Indian Journal of Biotechnology and Pharmaceutical Research
Journal homepage : www.ijbpr.in
ISSN: 2347-3266
2021, 9(1): 01 – 09

Research Article

Analytical Method Development and Validation of Assay for Carvedilol Tablets by RP-HPLC

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Carvedilol in Bulk and Pharmaceutical tablet Formulation. The present study involves the development and validation of a simple, rapid and economical, stability-indicating RP-HPLC method for the assay of CARVEDILOL in pure and tablet dosage forms. A mobile phase of acetonitrile and water in a ratio of 60:40, v/v, has been used for the assay of CARVEDILOL at pH 2.5 giving the retention time (tR) of around 3 min. The method has been validated according to the guidelines of International Council for Harmonization (ICH) for parameters such as linearity, range, accuracy, precision, sensitivity, robustness and specificity. The results indicate that the method is linear in the range of 25-150% (6.25-37.50 µg/ml), highly accurate (100.1%), precise (<1%), robust (<1.5%) and statistically comparable to the official USP methods. Conclusion: The stress degradation studies yielded a number of degradation products with varying tR values, all different from the principle peak of CARVEDILOL thus indicating that the method is highly specific and reliable for the assay of CARVEDILOL.

KEY WORDS: Carvedilol tablets, USP method, validation, RP-HPLC, UV detection, Recovery, Precise

Introduction

Carvedilol is a third-generation nonselective chemical drug for controlling different problems of a heart having the chemical formula (±)-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy) ethyl] amine (Fig-1). It has been identified as the most effective and non-selective α1 and β-adrenoreceptor (β1, β2) antagonist in the treatment of systolic heart failure, possesses both reactive oxygen species (ROS) scavenging and ROS suppressive effects.

Received: 04.02.2021, Revised: 13.02.2021, Accepted: 22.02.2021

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It showed protective effects against daunorubicin-(DNR-) induced cardiac toxicity by reducing oxidative stress and apoptosis. Its antioxidant effects are attributed to its carbazole moiety. Carvedilol is taken twice daily because it is extensively metabolized and therefore loses its effectiveness due to a short half-life.

Norepinephrine stimulates the nerves that control the muscles of the heart by binding to the α1, β1- and β2-adrenergic receptors. Carvedilol blocks the binding to those receptors, which slows the heart rhythm and reduces the force of the heart's pumping. This lowers blood pressure and reduces heart failure. Relative to other beta-blockers, Carvedilol has minimal inverse agonist activity. Therefore, its concentration plays a decisive role in controlling heart failure. Variation in its

concentration should be measured for proper prescription of this medicine both in patients' blood and drug formulations. It is a non-selective a1-blocker that has vasodilating and antioxidant properties. CARVEDILOL is used in the management and treatment of hypertension, mild to moderate Congestive Heart Failure (CHF), angina pectoris, and myocardial infarction [1-5]. It appears as a white crystalline powder that is insoluble in water. A number of workers have employed various techniques for the analysis of CARVEDILOL which include chromatographic methods such as gas chromatography [6, 7], High-Pressure Liquid Chromatography (HPLC) [8], spectrometric methods such as UV-visible spectrometry [16], spectrofluorimetry NMR spectrometry, Raman spectrometry, capillary electrophoresis phosphorimetry, and potentiometric titration. Potentiometric titration lacks sensitivity and selectivity compared to those of the chromatographic methods. However, the HPLC methods reported in USP are time-consuming and require stringent procedures of sample preparation. One of those preparations requires the addition of buffer in the mobile phase at pH 2.0. Buffers are known to affect the efficiency of columns and pumps of the HPLC system and a highly acidic pH of ≤ 2 can affect the performance of conventional silica-based columns. Moreover, for the assay of CARVEDILOL according to USP methods, the column must be set at higher temperatures (e.g. 40 or 55°C), which is an additional requirement. Although the literature gives information regarding the assay of CARVEDILOL through HPLC methods [8] there is no single method reported to the best of our knowledge that is at the same time, along with good accuracy and precision, is simple, economical method for the assay of both pure and tablet dosage forms of CARVEDILOL. The present work aims to develop a single, simple, rapid, economical, accurate, precise HPLC method for the determination of CARVEDILOL, which could be used for both pure form and tablet dosage forms with a non-astringent procedure of sample preparation. The developed method has been validated as per International Council for Harmonization guideline with respect to linearity, accuracy, precision, robustness, specificity, and system suitability. The proposed method has also been compared with the official USP methods in order to evaluate its working efficiency.

Materials and Methods

CARVEDILOL was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals used in this study were of analytical grade having the highest degree of purity. These include acetonitrile (99.9%, Sigma-Aldrich, USA), sodium hydroxide (98.0%, Sigma-Aldrich, USA), hydrogen peroxide solution (34.5-36.5%, Sigma-Aldrich, USA), hydrochloric acid (37%, Riedel-de Haen, Germany), orthophosphoirc acid (85.0- 88.0%, Sigma-Aldrich, USA), triethylamine (99.5%, LabScan Analytical Sciences, Poland), methanol (99.9%, Sigma-Aldrich, USA), monobasic potassium phosphate (99.5- 100.5%, Merck, Germany), and sodium dodecyl sulfate (95.0%, Merck, Germany).

Assay of CARVEDILOL

The assay was performed by weighing 25 mg of CARVEDILOL and transferring it into a 100 ml volumetric flask. The stock solution was prepared by adding acetonitrile and water in a ratio of 60:40, v/v. The pH of the solution was set at 2.5 using orthophosphoric acid. The solution was thoroughly shaken and sonicated for 5 min. A 1:10 dilution (25 µg/ml) was made in the same solvent system and subjected to chromatographic analysis. Fresh solutions were prepared each time and all assays were performed in triplicate.

High Performance Liquid Chromatography (HPLC)

The assay of CARVEDILOL was carried out on a Shimadzu LC 20 AT HPLC (Japan) with prominence diode array detector (Model SPD-M20A). The sample (10 µl) was injected by prominence autosampler (Model SIL-20ACHT) and prominence degassing unit (Model DGU-20A5R) was used for removing bubbles from the solvent system. The whole assay was carried out at room temperature (25±1°C). A C18 column of 5 µ, 100Å, 4.6×250 mm (SiliaChrom® XDB1 C18, Can- ada) and mobile phase of acetonitrile-water in 60:40 v/v ratio was used for the analysis of CARVEDILOL

with a detection wavelength of 240 nm and flow rate of 0.7 ml/min.

Validation of the Analytical Method

The HPLC method for the determination of CARVEDILOL was validated according to the guideline of ICH [46]. The validation parameter includes system suitability, linearity, accuracy, precision, limit of detection and quantification, specificity, robustness and solution stability which are described as follows:

System Suitability

Prior to the analysis, the system suitability was carried out by injecting six replicate solutions of CARVEDILOL at a concentration of 25.0 µg/ml. Parameters such as retention time (tR), peak area reproducibility, theoretical plates (N) and peak symmetry were determined.

Linearity

The linearity of the method was determined by preparing a calibration curve of peak area versus the concentration of CARVEDILOL. Solutions in the concentration range of 25-250% (6.25-62.50 µg/ml) were used to prepare the calibration curve. The linearity was statistically evaluated for the determination of correlation coefficient, slope of the regression line, y- intercept and the residual sum of squares.

Range

The range for the analysis of CARVEDILOL was selected from the data obtained for linearity. The concentrations showing a linear pattern over the studied concentration range were included in the assay range of the test method.

Accuracy

The accuracy of the method was evaluated by preparing solutions of known concentrations of 50% (12.5 µg/ml), 100% (25.0 µg/ml), and 150% (37.5 µg/ml) of CARVEDILOL from the stock solution (25 mg/100 ml). Each solution was prepared in triplicate and subjected to HPLC analysis for the determination of the accuracy of the proposed method. Similarly, the accuracy was further confirmed with the recovery results of the official USP method [30].

Precision

The precision of the method was determined by performing repeatability and intermediate precision. Repeatability (intra-day precision) was performed by preparing six individual samples, each at a concentration of 100% (25.0 µg/ml). Intermediate precision (inter-day precision) was per- formed similarly but by different analyst on different days. The precision of the test method was determined by calculating the % relative standard deviation (%RSD).

Sensitivity

The sensitivity of the method was calculated by deter- mining the Limit Of Detection (LOD) and the Limit Of Quantitation (LOQ). Both LOD and LOQ were calculated from the standard deviation of the response and slope using the following formulas:

$$LOD = 3.3 \times \sigma / S \quad LOQ = 10 \times \sigma / S$$

Where σ is the standard deviation and S is the slope of the standard curve.

Robustness

Robustness was evaluated by making deliberately small changes in the parameters of the assay method. The accuracy and precision of the method was determined with respect to the changes in flow rate (±0.2 ml/min), wavelength (±2 nm) and pH (±0.1 unit).

Solution Stability

The stability of the stock solution was determined at room (25±1°C) and refrigerator temperature (2-8°C) up to 48 h. The solutions were stored in tightly sealed containers, protected from light and subjected to analysis after making appropriate dilutions.

Application of the Test Method to Tablet Dosage Form of CARVEDILOL

The test method was also applied to three different commercial brands of CARVEDILOL and the

recovery was calculated for 50, 100 and 150% concentrations. A total of 20 tablets of each brand were weighed and powdered in a mortar with pestle. An amount equivalent to 25 mg was weighed accurately and diluted in the similar manner as described previously. The analysis for each concentration was performed in triplicate.

Results and Discussion

Confirmation of Purity of CARVEDILOL

It is necessary to confirm the purity of the compound be- fore the validation of an analytical method in order to make sure that no degradation products and unwanted impurities are present prior to analysis. The purity confirmation has been carried out by FTIR spectrometry and the spectrum obtained was compared with the USP reference standard (Fig. 1). On comparison of the sample with the reference standard, it has been observed that there is no difference in the spectra between the two indicating that there are no un- wanted substances present in the sample.

Validation of the Analytical Method

The validation of HPLC method was carried out as per the guideline of ICH [46]. The details of the studied parameters are discussed as follows.

System Suitability

System suitability is an important part of the validation of an analytical method. The parameters measured for the suit- ability of the system are reported in Table 1. According to Swartz and Krull [47] if the %RSD of a method is less than 2% and theoretical plates (N) are greater than 2000 with a tailing factor of less than or equal to 2, the method could be considered as suitable for the analysis of a substance.

Linearity and Range

The method has been found linear for the assay of CARVEDILOL in the concentration range of 25-150% (6.25-37.50 µg/ml) (Fig. 2). The response has been noted to become slightly non- linear for concentrations of more than 150%. The statistical interpretation of the data (Table 2) indicates negligible scattering of the points around the calibration curve as evident from the R2 value of 0.99997. Moreover, the y-intercept value is close to zero which indicates good peak purity of the sample. A typical chromatogram of CARVEDILOL is shown in Fig. (3).

Accuracy

The accuracy of the method shows agreement between the added and found values for the series of measurements.

taken for CARVEDILOL. The results for the accuracy of CARVEDILOL assay through the proposed method are reported in Tables 3 and 4. Standard deviation and relative accuracy error values are small that indicate good accuracy of the current method for CARVEDILOL analysis.

Precision

The precision of analytical method validation is defined as the intimacy or degree of scatter between the measurements obtained from the various sampling of the homogenous sample under recommended conditions. The results for the precision of the test method for the assay of CARVEDILOL are re- ported in Tables 3 and 4. The results showed that the %RSD is less than 1% which indicates that the test method is highly reliable for the determination of CARVEDILOL

Sensitivity

Limit of Detection (LOD)

LOD is defined as an analytical procedure which tends to measure the lowest quantity of sample which could be detected but not necessarily quantified. The LOD of the test method has been found to be 0.53 µg/ml (Table 2), which indicates that the proposed method is highly sensitive for the detection of CARVEDILOL.

Limit of Quantification (LOQ)

LOQ is defined as the quantitative determination of the lowest possible concentration of an analyte with suitable precision and accuracy. The LOQ of the test method has been found to be 1.59 µg/ml (Table 2), which indicates that the proposed method is

sensitive enough for the quantitative determination of CARVEDILOL.

Robustness

Robustness is the capability of any analytical procedure to remain unaffected by any small and deliberate changes in the test method. It basically indicates the suit- ability and reliability of the method during the normal use. The accuracy and precision of the test method after making deliberate changes in flow rate, wavelength and pH are reported in Table 5. The results indicated that the studied changes do not affect the accuracy of the method which has been found in the range of 99.3-100.2% with a precision of ≤1.5% in each case. Additionally, Student's t-test has also been applied to statistically compare the difference between % recoveries of the original and altered parameters (Table 5). The results showed non-significant differences between the two means as calculated values for all parameters are found to be less than tabulated thus further confirming the reliability and robustness of the method during normal usage.

Table 1. System suitability parameters for Carvedilol

Parameter	Response
t_R (min)	2.99
RSD (%)	0.06
N	7794
Tailing factor	1.15

Table 2. Analytical parameters for the determination of Carvedilol

Parameter	Value
λmax	240 nm
Linearity	
Range	6.25-37.50 µg/ml
Correlation coefficient	0.99997
Slope	88391
SE of slope	6167
Intercept	13332
SE of intercept	5741
SD of intercept	14063
Recovery range (%)	99.1-100.5
Accuracy (%) ± SD	99.9±0.5
Precision (%RSD)	0.5
LOD (µg/ml)	0.5
LOQ (µg/ml)	1.6

Table 3. Accuracy and precision of the proposed method at three different concentrations of Carvedilol

Concentration (%)	Amount Added (mg)[a]	Amount Found (mg)	Recovery (%)	Mean % Recovery ± SD (%RSD)	Relative Accuracy Error (%)[b]
50	25.3	25. 1	99.2	99.3±0.2 (0.2)	-0.1
	24.9	24.8	99.6		+0.3
	25.3	25.1	99.2		-0.1
100	25.2	25.2	100.0	100.9±1.0 (1.0)	-0.90
	25.3	25.5	100.8		-0.1
	25.3	25.8	102.0		+1.1
150	24.9	24.9	100.0	100.0±0.4 (0.4)	0.0
	25.1	25.2	100.4		+0.4
	25.4	25.3	99.6		-0.4
			Mean =	100.1±0.4 (0.4)	

Table 4. Robustness of the proposed method at 100% concentration (25.0 µg/ml) of Carvedilol

Robustness Parameter	Accuracy (%)[a] ± SD	Precision (%RSD)	Student *t* Test[b]
Change in flow rate (0.7±0.2 ml/min)			
0.5 ml/min	100.2±1.5	1.5	0.712
0.9 ml/min	100.0 ± 1.4	1.4	0.906
Change in wavelength (240±2 nm)			
238 nm	100.1 ±1.4	1.4	0.825
242 nm	100.0 ±1.4	1.4	0.921
Change in pH (2.5±0.1 unit)			
2.4	100.1 ± 0.1	0.1	1.422
2.6	99.3 ± 0.9	0.9	2.172

Table 5. Stability of solutions of Carvedilol

TIME(HOURS)	PEAK AREA	CUMULATIVE % RSD
Initial	5121901	
4	5131913	1.5
8	5111908	1.1
12	5111901	1.7
16	5121900	0.9
20	5121892	1.6
24	5111908	1.5

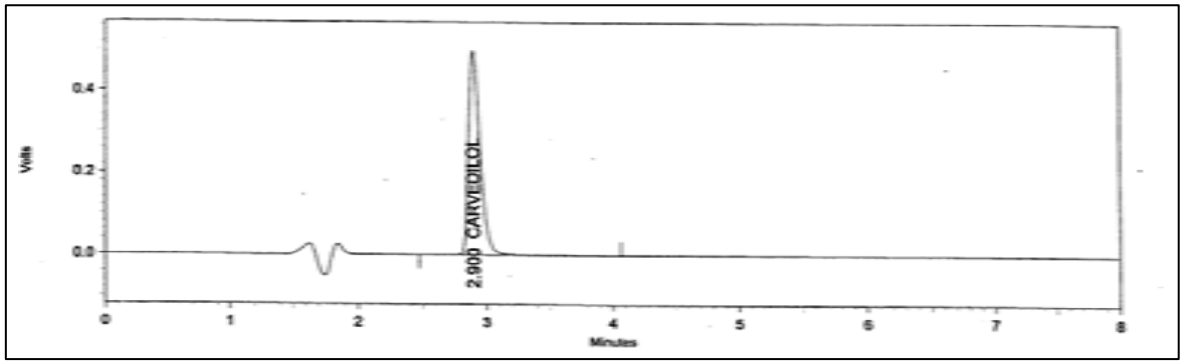

Figure 1: Chromatogram of Carvedilol

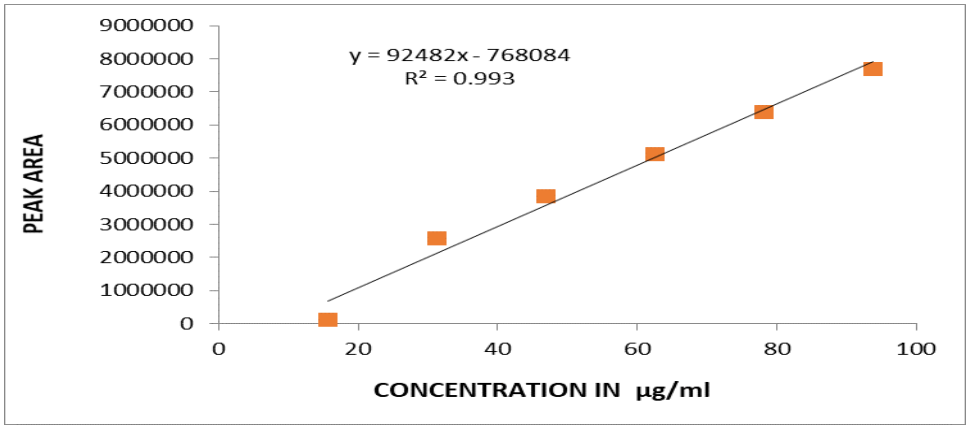

Figure 2: Calibration curve of Carvedilol

Table 7. Summary of Carvedilol

S. No.	TEST	ACCEPTANCE CRITERIA	RESULT
1	System suitability	a. %RSD for std injection is NMT 2.0%	0.1
		b. Tailing factor for carvedilol peak is NMT 2.5	1.5
2	Specificity	a. All individual peaks should be well separated. b. The interference of carvedilol peak (main peak)from the other peaks should be Nil. c. The carvedilol purity is NLT 0.99	Complies
3	Accuracy	a. The % recovery is in between 95% and105% b. The %RSD for all recovery values should be NMT 2%.	98.4% and 102.4% 0.1%
4	Linearity	The correlation co efficient should be NLT 0.99	0.99
5	Precision	a. %Content of carvedilol should be in between 90.0 to 110.0 (Analyst 1).	98.7%
		b. %Content of carvedilol should be in between 90.0 to 110.0(Analyst 2)	99.6%
		c. %RSD not more than 2.0%(Analyst 1)	0.77%
		d. %RSD not more than 2.0% (Analyst 2)	1.31%
		e. The %RSD between the average results of two analysts should be not more than 2.0%	1.04%
6	Robustness	a. % Content of carvedilol should be in between 90.0 to 110.0 b. %RSD not more than 2.0%	100.2% 0.52%
7	Solution stability	a.% RSD not more than 2.0%	1.5% for standard

Conclusions

A simple, rapid and economical RP-HPLC method for the determination of CARVEDILOL has been developed. The proposed method has been validated as per the guideline of ICH and was found to be highly accurate, precise and robust for the assay of CARVEDILOL in both pure and tablet dosage forms. The forced degradation studies showed that the degradation products do not interfere with the peak of CARVEDILOL. Thus, indicating that the method is a stability-indicating method and can readily determine CARVEDILOL in the presence of

other compounds such as formulation excipients and degradation products. The test method has been compared with the official USP methods for pure and tablet dosage forms. The developed method was found to be statistically equivalent to the official methods for the determination of CARVEDILOL.

The developed method is a simple and cost-effective method which includes a trouble-free preparation of mobilphase with rapid detection of the active drug. No buffers or surfactants have been used in the mobile phase that are known to shorten the life of HPLC columns. The proposed single method can be applied with high accuracy and precision to the assay of CARVEDILOL in both pure and pharmaceutical dosage forms as compared to the official USP methods, which are different for the pure drug and dosage forms of CARVEDILOL and are time consuming. It is suggested that the determination of CARVEDILOL should also be carried out in binary mixtures using the test method. This method should also be applied for the kinetic studies of CARVEDILOL

Acknowledgements

Authors thank to the management of Shri Ram Group of Pharmacy, Jabalpur for providing necessary facilities to carry out this work.

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How to Cite This Article:

Gupta S, Patel A and Diwedi N. Analytical method development and validation of assay for Carvedilol tablets by RP-HPLC. Indian J. Biotech. Pharm. Res. 2021; 9(1): 01 – 09.