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Analysis of Carvedilol in Pharmaceutical Preparations by Spectrofluorometric Method

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Research Article

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ABSTRACT

The goal of this research is to develop a spectrofluorometric method for analyzing carvedilol in pharmaceutical preparations and apply this method to the formulations. The method's calibration curve was plotted between 25 and 500 ng/mL. The mean calibration equation from six replicate experiments is y=1.8736x+7.7291. The correlation coefficient value was higher than 0.997 for the mean calibration curve. The trueness results were better than 2.09% and the precision results were less than 2.73% for carvedilol. The detection and quantitation limits were determined as 2.196 and 6.654 ng/mL, respectively. In addition, the method was used to study carvedilol in pharmaceutical preparations. The method had recovery values >98.4% for all samples in pharmaceutical preparations. The detection wavelength was optimized to maximize the sensitivity of the method. This method has good sensitivity with satisfactory results. Therefore, the method can be used in carvedilol analysis.

Keywords: Cocklebur, Biodiesel, Fatty acids, Linoleic acid.

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Introduction

Carvedilol is an antagonist of α1 and β1, β2 receptors as cardiovascular agent [1-3]. In addition, it is used to treat congestive heart failure, myocardial infraction, high blood pressure and ischemic heart disease. The chemical formula structure of carvedilol is (±)-1-(carbazol–4-yloxy)-3-((2-(o-methoxyphenoxy)ethyl)amino)-2-propanol (Figure 1).

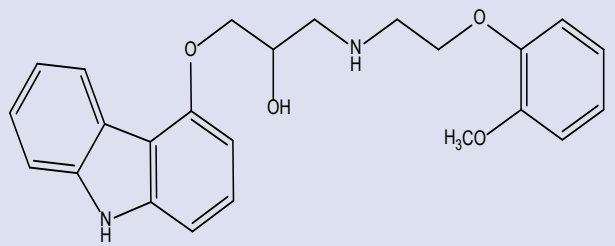

Figure 1. Structure of carvedilol

Carvedilol is available 12.5 mg and 25 mg varying doses in tablet formulations. Carvedilol has got $C_{24}H_{26}N_2O_4$ molecular formula and of 406.474g/mol molecular mass [4]. Since it is polar, its solubility in polar solvents is good [5].

In literature research, UV-Visible spectrophotometry [6-13], spectrofluorometry [14], HPLC [15-21] and capillary electrophoresis [22,23], either as a single entity or with other drugs in methods for determining carvedilol in pharmaceutical formulations or biological fluids have been reached.

These methods, except spectrophotometric methods, offered the required sensitivity and selectivity for the analysis of carvedilol in biological fluids, however their sophisticated instrumentation and high-analysis cost limited their use in quality control laboratories for analysis of carvedilol in its pharmaceutical dosage forms. Moreover, these instruments are not available in most quality control laboratories specially, third world countries. In general, spectrofluorometry is considered one of the most convenient analytical techniques, because of its inherent simplicity, low cost, and wide availability in most quality control laboratories. For these reasons, the goal of this research is to develop a spectrofluorometry method for analyzing carvedilol in pharmaceutical preparations and apply this method to the formulations. The developed method was then validated with respect to the ICH Topic Q 2 (R1) guideline [24].

The presented method is based on a simple and single analysis step in a short time using inexpensive chemicals. At the same time, the approach was also used to examine carvedilol levels in pharmaceutical preparations.

Materials and Methods

Chemicals

Carvedilol standard (98≥ purity) and methanol were obtained from Sigma (Germany). From a pharmacy, Dilatrend, Carvexal and Coronis tablet that included 25 mg carvedilol was purchased.

Spectrofluorometry System

Fluorescence analyses were performed with SHIMADSU RF-5301 PC spectrofluorometer system. In this work, a Xenon lamp was used. Excitation and emission wavelenghts were as λ_{exc}=285 nm and λ_{em}=335 nm. Slit width was selected as 5.0 nm on spectrofluorometer system equipped with a 1 cm quartz cells.

Preparation of Standard Solutions

Methanol was used to make a 1000 ng/mL carvedilol solution. Carvedilol standard solutions were diluted with methanol. Standard calibration samples were prepared 25-500 ng/mL (25, 50, 100, 200, 300, 400 and 500 ng/mL). The carvedilol solutions were all kept at 4 ^{0}C. Carvedilol quality control standard solutions were produced 75, 150 and 450 ng/mL.

Statistical Analysis

The statistical analyses were done with SPSS V.15.0 version at computer program. Regression analyses were used in the preparation of the carvedilol standard line and calculations. For statistical significance, the results were given with the mean ± standard error.

Results and Discussion

Development and Optimization of the Method

In this work, the various solvent systems (acetonitrile and methanol) were investigated for spectrofluorometry method. Methanol was selected as the solvent for sensitivity and stability. Excitation and emission spectra were recorded at λ_{exc}=285 nm and λ_{em}=335 nm, respectively.

Validation of the Method

Spectrofluorometry method was validated with validation parameters according to CDER. These parameters were specificity, linearity, precision, trueness, recovery, limit of detection (LOD), limit of quantification (LOQ) and stability.

Specificity

All the standard, quality control and tablet solutions were recorded at λexc=285 nm and λem=335 nm, respectively. The emission spectrum of carvedilol solutions showed maximum values. The spectrums of carvedilol standard were given in Figure 2.

The emission wavelenghs of standard, quality control and tablet solutions did not changed at λem=335 nm. The effects of common excipients and additives were tested for their possible interferences in the assay of carvedilol. The simulated and placebo samples were prepared and analyzed.

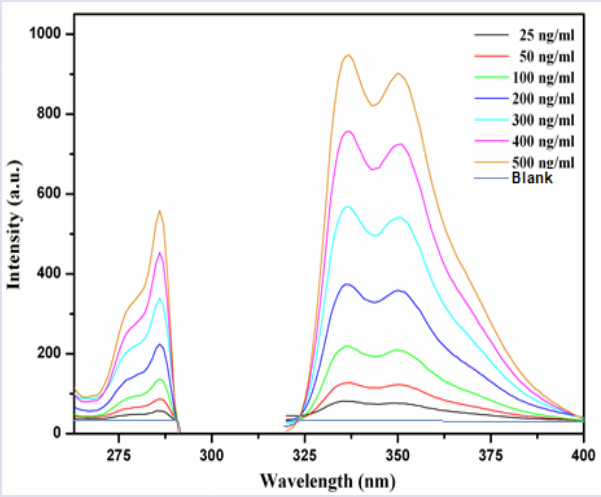

Figure 2. The spectrums of carvedilol.

It has not been determined any interference of these substances at the levels found in dosage forms. Excipient that was used in this preparation was the most commonly used by the pharmaceutical industry. The presence of titanium dioxide, talc, lactose, starch, and magnesium stearate did not appear interfere in the results of the analysis. Endogenous interference substances were not observed in spectra. According to the analysis results the method can be specific.

Linearity

Standard calibration curve was drawn according to emission value (*y*) of carvedilol versus carvedilol concentration. It was found to be linear over the 25-500 ng/mL concentration range for carvedilol. The mean calibration equation from three replicate experiments is y=1.8736x+7.7291. The correlation coefficient value was higher than 0.997 for the mean calibration curve. The results are listed in Table 1.

Table 1. Linearity values of carvedilol

Parameters	Carvedilol
λ_{exc} (nm)	285
λ_{em} (nm)	335
Linearity range (ng/mL)	25-500
Slope	18.736
Intercept	77.291
Correlation coefficient	0.997
Standard deviation of slope	0.002
Standard deviation of intercept	1.247
LOD (ng/mL)	2.196
LOQ (ng/mL)	6.654

Precision and trueness

The %RSD value values were used to assess the proposed method's precision. Six replicates for each of three different concentrations were analyzed to determine intra-day precision. The same samples were analyzed in three successive days to measure the intermediate precision. In addition, the percentage relative error was used to assess the method's trueness. The results are listed in Table 2.

Table 2. Precision and trueness of carvedilol

	Intra-day			Inter-day		
Added (ng/mL)	Found ± SD[a]	Precision % RSD[b]	Trueness[c]	Found ± SD[a]	Precision % RSD[b]	Trueness[c]
75	74.6 ± 1.424	1.91	-0.53	75.2 ± 2.052	2.73	0.27
150	148.5 ± 3.121	2.10	1.00	152.2 ± 3.149	2.07	1.47
450	445.2 ± 4.243	0.95	-2.09	453.4 ± 5.325	1.17	0.76

In addition, the percentage relative error was used to assess the method's trueness. The results are listed in Table 2.

The precision and trueness for carvedilol from standard solution samples were gratifying. %RSD value is obtained as lower than 2.73%. In addition to this, trueness is detected to be within ± 2.09% with relative error. From the results obtained, it is understood that both the precision and the trueness of this method are good.

Recovery

The percentage recovery was checked to study the formulation interference effects at three different concentrations. The recoveries were performed by adding known amount of pure drugs to pre-analyzed samples of carvedilol tablets. The percentage recoveries were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. The results are listed in Table 3.

Table 3. Recovery of carvedilol in tablets (n=6)

Tablet	Added (ng/mL)	Found ± SD	Recovery (%)	RSD (%)
Dilatrend (150 ng/mL)	50	49.2 ± 1.312	98.4	2.67
	150	149.2 ± 3.124	99.5	2.09
	250	253.0 ± 6.847	101.2	2.71
Carvexal (150 ng/mL)	50	49.3 ± 1.097	98.6	2.23
	150	152.4 ± 3.473	101.6	2.28
	250	247.4 ± 3.146	98.9	1.27
Coronis (150 ng/mL)	50	49.6 ± 1.073	99.2	2.16
	150	147.8 ± 3.624	98.5	2.45
	250	252.7 ± 4.369	101.1	1.73

Also, the proposed method was compared with the official method [25]. Besides, the results of the proposed method were compared with the reported method [26]. The results revealed no significant difference between the proposed and reference methods using F test at the 95% confidence level (Table 4). Also, the limit of quantitation of the proposed method is lower than those of the official method [25].

Table 4. Comparison of the methods

Parameters	Spectrofluorometry	Official method [25]	Reported method [26]
Trueness %	99.68	99.98	99.93
SD	0.847	0.012	-
% RSD	0.849	0.012	0.28
Variance	0.717	$1.44x10^{-4}$	
Standart error	0.346	$4.89x10^{-3}$	
Calculated F-value (F_c)	1.76		
Tabulated F-value (F_t)	3.00	$F_t > F_c$: (P > 0.05)	

LOD and LOQ

The LOD and LOQ values were calculated using calibration standards as 3.3 σ/S and 10 σ/S, respectively [27,28]. (Where, σ: Standard deviation of the response, S: Slope of the calibration curve). The LOD and LOQ for the method were obtained as 2.196 and 6.654 ng/mL, respectively.

Stability

Carvedilol stock solution stability was evaluated for at least 72 hours at room temperature. In addition, carvedilol standard sample solutions were stable at room temperature, 4 and -20 ^{0}C refrigeration temperature for 72 h. The trueness of carvedilol stability are within the acceptance range of 90-110%. No significant degradation product of carvedilol in these conditions. The results are also listed in Table 5.

Table 5. Stability of carvedilol in different temperatures (n=6)

Added (ng/mL)	Room temperature 24 h (Mean ± SD)	Room temperature 72 h (Mean ± SD)	Refrigeratory +4 °C, 72 h (Mean ± SD)	Frozen -20 °C, 72 h (Mean ± SD)
150	98.6 ± 2.65	98.6 ± 2.74	101.9 ± 1.47	98.6 ± 3.32
300	98.9 ± 1.62	98.9 ± 2.04	100.6 ± 1.79	98.2 ± 3.62
450	99.4 ± 3.61	101.2 ± 3.41	98.6 ± 2.64	101.9 ± 3.71

Procedure for Pharmaceutical Preparations

The preparation of tablet sample solution was done by taking twenty tablets of carvedilol. Tablets were powdered in a mortar pestle. After, an amount of the powdered sample equivalent to 25 mg of drug was taken in a 25 mL volumetric flask and then solubilized with 25 mL methanol. Standard sample was prepared as 1.0 mg/mL. The tablet solution was filtered by Whatman No 42 paper. Then, it was diluted to get in the range of 100 and 400 ng/mL with methanol (Figure 3).

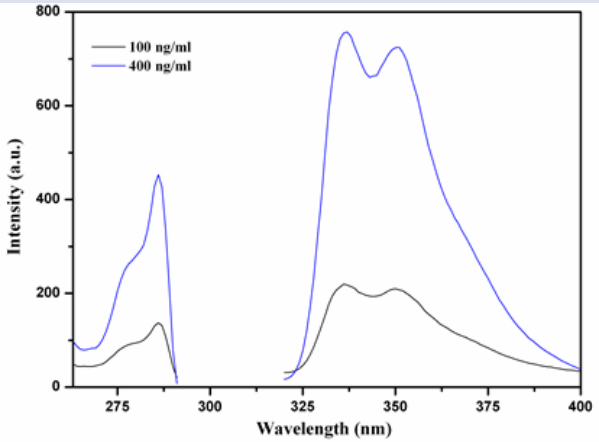

Figure 3. The spectrums of Dilatrend tablet containing carvedilol.

Conclusions

In this work, a simple, new and fast spectrofluorometry method has been completely developed in order to analyze carvedilol in pharmaceutical preparations. Furthermore, the validation parameters were used to validate the procedure. The method was found to easy for the analysis of carvedilol. The carvedilol recoveries in tablets was in good agreement with their respective label claims. The method described has been effectively and efficiently used to analyze carvedilol pharmaceutical tablets without any interference from the pharmaceutical excipients. On the other hand, no extraction procedure is used. Therefore, the spectrofluorometric run time of 1 min allows the analysis of a large number of samples in a short period of time.

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Conflicts of Interest

The author states that did not has conflict of interests.

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