AMERICAN JOURNAL OF PHARMTECH RESEARCH

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RP-HPLC Method for Estimation of Carvedilol in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, rapid and specific RP-HPLC method has been developed and validated for determination of Carvedilol in bulk and tablet formulations. Chromatographic separation was performed by Phenomenex Luna C-18 (250 x 4.6mm, 5µm particle size) column with a mobile phase consisting of a mixture of phosphate buffer, acetonitrile and methanol in the ratio (30:45:25 v/v/v), pH adjusted to 4.8 with ortophosphoric acid. The mobile phase was was filtered through a 0.45µ cellulose nitrate filter, sonicated for 15 min and delivered at a flow rate of 1ml/min. Detection was performed at a wave length of 241 nm at ambient temperature. Linearity was obtained in a concentration range of 30 to130 µg/ml with a correlation coefficient (r^2) of 0.999. The limit of detection and limit of quantification were 1.08 and 3.24 µg/ml, respectively. No interference of excipients in determining tablet formulation; identical results were obtained like that of the standard sample. The proposed RP-HPLC method is simple, accurate, precise, rapid and economical to be employed for routine analysis of carvedilol in pharmaceutical dosage forms.

Keywords: Carvedilol, RP-HPLC, Validation, Tablet formulation

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Received 14 March 2012, Accepted 28 March 2012

Please cite this article in press as: Ketema G *et al.*, RP-HPLC Methods For Estimation of Carvedilol in Pharmaceutical dosage forms. American Journal of PharmTech Research 2012.

INTRODUCTION:

Carvedilol is a non- selective lipophilic β1- and β2- adrenoreceptor antagonist with antioxidant and antiproliferative effects. It has vasodilating properties that are attributed mainly to its blocking activity at α1- receptors.[1-3] Chemically carvidol is described as [3-(9H-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy)ethyl]amine(Figure1), with a molecular formula of $C_{24}H_{26}N_2O_4$ [4].

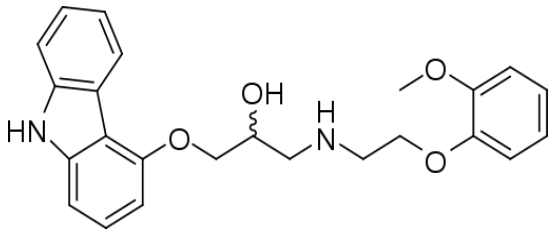


Figure -1: The chemical Structure of Carvedilol

Literature survey reveals that there is no RP-HPLC methods reported for quantification of carvedilol in pharmaceutical formulation. However, a few methods have been used for quantification of carvedilol in biological fluids [5-9]. High-performance liquid chromatography (HPLC) with fluorescence [5] and mass spectrometer [6] detectors, RP-HPLC and HPTLC with UV detector [7-8] and gas chromatography (GC)-MS detector [9] have been reported. Therefore, the present study aims to develop and validate a simple, fast and economical RP-HPLC method for estimation of carvedilol in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with LC-20AT double pumps; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV/VIS detector, SPD-20A and the output signal was monitored and integrated by spinchrom software. Shimadzu UV-1800 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by UV prob-2.3 version software. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase. All the chemicals and drugs were weighed by using Shimadzu electronic analytical balance (A x 200) and pH of the mobile phase was adjusted by using ELICO LI120 digital pH meter.

Reagents and Chemicals

The drug samples, Carvedilol working standard was obtained as gift sample by Orchid Health Care Pvt. Ltd, Chennai. The pharmaceutical formulation was procured from local market.

Acetonitrile and Methanol (HPLC Grade), Triethylamine and orthophosphoric acid (AR Grade) used were purchased from Merck Specialities Private Limited, Mumbai, India. Millipore's distilled water was used to carry out the analysis.

Preparation of standard stock solution:

Standard stock solution of carvedilol pure drug (1mg/ml) was prepared by accurately weighing about 25 mg of each drug in 25 ml volumetric flask. The drugs were dissolved with methanol and volume made up to the mark with same solvent. Appropriate volumes of these solutions were further diluted with mobile phase to get appropriate concentrations.

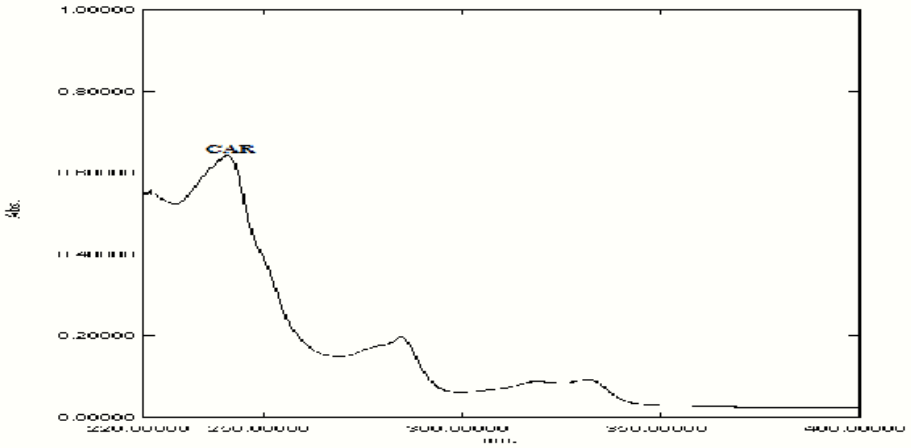
Preparation of sample solution

Carvedilol tablets of three different brands were purchased from local pharmacy. Ten tablets were weighed and average weighed calculated. The tablet powder equivalent to 25 mg of Carvedilol is transferred in to a 25 mL volumetric flask. 15 mL of methanol was added and sonicated for complete solubility; the volume was made up to the mark with methanol. Then the solution was filtered through 0.45µ membrane filter and further diluted to the appropriate concentration.

RESULT AND DISCUSSION

Estimation of absorption maximum by UV

The absorption spectrum of carvedilol obtained by scanning the sample in the UV region (220-400 nm) in spectrum mode showed that the drug has maximum absorbance at 241 nm (Figure2). Analysis was carried out by adjusting the UV detector of the HPLC system at 241nm.



Figur-2: Absorption spectrum of Carvedilol

Development of the method

Phenomenex Luna C-18 (250 x 4.6mm, 5µm particle size) was the column used for separation. To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory

peak symmetry was obtained with mobile phase consisting of 50 mM KH_2PO_4: acetonitrile: methanol (30:45:25 v/v/v). The mobile phase was filtered through a 0.45µ nylon filter, sonicated for 15 min and delivered at a flow rate of 1.0 ml/min. Quantification was achieved with UV detection at 241 nm based on peak area at ambient temperature. A representative chromatogram is shown in (Figure 3).

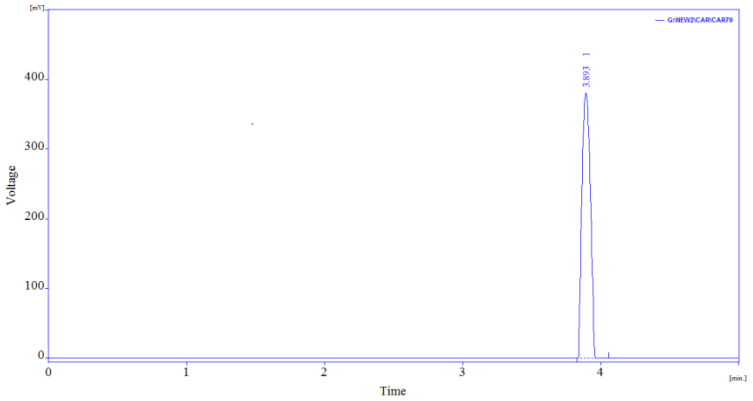


Figure-3: A typical HPLC chromatogram of carvedilol

Validation of the proposed method

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for system suitability, linearity, precision, accuracy, specificity, limit of detection, limit of quantification, and robustness [10].

System suitability parameter

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 1.

Table -1: System suitability test parameters of the proposed method

Sr.No.	Parameters	Results(n=3)	
		Mean±SD	%RSD
1	Retention time	3.88 ± 0.015	0.386
2	Peak area	3050 ± 6.889	0.226
3	Theoretical plate	29804 ±74	0.248
4	Tailing factor	1.243± 0.0203	1.633

All these parameters were evaluated based on regulatory requirements. The results obtained meets the system suitability requirements and indicated that the system is suitable for analysis

Linearity

Calibration curve was constructed by plotting average peak areas against concentration and a regression equation was computed. Serially diluted calibration solutions containing 30, 50, 70,

90, 110,130 µg/ml of carvedilol were injected (n=3) separately in to the optimized chromatography system and chromatogram were recorded. Linear data and calibration graph were given in Table-2 and Figure-3.

Table-2: Linearity data of carvedilol

Conc.(µg/ml)	n=3			Mean Peak area	SD	%RSD
30	1623	1656	1640	1639.66	16.50	1.006
50	2580	2539	2595	2571.33	28.98	1.127
70	3519	3520	3490	3509.66	17.04	0.485
90	4460	4563	4453	4492.00	61.59	1.371
110	5400	5411	5419	5410.00	9.54	0.176
130	6353	6340	6351	6348.00	7.00	0.110

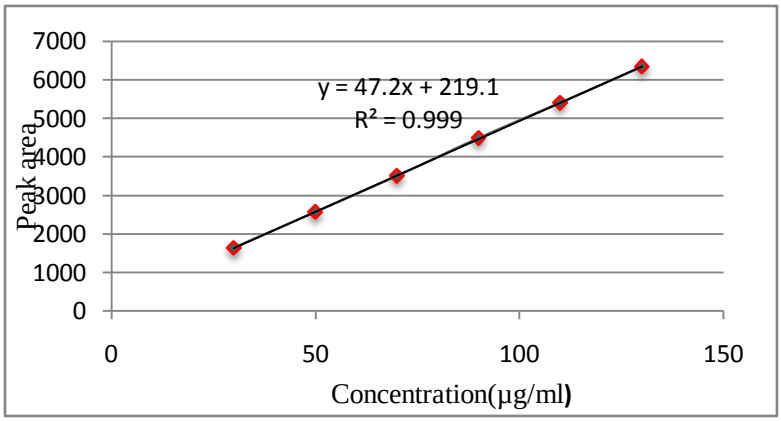


Figure -4: Linearity graph of carvedilol

According to the guide lines, the correlation coefficient (R^2=0.999) meets the acceptance criteria, which indicates that the peak responses are linear. This concludes that the method is linear throughout the range selected (from 30 to130 µg/ml). Figure-5 shows the overlain chromatograph for selected range of concentrations.

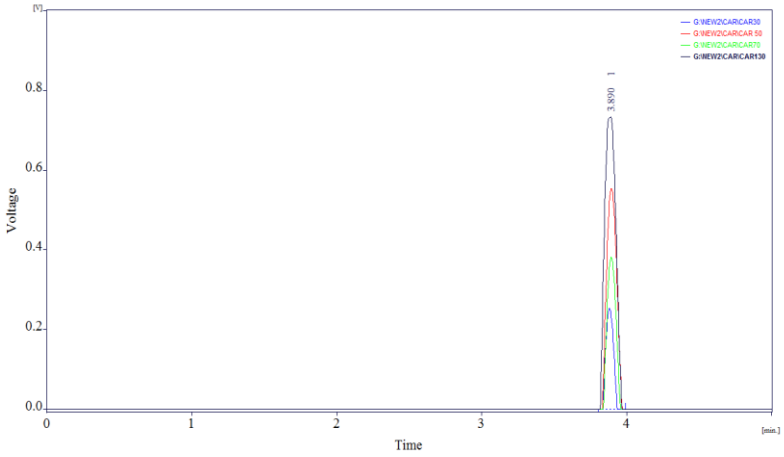


Figure-5: The overlain chromatograph of Carvedilol

Precision

Precision of the method was evaluated by determining repeatability, intraday precision and interday precision and expressed in terms of % relative standard deviation (%RSD). In Repeatability study, dilutions at three levels (50, 70, and 90 µg/ml) was analyzed in triplicate and % RSD was found to be 0.45-0.56. Intraday variation ranges from 0.48 -1.34 % where as interday precision was found to be 0.63-1.55%. Results for repeatability, intraday and interday precision are shown in Table -3. As percentage relative standard deviation for each injection was less than 2%, the method is precise.

Table- 3: Results of precision study

Concentration(µG/ML)	Intraday %RSD (N=5)	Interday %RSD(N=5)
30	0.56	1.45
50	1.23	0.87
70	0.91	0.63
90	0.48	1.02
110	1.34	0 .69
130	1.20	1.55

Accuracy

The accuracy of the test method was done by preparing recovery sample of carvedilol (30, 60, and 90 µg/ml). It was determined at three different level 50%, 100% and 150% of the target concentration in triplicate and the % recovery was calculated. The results were presented in Table -4:

Table- 4: Results of the study of Accuracy

Level	Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery	Mean ± SD	%RSD
50%	30.4	31.1	102.30	101.65±0.653	0.642
	30.1	30.4	100.99		
	30.1	30.6	101.66		
100%	60.3	61.2	101.49	100.55±0.912	0.908
	60.5	60.3	99.67		
	60.2	60.5	100.50		
150%	90.4	91.4	101.11	100.44±0.616	0.613
	90.3	90.6	100.33		
	90.2	90.1	99.89		

The result shows that the %RSD is not more than 2% and the recovery lies between 100.44±0.616 and 101.65±0.653 for each level, this confirms that the result is within the acceptable limit and hence the method is accurate.

Limit of detection (LOD) and Limit of quantification (LOQ)

As per ICH guideline [10], both LOD and LOQ were determined by calculate the standard deviation of the response (peak area) of the standards and divide by the average of the slopes. Multiply by 3.3 to get LOD and 10 for LOQ. Accordingly, the LOD was found to be 1.08 µg/ mL and LOQ was found to be 3.24 µg/ mL.

Specificity

The specificity of the method was determined by comparing test results obtained from analysis of sample solution containing excipients with that of test results those obtained from standard drug. The peak purity for the standard and formulation samples was found to be similar; which means that there is no interference from mobile phase, diluents and excipients. From the result it can be concluded that the method is specific for the analysis of carvedilol in its tablet dosage form. Figure-6 shows the chromatograph of formulation sample

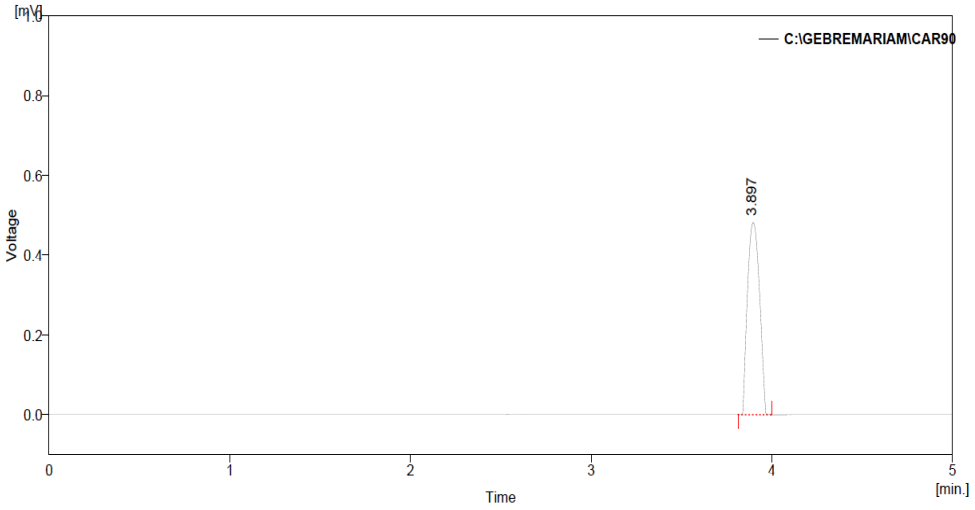


Figure-6: Atypical chromatograph of formulation sample

Robustness

According to ICH guide line, the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [11]. It was checked by making slight deliberate changes in chromatographic conditions (mobile phase ratio, pH of buffer, flow rate) and the results obtained are summarized in Table-5.

Since there are no marked changes in the system suitability parameters (%RSD in all cases is below 2%), the method was capable to remain unaffected by small variation of the above tested variables. which demonstrated that the developed RP-HPLC method is robust.

Table- 5: Robustness results of the method (n=3)

Parameter	Retention time	Peak area	Theoretical plate	Tailing factor
Actual conditions (1mL/min, Buffer:ACN: MeOH=30:45:25 v/v/v, pH 4.8)	3.883	3050	29804	1.243
Flow rate:0.9 ml/min	3.998	3065	29812	1.245
Flow rate:1.1 ml/min	3.775	3043	29943	1.22
Mobile phase (30:43:27v/v/v)	3.897	3067	29823	1.243
Mobile phase(30:47:23 v/v/v)	3.863	3103	29782	1.233
Mobile phase pH:4.6	3.889	2980	29798	1.241
Mobile phase pH:5.0	3.885	3127	29854	1.254
Mean	3.884	3062.143	29830.86	1.240
SD	0.065	46.849	54.340	0.011
%RSD	1.679	1.530	0.180	0.860

Solution stability

The stability of both the standard and sample solutions which kept for 24 hours, at room temperature, and without protecting from light was determined against freshly prepared standard solution. The percentage change in the assay results obtained after 24 hours for both the standard and sample solutions was not more than 2%. This shows that both standard and sample solution were stable up to 24 in the above tested conditions.

CONCLUSION

From the result obtained it can be concluded that the method is fast, precise, accurate and specific to successfully determined carvedilol in tablet formulation and the method can be employed for routine quality control of the drug.

ACKNOWLEDGMENT

The author wants to acknowledge Orchid Health Care Pvt. Ltd, Chennai for providing gift sample of Carvedilol working standard. The author is also thankful to Pharmacognosy department, College of pharmacy, Andhra University for providing the necessary analytical instruments and facilities for the research work.

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