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Der Pharmacia Sinica, 2014, 5(6):29-35

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

UV spectrophotometric estimation of carvedilol hydrochloride by first order derivative and area under curve methods in bulk and pharmaceutical dosage form

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ABSTRACT

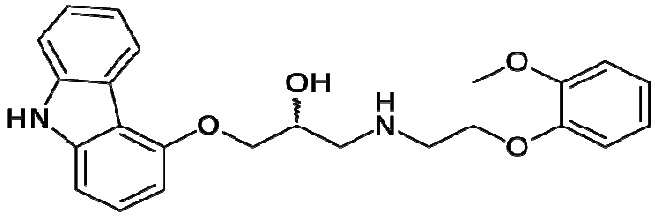
Simple and precise UV spectrophotometric methods by first order derivative and area under curve [AUC] - have been developed and validated for the estimation of carvedilol hydrochloride in bulk and its tablet formulation. The standard and sample solutions of carvedilol hydrochloride were prepared in methanol. Carvedilol hydrochloride was estimated at 233.7 nm for the first order derivative UV-spectrophotometric method (A), while in area under curve (AUC) method (B) the zero order spectrum of carvedilol hydrochloride was measured in between 240 nm to 244 nm. Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 1.5130 for first order derivative method. Similarly in AUC method, Beer's law was obeyed in the concentration range of 1 to 14 µg / ml with coefficient of correlation value 0.6606. These methods were tested and validated for various parameters according to ICH guidelines. The precision expressed as relative standard deviation were of 1.2145 % and 0.8929 % for the above two methods respectively. The proposed methods were successfully applied for the determination of carvedilol hydrochloride in pharmaceutical formulation. Results of the analysis were validated statistically and were found to be satisfactory. The proposed methods are simple, easy to apply, low-cost and require relatively inexpensive instruments.

Keywords: Carvedilol hydrochloride, UV spectroscopy, Derivative spectroscopy, Area under curve method, methanol.

INTRODUCTION

Its chemical name is (±)-1-(isopropylamino)-3-[*p*-(2-methoxyethyl)phenoxy]-2-propanol hydrochloride .Carvedilol hydrochloride is official in USP [1], EP [2] . Literature survey reveals the Spectrophotometric [3-8] HPLC [9-16],UPLC[17] methods for the estimation ofcarvedilol hydrochloride. Simple, rapid and reliable UV spectrophotometric methods are developed for the determination of carvedilol hydrochloride. These methods can be used for the routine analysis. In the proposed methods optimization and validation of this method are reported.

Structure of carvedilol



MATERIALS AND METHODS

Shimadzu UV-1800 was used with 10 mm matched quartz cell to measure absorbance of solution.

A Shimadzu analytical balance with 0.01 mg was used.

Chemical and reagents

Reference standard of carvedilol hydrochloride was obtained from reputed firm with certificate analysis. All spectral absorbance measurements were made on Shimadzu UV-1800 with 10 mm matched cell.

PREPARATION OF STANDARD SOLUTION

About 10 mg of standard carvedilol hydrochloride was weighed accurately and transferred in 100 ml of volumetric flask. About 30 ml of absolute alcohol was added and sonicated for 15 minutes. The volume was adjusted up to the mark with absolute alcohol to give concentration as 100 µg /ml.

Estimation from tablets

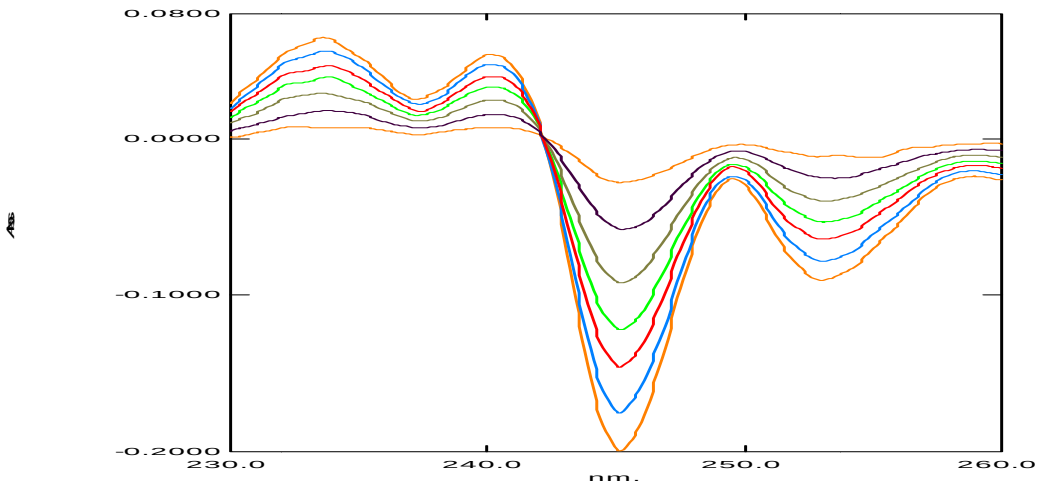
Twenty tablets were weighed accurately and average weight of each tablet was determined. Powder equivalent to 10 mg of carvedilol hydrochloride was weighed and transferred in 100 ml of volumetric flask. A 30 ml of absolute alcohol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with absolute alcohol to give concentration as 100 µg /ml. Such solution was used for analysis.

Experimental

Method A: First order derivative method

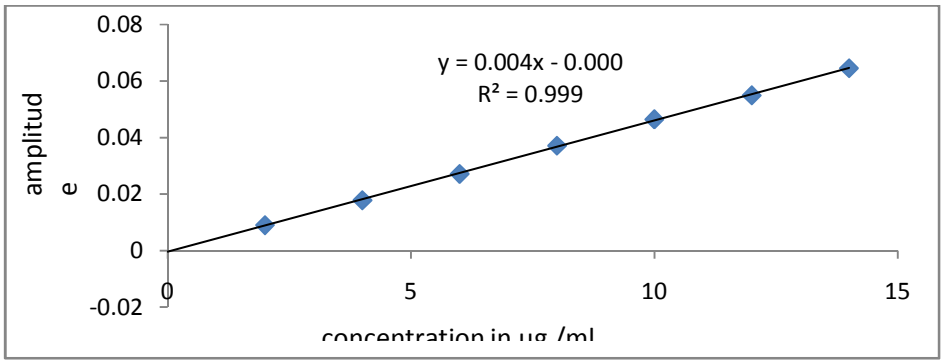
For the selection of analytical wavelength, 10 µg /ml solution of carvedilol hydrochloride was scanned in the spectrum mode from 300 nm to 200 nm by using absolute alcohol as blank. The first order derivative spectrum was obtained by using derivative mode by UV probe 2.42 software. From the spectrum, the amplitude of the derivative spectrum was measured at 233.7 nm (Fig. 2).

Fig. 2. Overlay spectra of First order derivative spectrum of carvedilol hydrochloride (2-14 µg/ml) showing absorbance at 233.7 nm



Into series of 10 ml graduated flask, varying amount of standard solutions of carvedilol hydrochloride was pipette out and volume was adjusted with absolute alcohol as solvent. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectra were obtained by using derivative mode. Amplitudes of the resulting solutions were measured at between 233.7 nm by using absolute alcohol as blank. The calibration curve was prepared in the concentration range of 1 to 14 µg/ml. (Fig. 3)

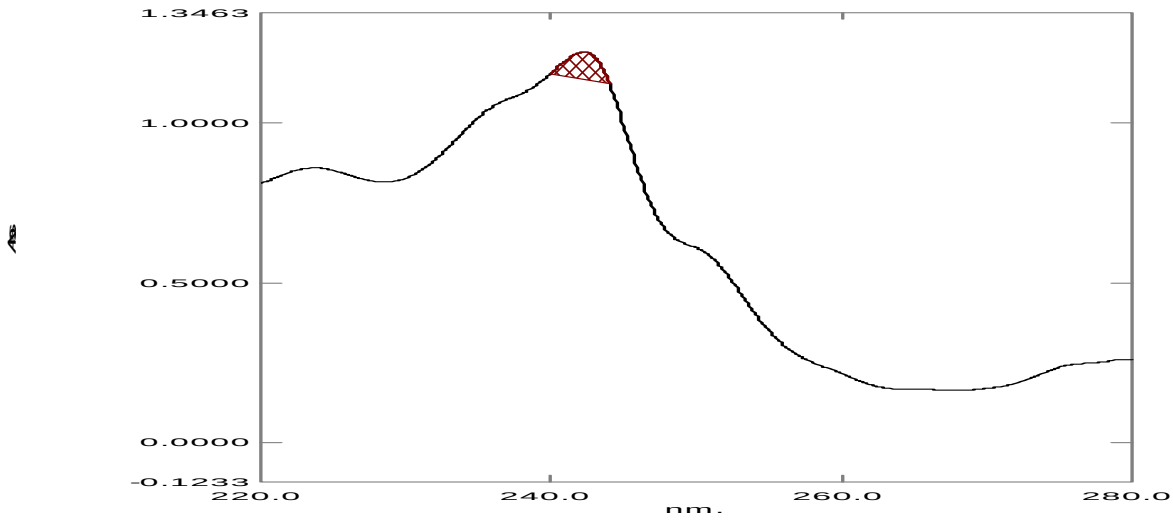
Fig. 3. Calibration curve for carvedilol hydrochloride at 233.7 nm by first order derivative Spectroscopy



Method B: Area under curve (AUC) method

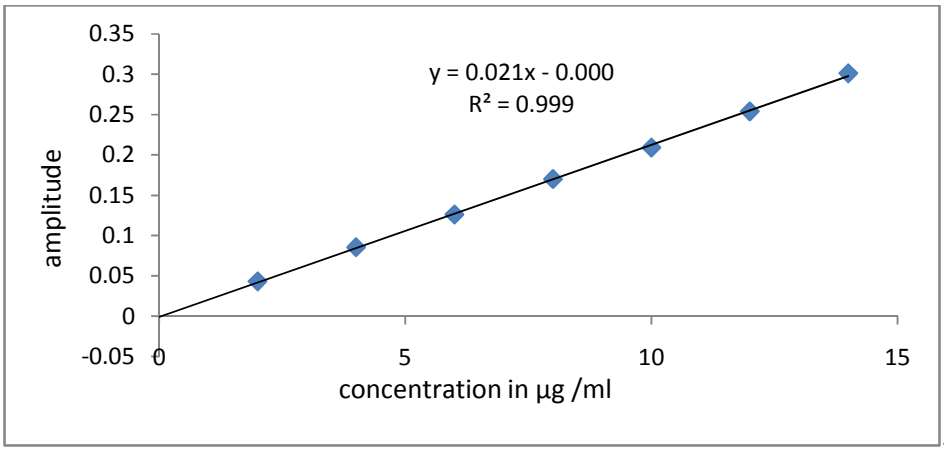
Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ_1 and λ_2. The area under curve between λ_1 and λ_2 were calculated by UV probe 2.42 software. In this method, 10 µg/ml solution of carvedilol hydrochloride was scanned in the spectrum mode from 300 nm to 200 nm. From zero order spectrum the AUC calculation was done. The AUC spectrum was measured between 240 nm to 244 nm (Fig. 4).

Fig. 4. Area under curve spectrum of carvedilol hydrochloride (10 µg/ml) showing area from 240 nm to 244 nm.



Into series of 10 ml graduated flask, varying amount of standard solutions of carvedilol hydrochloride were pipette out and volume was adjusted with absolute alcohol. Solutions were scanned between 300 nm to 200 nm in spectrum mode. The AUC calculations were done and the calibration curve for carvedilol hydrochloride was plotted in the concentration range of 1 to 14 µg/ml (Fig. 5).

Fig. 5. Calibration curve for carvedilol hydrochloride by area under curve spectroscopy



Results of analysis are given in table 1.

Table 1: Values of results of optical and regression of drug

Parameter	First order derivative method	Area under curve (AUC) method
Detection Wavelength (nm)	233.7	240-244
Beer Law Limits (µg/ml)	1-14	1-14
Correlation coefficient(r^2)	0.9997	0.9994
Regression equation (y=b+ac)		
Slope (a)	0.0046	0.0213
Intercept (b)	-0.0004	-0.0008

Validation

Accuracy

Accuracy of the proposed methods was carried as on the basis of recovery studies. It is performed by the standard addition method. Recovery studies were performed by adding standard drug at different levels to the pre-analyzed tablets powder solution and the proposed method was followed. From the amount of the drug estimated, the percentage recovery was calculated. The results of the analysis are shown in table (2, 3).

Table 2: Results of recovery of carvedilol hydrochloride for first order derivative method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	1.9968	99.843	0.0587	2.9415
2	2	4.0156	100.392	0.0679	1.6932
2	4	5.9811	99.686	0.0529	0.8856
2	6	8.1695	102.119	0.0434	0.5316
				Mean=0.0557	Mean=1.5130

Table 3: Results of recovery of carvedilol hydrochloride for area under curve (AUC) method

Amount of Sample Added in (µg/ml)	Amount of Standard Added in (µg/ml)	Total amount recovered	Percentage recovery(%)	Standard deviation	Percentage of relative standard deviation (C.O.V.)
2	0	2.0209	101.049	0.0156	0.7760
2	2	4.0189	100.473	0.0152	0.3790
2	4	5.9823	99.706	0.051	0.8553
2	6	8.0013	100.016	0.0505	0.6321
				Mean=0.0331	Mean=0.6606

Precision

The method precision was established by carrying out the analysis of homogenous powder blend of tablets. The assay was carried out of drug by using proposed analytical method in six replicates. The values of relative standard deviation lie well within the limits indicated the sample repeatability of the method. The results obtained are tabulated in table 4.

Table 4: Precision- method precision

Experiment no.	carvedilol hydrochloride taken in µg/ ml	Carvedilol hydrochloride in µg/ ml	
		First order derivative method	Area under curve method
1	10	10.219	9.905
2	10	10.131	9.952
3	10	10.109	10.00
4	10	9.956	9.857
5	10	9.890	9.810
6	10	10.109	10.047
	Standard deviation	0.1223	0.0886
	%RSD	1.2145	0.8929

Inter-day and intra-day precision

An accurately weighed quantity of tablets powder equivalent to 10 mg of carvedilol hydrochloride was transferred to 100 ml of volumetric flask. A 30 ml of absolute alcohol was added and sonicated for 15 minutes and filtered. The filtrate and washing were diluted up to the mark with absolute alcohol to give concentration as 100 µg /ml. Such solution was used for analysis.

For first order derivative method

Solution was scanned between 300 nm to 200 nm in spectrum mode. The first order derivative spectrum was obtained by using derivative mode. Amplitude of the resulting solution was measured at 233.7 nm by using absolute alcohol as blank. The amplitude of final solution was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 233.7 nm for first order derivative (method A). Similarly the amplitude of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of carvedilol hydrochloride was estimated by comparison with standard at 233.7 nm for first order derivative, table 5.

For area under curve method

Solution was scanned between 300 nm to 200 nm in spectrum mode. The area under curve of resulting solutions was measured at between 240 nm to 244 nm by using absolute alcohol as blank. The area under curve of final solutions was read after 0 hr., 3 hrs. and 6 hrs. in 10 mm cell at 240 nm to 244 nm (method B). Similarly area under curve of the same solution was read on 1^{st}, 2^{nd} and 5^{th} day. The amount of carvedilol hydrochloride was estimated by comparison with standard at 240 nm to 244 nm, table 5.

Table 5: Summary of validation parameter for intra-day and inter-day

Sr. no.	Parameters	First order derivative method	Area under curve (AUC) method
(A)	Intra-day precision (n=3) Amount found ± % RSD	99.686% 0.8856	100.473% 0.37907
(B)	Inter-day precision (n=3) Amount found ± % RSD	98.484% 0.1366	99.706% 0.8553
(c)	Ruggedness Analyst to analyst(n= 3) %RSD	0.5316	0.6606

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

$LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability .The values of LOD and LOQ are given in table 6.

Table 6: Values of results of LOD and LOQ

parameters	First order derivative method	Area under curve (AUC) method
Limit of Detection (µg/ml)	0.4309	0.05128
Limit of Quantification (µg/ml)	1.3060	0.1553

Ruggedness

The ruggedness of the method is defined as degree of reproducibility of results obtained by analysis of carvedilol hydrochloride sample under variety of normal test conditions such as different laboratories, different analysts and different lots of reagents. Quantitative determination of carvididol hydrochloride was conducted spectrophotometrically on one laboratory. It was again tested in another laboratory using different instrument by different analyst. The assays obtained in two different laboratories were well in agreement. It proved ruggedness of the proposed methods.

RESULTS AND DISCUSSION

The first order derivative and area under curve UV-spectroscopic methods are useful for routine analysis of carvedilol hydrochloride in bulk drug and formulation. The derivative spectroscopy method applied has the advantage that it locates hidden peak in the normal spectrum. It eliminates the interference caused by the excipients and the degradation products present, if any, in the formulation. The method was validated according to International Conference on Harmonization guidelines for validation of analytical procedures. Carvedilol hydrochloride has the absorbance maxima at 233.7 nm (method A) and in the AUC spectrum method areas were measured between 240 nm to 244 nm (method B). The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1 to 14 µg/ml and given in table1. Recovery studies were carried out by adding the pure drug to the previously analyzed tablet powder sample and shown in table 2, 3. The percentage recovery value indicates non interference from excipients used in formulation. The reproducibility and accuracy of the method were found to be good, which was evidenced by low standard deviation.

CONCLUSION

The most striking features of two methods are its simplicity and rapidity, not requiring tedious sample solutions preparations which are needed for other instrumental methods. From the results obtained it can be concluded that the proposed methods are fully validated and found to be simple, sensitive, accurate, precise, reproducible, rugged and robust and relatively inexpensive. So, the developed methods can be easily applied for the routine quality control analysis of carvedilol hydrochloride in pharmaceutical formulation.

Acknowledgment

Authors express sincere thanks to the Principal, Dr. Tushar M. Desai of D. G. Ruparel college.

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