flask, dissolved, sonicated and diluted to the mark with 0.1 N HCl to obtain standard stock solution having concentration of

CAR (200 µg/ml) . Working standard solution (50 µg/ml) was prepared by appropriate dilution of stock solution in 0.1 N HCl.

Development of the methods

For selection of wavelengths, standard solution of CAR 10 µg/ml was prepared from working standard solution (50 µg/ml) in 0.1 N HCl for method A, B and C. The simple UV, first derivative and AUC spectra of solution was recorded in the scanning range of 200-400 nm.

Method A is simple UV spectrophotometric method. In this method the simple UV spectrum of CAR in 0.1 N HCl was obtained which exhibits absorption maxima (λ max) at 241.2 nm. Aliquots of working solution (0.1 – 1.2 ml) were transferred into a series of 5 ml of volumetric flask and diluted upto mark with 0.1 N HCl. The absorbences of the resulting solutions were measured at 241.2 nm against 0.1 N HCl as blank. Calibration curve was prepared by plotting absorbance versus concentration. The calibration curve was linear in concentration range of 1 – 12 µg/ml.

Method B is the 1st derivative spectrophotometric method. In this method the simple UV spectrum of CAR in 0.1 N HCl was obtained and derivatised to 1st order. Maxima occur at 290.8 nm and minima at 251 nm. Aliquot of working solutions of CAR (0.2 – 2 ml) were transferred into series of 5 ml volumetric flask. These solutions were diluted with 0.1 N HCl up to the mark and first derivative spectra were obtained which shows absorbance maxima at 290.8 nm and minima at 251 nm. A calibration curve was prepared by plotting the absorbance difference between maxima and minima versus concentration. The calibration curve was linear in concentration range of 2 – 20 µg/ml.

Method C is the Area under Curve method. In this method the simple UV spectrum of CAR in 0.1 N HCl was obtained and area between two selected wavelengths measured. Area measured between 246 nm and minima at 228.6 nm. Aliquot of working solutions of CAR (0.2 – 2 ml) were transferred into series of 5 ml volumetric flask. These solutions were diluted with 0.1 N HCl up to the mark and spectra were obtained which shows area between 246 nm and 228.6 nm. A calibration curve was prepared by plotting the area versus concentration. The calibration curve was linear in concentration range of 2 – 20 µg/ml.

Validation of the proposed methods

Linearity (Calibration curve)

A calibration curve was plotted over concentration range of 1 – 12 µg/ml of CAR for zero order spectrophotometric method (Method A) and 2 – 20 µg/ml of CAR for first derivative spectrophotometric method (Method B) and AUC method (Method C). Accurately measured standard working solutions of CAR (0.1 – 1.2 ml) were transferred into a series of 5 ml of volumetric flask and diluted upto mark with 0.1 N HCl. The absorbences of the resulting solutions were measured at 241.2 nm and was plotted versus concentration to obtain calibration curve and regression equation was calculated (Method A). Accurately measured standard working solutions of CAR (0.1 – 2.0 ml) were transferred into a series of 5 ml of volumetric flask and diluted upto mark with 0.1 N HCl. First derivative curves of these solutions (Method B) were obtained, which shows maxima and minima at 251 and 274 nm, respectively. Area of the zero order spectra's were calculated and the calibration curve of area against concentration was plotted (Method C).

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbances of solutions (n=6) of CAR (6 µg/ml for method A and 10 µg/ml for method B and C, respectively) without changing the parameters for the method. The repeatability was expressed in terms of percentage relative standard deviation (% RSD).

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of CAR (2, 6 and 10 µg/ml for method A) and (6, 12 and 18 µg/ml for method B and C). The results were reported in terms of percentage relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of CAR by the standard addition method. Known amounts of standard solutions of CAR were added at 50, 100 and 150 % level to prequantified sample solutions of CAR (4 µg/ml for method A and 6 µg/ml for method B and C). The amounts of CAR was estimated by applying obtained values to the respective regression line equations.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[11]

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of CAR in tablet formulations

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 10 mg of CAR was weighed and transferred to 100 ml volumetric flask.0.1 N HCl (50 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with 0.1 N HCl. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with 0.1 N HCl to get a final concentration of CAR 4 µg/ml and 6 µg/ml, respectively. The absorbances of final solution were recorded at selected wavelengths for determination of CAR. The analysis procedure was repeated three times with tablet formulation.

RESULTS AND DISCUSSION

Method A is simple UV spectrophotometric method. In this method the simple UV spectrum of CAR in 0.1 N HCl was obtained which exhibits absorption maxima (λ max) at 241.2 nm (Figure 1). The calibration curve was linear in concentration range of 1 – 12 µg/ml. Method B is the 1st derivative spectrophotometric method. Maxima occur at 290.8 nm and minima at 251 nm (Figure 2). The calibration curve was linear in concentration range of 2 – 20 µg/ml. Method C is the Area under Curve method. In this method the simple UV spectrum of CAR in 0.1 N HCl was obtained and area between two selected wavelengths measured. Area measured between 246 nm and minima at 228.6 nm (Figure 3). The calibration curve was linear in concentration range of 2 – 20 µg/ml.

The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine analysis of CAR in pharmaceutical formulations. The linearity ranges was found to be 1-12 µg/ml for method A and 2 – 20 µg/ml for method B and C. Characteristic parameters for regression equation and correlation are given in Table 3. Precision was calculated as repeatability (relative standard deviation) and intra and inter day variation (% RSD) for CAR. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The LOD and LOQ for CAR were found to be 0.22 and 0.67, 0.52 and 1.58, 0.65 and 1.97 µg/ml for method A, B and C, respectively indicates sensitivity of the proposed methods. The methods were successfully used to determine the amounts of CAR present in tablets. The results obtained are in good agreement with the corresponding labeled amount (Table 2). By observing the validation parameters, the methods were found to be sensitive, accurate and precise (Table 3). Hence the methods can be employed for the routine analysis of CAR in tablet formulations.

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TABLE 1: RECOVERY DATA FOR PROPOSED METHODS

Method	Level	Amount taken (µg/ml)	Amount added (%)	% Recovery ± S.D. (n = 3)
A	1	4	50	98.71 ± 0.14
	2	4	100	100.09 ± 0.16
	3	4	150	101.16 ± 0.18
B	1	6	50	100.88 ± 0.68
	2	6	100	99.97 ± 0.13
	3	6	150	99.83 ± 0.20
C	1	6	50	99.66 ± 0.10
	2	6	100	99.83 ± 0.45
	3	6	150	100.39 ± 0.50

Method A is the simple UV method, Method B is the first derivative method and Method C is Area under Curve method. n is number of determination and S.D. is standard deviation.

TABLE 2: RESULTS OF ANALYSIS OF TABLET FORMULATIONS

Tablet	Label claim (mg)	Parameter	% amount found (n = 3)		
			Method A	Method B	Method C
Brand A	25	Mean	99.81	99.88	100.39
		S.D.	0.22	0.34	0.86
Brand B	25	Mean	99.51	99.80	99.47
		S.D.	0.47	0.36	0.60

n is number of determination and S.D. is standard deviation.

TABLE 3: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATION PARAMETERS FOR THE PROPOSED METHODS

Parameters	Simple UV method	1st derivative method	Area under Curve method
Absorption Maxima	241.2 nm	290.8 nm	246 nm
Absorption minima	-	251 nm	228.6 nm
Beer's Law Limit (µg/ml)	1 – 12	2 – 20	2 – 20
Regression equation (y = a + bc)			
Slope (b)	0.1020	0.0039	0.2628
Intercept (a)	0.0436	0.0010	0.0602
Correlation Coefficient (r^2)	0.9987	0.9995	0.9991
Accuracy (n = 3)	99.99 ± 0.16	100.23 ± 0.34	99.96 ± 0.35
Repeatability (% RSD[a], n=6)	0.31	0.20	0.17
Precision (% RSD) (n = 3)			
Interday	0.60 – 1.72	0.83 – 1.79	0.69 – 1.17
Intraday	0.45 – 1.24	0.20 – 0.82	0.28 – 1.04
LOD[b] (µg/ml)	0.22	0.52	0.65
LOQ[c] (µg/ml)	0.67	1.58	1.97

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

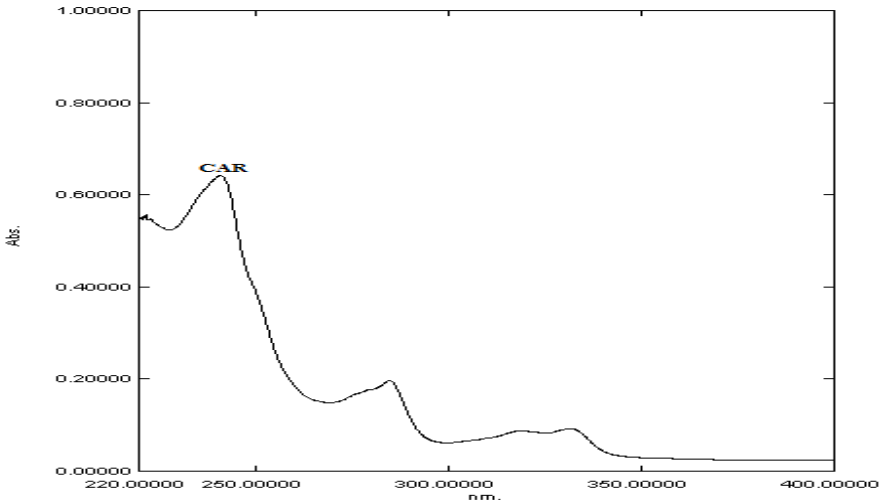

FIGURE 1: Simple UV spectrum of Carvedilol in 0.1 N HCl (Method A)

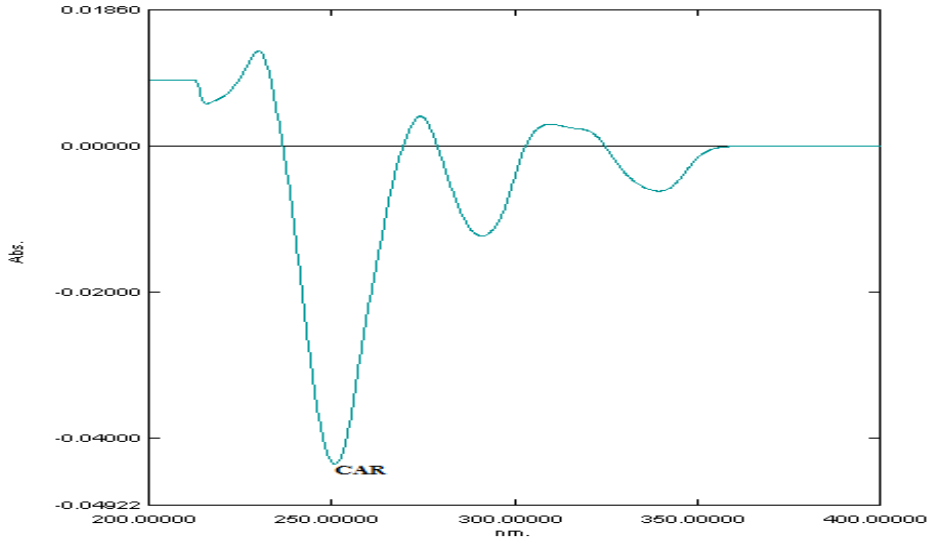

FIGURE 2: 1st derivative spectrum of Carvedilol in 0.1 N HCl (Method B)

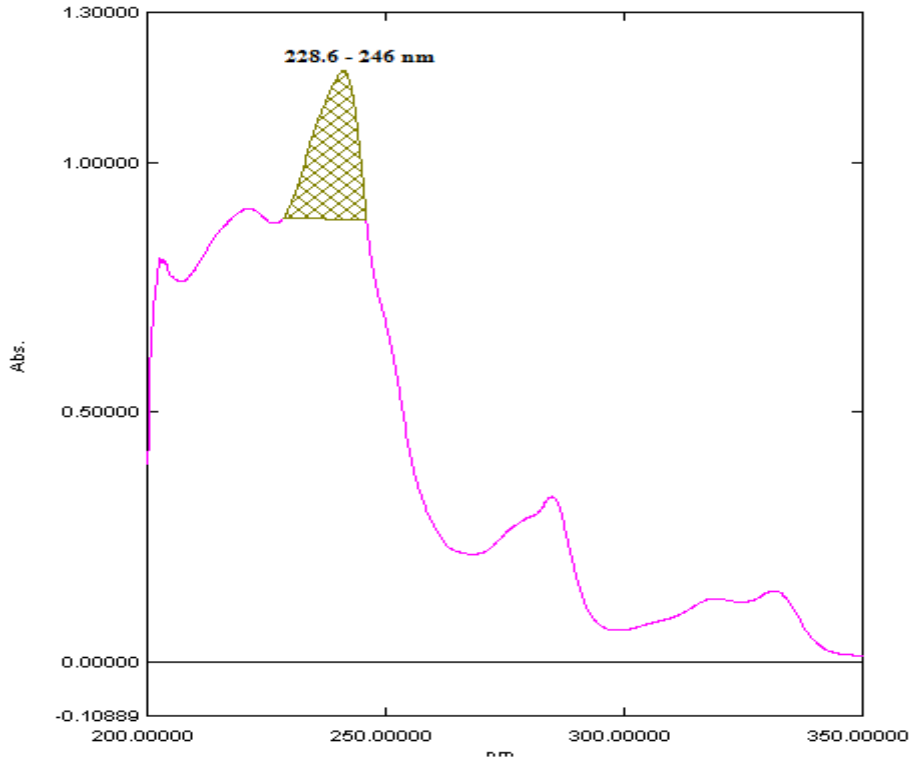

FIGURE 3: Area Under Curve of Carvedilol in 0.1 N HCl (Method C)

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