RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF CARVEDILOL IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective of the present study was the development and validation of a simple RP-HPLC method for the estimation of carvedilol in bulk and pharmaceutical dosageforms. The analysis was carried out by using phenomenex C-18 column in isocratic mode with the mobile phase consisting of Acetonitrile and phosphate buffer in the ratio of 69:31 v/v at a flow rate of 1ml/min. The eluent was detected for 242 nm . The retention time of the drug was 3.57min. The proposed method was statistically validated and found that it is simple, accurate, precise, robust, and suitable for the routine analysis of pharmaceutical formulations.

KEY WORDS: Carvedilol, HPLC, Validation.

1.INTRODUCTION

Carvedilol is a Beta receptor blocking agent and posses vasodialation and antioxidant activity. It is used in the treatment of hypertension, angina pectoris and congestive heart failure. Chemically it is (±)-1-(carbazol-4-yloxy)-3-((2-(*o*-methoxyphenoxy) ethyl) amino)-2-propanol (Ahmed,2007). Carvedilol is a racemic compound. Practically it is insoluble in water and soluble in organic solvents like chloroform and methanol. According to the literature, very few HPLC methods have been reported (Ramesh,2007; Patel,2006; Jelena,2007; Behn,2001; Eisenberg,1989). The present method is simple, rapid, accurate and precise for the estimation of carvedilol in pharmaceutical dosage forms.

2.EXPERIMENTAL

Materials: A High performance liquid chromatograph (Shimadzu-10ATVP) equipped with two pumps (Model-10ATVP) and Shimadzu –UV-Visible detector (SPD-10ATVP), Phenomenex P/N0-00G-4274-EO C-18 column, Luna 5µ silica(2)100, Size: 250×4.6mm, Bath sonicator Sonica®, Ultrasonic cleaner Spincotech PVT Ltd., HPLC grade Acetonitrile, Methanol, water, potassium dihydrogen ortho phosphate were purchased from SD Fine Chemicals, India. carvedilol was procured as a gift sample from Orchid Pharmaceuticals.

Preparation of the standard and sample drug solutions: To prepare standard stock solution 10mg of carvedilol was accurately weighed in to a 10ml volumetric flask and dissolved in methanol and volume was made up to 10ml with the same. From this subsequent dilutions ranging from 0.5 to 20µg/ml were made with phosphate buffer saline of pH-7.4 . The sample drug solution was prepared by taking not less than 20 tablets each containing 6.25mg of carvedilol. The tablets were weighed and powdered. The quantity of powder equivalent to 10mg of carvedilol was weighed, transferred to a 10ml volumetric flask and extracted with methanol. The solution was sonicated for 15min. The extracts were filtered through whatmann filter paper No: 41 and residue was washed with methanol. The extracts and washings were pooled and transferred to a 10ml volumetric flasks and volume was made up to 10ml with methanol.

Determination of λ-max: From the standard dilutions 10 µg/ml solution was scanned using UV/Visible spectrophotometer between 200 to 400nm. λ-max of

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242 nm was selected where drug showed maximum absorbance.

Chromatographic conditions

Preparation of mobile phase: Acetonitrile: phosphate buffer (pH-5.2±0.2) in the ratio of (69:31) mixture was selected as mobile phase. Both the solvents were filtered by using membrane filter pore size 0.45µ and degassed with bath sonicator (Sonica®, Ultrasonic cleaner).

Preparation of calibration curve: Working standard solutions of carvedilol were prepared in the concentration range of 0.5µg to 20 µg per ml. A 20µl of this solution was injected each time in to the column at a flow rate of 1ml/min. The detection of the carvedilol was monitored at 242 nm. Each of the dilution was injected for 3 times into the column and corresponding chromatograms were obtained. The retention time was found to be 3.57minutes. Graphs were plotted between the mean peak areas of the drug with respect to concentration.

Validation of the method: The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day precision, and robustness.

Assay of the marketed products: 10µg/ml of the filtered sample solution was analyzed by the developed method .The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded.

3.RESULTS AND DISCUSSIONS

The mobile phase consisting of acetonitrile and Phosphate buffer of pH 5.2 in the ratio of 69:31 retained a good symmetric peak at 3.57 minutes. A typical chromatogram was shown in figure 1. The calibration graph was plotted with concentration vs peak area and the standard graph was shown in figure 2. The linear regression data (n=3) showed a good linear relationship over a concentration range of 0.5-20 µg/ml. The correlation coefficient was found to be 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) was determined by using the formula 3.3σ/S and 10σ/S respectively. Where σ is the standard deviation of the response and S is the slope of the calibration curve. The results were given in the table 1. The intra-day precision was determined by analyzing standard solutions in the concentration range of 0.5 to 20 µg/ml for three times on the same day while inter-day precision was determined by analyzing corresponding standard daily for three days over a period of one weak, and percentage relative standard deviation (RSD) was calculated. The RSD was found to be less than 2 for both iner-day and intra-day precision. To confirm specificity of the proposed method the sample was injected in to the HPLC column. It was observed that excipients present in the formulation did not interfere with the drug peak. All the validation parameters were given in table 1.

Recovery studies of the drug were carried out for the accuracy parameter. These studies were carried out at three levels i.e., multiple level recovery studies to the sample solution 50%, 100% and 150% of the standard drug solutions were added, dilutions were made and analyzed by the method. The percentage recovery and the percentage RSD were calculated and found to be within the limits. The assay value of the marketed formulation was found to be within the limits. The low RSD value indicated suitability of this method for routine analysis of carvedilol in pharmaceutical dosage forms. The results were shown in table 2 and table 3. Robustness of the method was studied by making small deliberate variations in method parameters. Sample dilutions were made in phosphate buffer of pH 7.4 containing 1% tween-80. Results were as shown in table 4.

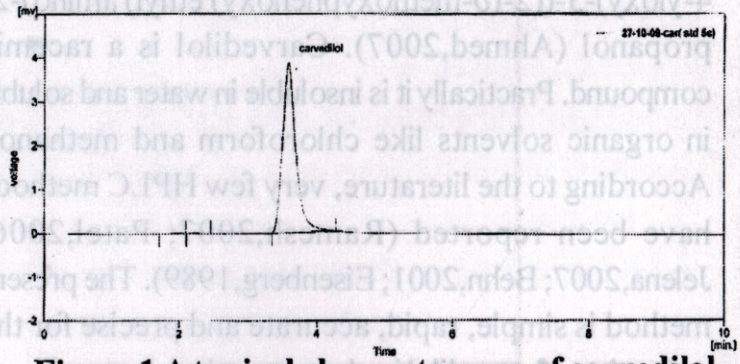

Figure 1 A typical chromatogram of carvedilol

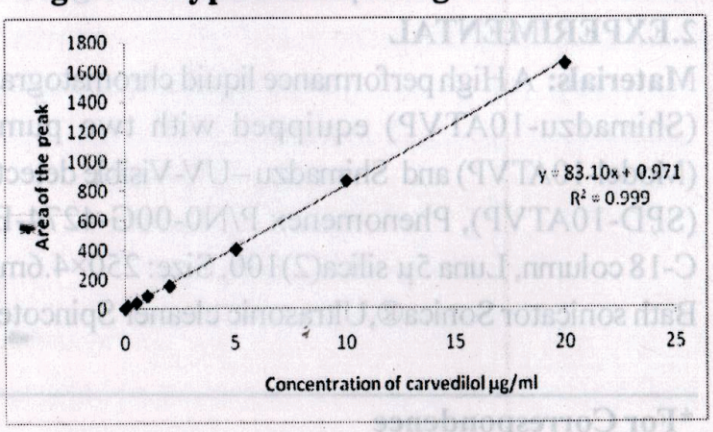

Figure 2 Standard graph of carvedilol in PBS pH 7.4.

Table 1: Validation parameters

parameter	value
Retention time	3.57 min
Linearity	0.5 – 20 µg/ml
Correlation coefficient	0.9997
LOD	0.052
LOQ	0.158
Precision intra-day	0.76
Precision inter- day	1.2
No of theoretical plates	2126

Table 2: Recovery studies of carvedilol

Drug	%Level	n	Conc. µg/ml	Amt. recovered µg/ml	%recovery	%RSD
Carvedilol	50	3	5	5.05	101	1.38
	100	3	10	9.95	99.5	0.86
	150	3	15	15.20	101.3	1.02

Table 3: Assay of the marketed formulation

drug	n	Label claim Mg/tablet	Amt found Mg/tablet	Mean %recovery	%RSD
carvedilol	3	6.25	6.29	100.64	0.75

Table 4: Robustness of the method

solvent	Retention time	Tailing factor	Theoretical plates
Buffer	3.57	1.3	2126
Tween-buffer	3.3	1.2	2266
methanol	3.2	1.2	2116

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