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Research Article

UV SPECTROPHOTOMETRIC DETERMINATION OF CARVEDILOL IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

In this study, new and rapid method indicating ultraviolet spectroscopic methods were developed and validated for the estimation of carvedilol in pure form and in their respective formulations. The adequate drug solubility and maximum assay sensitivity was found in methanol. The absorbance of carvedilol was measured at 241nm in the wavelength range of 200 - 350 nm. The linear calibration range was found to be 50% - 150%. This method was validated and applied to the determination of carvedilol in tablets. No interference was found from tablet excipients at the selected wavelength and analysis conditions. It was concluded that the developed methods are accurate, sensitive, precise, and reproducible. They can be applied directly for the estimation of drug content in pharmaceutical formulations.

Keywords: Carvedilol, UV Spectrophotometric Determination.

INTRODUCTION

Number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias.

This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Analytical methods play a vital role in new drug development preformulation and formulation studies, stability studies, quality control testing and in quality assurance programmes. Analytical testing of a pharmaceutical product is necessary to ensure its stability, safety and efficacy. Such testing needs an analytical method with reliable result adequate for intended purpose.

Carvedilol is in a group of drugs called beta-blockers. Beta-blockers affect the heart and blood circulation. Carvedilol is a racemic lipophilic aryoxypropanolamine that blocks α1- and ß-adrenergic receptors. Carvedilol significantly decreases systemic blood pressure, pulmonary artery pressure, and pulmonary capillary wedge pressure because of the vasodilatation that occurs with blocking of 1-receptors. Blocking of ß-receptors reduces the heart rate and increases diastolic filling time.

The combined effects of blocking both α- and ß-adrenergic receptors are decreases in preload, after load, and myocardial oxygen consumption. The primary cytochrome P-450 enzymes responsible for the metabolism of Carvedilol are microsomal CYP2D6 and CYP2C9. Inhibition of Carvedilol metabolism via the CYP2D6 isozyme causes greater 1-adrenergic blockade than dos metabolism via CYP2C9 and increases the risk of episodic hypotension. In contrast, inhibitors of CYP2C9 produce greater ß-adrenergic blockade, increasing the risk of more profound bradycardia or overt heart failure.

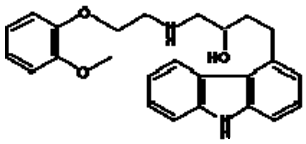

Fig. 1: Structure of Carvedilol

The aim of this study was to develop a fast, simple, reliable, selective, sensitive and inexpensive UV spectrophotometric method for the determination of Carvedilol in bulk drug and commercial pharmaceutical formulations as tablet. The proposed method was developed and validated according to the validation parameters. The developed method was applied to the determination of Carvedilol in pharmaceutical formulations without the necessity of sample pre-treatment. UV Spectrophotometric is selected because it is simplest and less time consuming. Compared to HPLC, as it takes more than an hour to make a single analysis and its calculation.

MATERIALS AND METHODS

Physico – Chemical Techniques

The spectrophotometric measurements were carried out using Perkin Elmer Lamada 25 odel UV – visible Spectrometer with UV detector and win Lab Software. IR spectrum of the Carvedilol in the region 4000 – 400cm^{-1} was recorded on a Perkin – Elmer 597 Spectrophotometer using KBr pellets.

Chemicals and Reagents

Carvedilol was kindly provided from Medopharm Pvt Ltd. The sample is tested as per the tests in the monograph and found to satisfy the limit. Methanol was purchased from Merck and was used as the solvent.

Standard Solutions

Standard stock solutions of Carvedilol were prepared in methanol. Working standard solutions were prepared by diluting stock solutions at the concentrations of 2mcg/ml – 6mcg/ml in methanol. Then the absorbance of these solutions was measured. In measurements methanol was used as a blank solution.

Sample Solution

Ten tablets of Carvedilol were accurately weighed and finely powdered and mixed. A portion of the powder equivalent to 20mg of Carvedilol was transferred into a 100 mL volumetric flask and 60 mL of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. 2ml of this solution was then diluted to 100ml volume with methanol.

Calculation of Assay Percentage

Carvedilol (in %)

$$\frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{W_{STD}}{100} \times \frac{2}{100} \times \frac{100}{W_{SPL.}} \times \frac{100}{2} \times \text{Label Claim} \times \text{A.Wt}$$

Where

W_{STD} = Weight of the Carvedilol working standard taken (mg).
W_{SPL}. = Weight of the sample taken (mg).
A.Wt = Average weight of Tablet (mg).

Solubility of carvedilol

Carvedilol is poorly soluble in acidic media. Thus,different solutions such as methanol ethanol and acetone were investigated and the UV spectrum of Carvedilol was measured. The UV spectra of Carvedilol standard in these solutions are given in Figure 2&3. In acetone solutions at different concentrations, well-defined peak was not observed for determination of Carvedilol. As results, well-defined peak was obtained in methanol and ethanol solutions. Therefore, the effect of methanol and ethanol in the solution was evaluated over the range 50-150 % for 72hrs. But, in these solutions the absorbance was not changed and almost identical maximum absorbance at wavelength of 241 nm. At the end of these studies, methanol was chosen for the working solution. The spectrum shows a well-defined peak at 241 nm in the measuring wavelength range 200 - 360nm.This wavelength was used for the determination of Carvedilol.

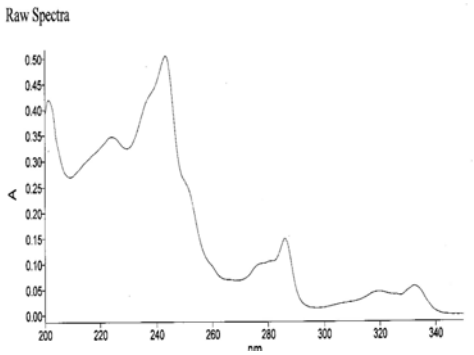

Fig. 2: Spectra of Carvedilol in ethanol

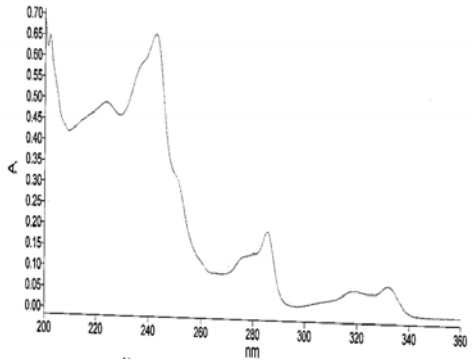

Fig. 3: Spectra of Carvedilol in ethanol

System Suitability

The system suitability of an analytical method is the degree of repeatability of the results in a series of experiments run during a single session operator with identical reagents and equipment.

Six standard solutions are prepared and the absorbance of resulting solutions is measured at the maximum of 241nm and the percentage RSD for the corrected absorbance is calculated. The result data shows that system suitability test is within the specified limit.

Specificity

The Specificity was the ability of an analytical procedure to measure accurately an analyte in presence of components that may be expected to present in sample matrix. Standard solution, sample solution, placebo solution, and standard solution spiked with placebo were prepared and the absorbance was found at 241nm.

Placebo solution

Placebo Powder equivalent to 20 mg of Carvedilol was weighed and transferred accurately (approximately 263mg) into 100ml volumetric flask, 60ml of methanol was added, Shaked for 20minutes and made up volume with methanol and is filtered. 2ml of the filtered solution was diluted to 100 ml made up volume with methanol.

Standard solution spiked with placebo

Placebo Powder equivalent to 20 mg of Carvedilol was accurately weighed transferred into 100ml volumetric flask along with 2ml of the standard stock solution, 60ml of methanol was added, Shaked for 20 minutes made up volume with methanol, and is filtered.

The result data shows that the diluent and placebo doesn't interfere in this assay method at the same wavelength as that of Carvedilol

Linearity

The linearity of an analytical method was its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line is calculated according to an established mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte.

The standard solutions are prepared over the range of 50 – 150 % and the absorbance of resulting solution was measured at the maximum of 241nm. A graph of concentration in ppm v/s absorbance was plotted, correlation coefficient, y-intercept and slope of regression line was recorded.

The result data shows that the correlation coefficient is 1.0000, hence the data shows that the specified method is linear.

Range

The range of an analytical method was the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with precision, accuracy and linearity using the method as written.

Test solution preparation

Six experiments are carried out for each level as follows:

Level I (50% i.e., 2mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 1ml of Standard Stock Solution was weighed and transferred in 100ml volumetric flask made up the volume with methanol and is filtered.

Level II (100% i.e., 4mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 2ml of Standard Stock Solution was weighed and transfered in 100ml volumetric flask made up the with methanol and is filtered.

Level III (150% i.e., 6mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 3ml of Standard Stock Solution was weighed and transfered in 100ml volumetric flask made up the with methanol and is filtered.

The absorbance of resulting solution and the standard solution was measured at the maximum at 241 nm. A graph of concentration in mcg/ml versus absorbance was plotted; correlation coefficient, y-intercept and slope of regression line are recorded.

The % recovery is calculated by using following formula

$$\text{Qty. recovered in ppm} = \frac{\text{Sample Abs x Standard concentration}}{\text{Standard Abs}}$$

$$\% \text{ Recovery} = \frac{\text{Quantity recovered in ppm}}{\text{Quantity added in ppm}} \times 100$$

The result data shows that the range is between 2mcg/ml – 6mcg/ml of Carvedilol with theoretical concentration. But the more accurate concentration to be used is 4mcg/ml which has the percentage recovery of 100.2%.

Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It was the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added.

Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added both above and below the normal levels expected in the samples. Accuracy was then calculated from the test results as the percentage of the analyte recovered by the assay. Dosage form assays commonly provide accuracy within 3-5% of the true value. (The specified limit is NMT 2% in % RSD).

Test solution preparation

Three experiments are carried out for each level as follows:

Level I (50% i.e., 2mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 1ml of Standard Stock Solution was weighed and transferred in 100ml volumetric flask made up volume with methanol and is filtered.

Level II (100% i.e., 4mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 2ml of Standard Stock Solution was weighed and transferred in 100ml volumetric flask made up with methanol and is filtered.

Level III (150% i.e., 6mcg/ml)

Placebo powder equivalent to 20mg of Carvedilol sample and 3ml of Standard Stock Solution was Weighed and transferred in 100ml volumetric flask made up with methanol and is filtered.

The absorbance of resulting solution and the standard solution was measured at the maximum at 241nm.

A graph of concentration in mcg/ml versus absorbance was plotted, correlation coefficient, y-intercept and slope of regression line are recorded.

The % recovery is calculated by using the following formula.

$$\text{Qty. recovered in ppm} = \frac{\text{Sample Abs x Standard concentration}}{\text{Standard Abs}}$$

$$\% \text{ Recovery} = \frac{\text{Quantity recovered in ppm}}{\text{Quantity added in ppm}} \times 100$$

The result data shows that the result is within the specified limit.

Precision

When the precision of an analytical method was the degree of agreement among individual test results method was applied repeatedly to multiple samplings of homogenous samples. This was usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision was a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances.

The standard and the sample solutions are prepared at the concentration of 4 mcg/ml. Six determinations of this concentration are carried out and the absorbance of resulting solution was measured at the maximum of 241 nm. The amount of Carvedilol and % RSDis calculated.

The result data shows that the result is within the specified limit.

Ruggedness

The ruggedness of an analytical method was the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay.

The testing of ruggedness was normally suggested when the method was to be used in more than one laboratory. Ruggedness was normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method. For the determination of ruggedness, the degree of reproducibility of test result was determined as function of the assay variable. This reproducibility may be compared to the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method. (The specified limit is NMT 2% in % RSD).

The standard and the sample solutions are prepared. Six determinations of the above concentration are carried out by varying the analyst (as analyst 1 and analyst 2) and the absorbance of resulting solution was measured at the maximum of 241 nm. The amount of Carvedilol and % RSD was calculated and compared.

Table 1: Results of Ruggedness

	ANALYST – I	ANALYST – II
Mean of Assay in Percentage	1.5	98.5
Standard Deviation	0.89	0.51
% RSD	0.88	0.51

The result data shows that the results are similar and within the limit.

Robustness

The robustness of an analytical method was a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied.

Table 2: Results of Robustness

Change in	Assay in Percentage		Mean	Standard Deviation	% RSD
	Initial	After Change			
Extraction time					
15 min	101.7	100.4	101.1	0.92	0.97
25 min	101.7	104.0	102.9	1.63	1.58
Storage Condition 4 – 8 c	101.7	99.8	100.8	1.34	1.33
Reagent make(Qualigens)	99.9	99.9	100.9	1.41	1.40
Stability of a solution after 24 hours	101.7	99.9	100.9	1.41	1.40

Table.3 Results of Validation Parameters

S.no	Parameters	Results obtained	Acceptance criteria
1	System suitability test	1.61%	%RSD ≤ 2.0 for Absorbance
2	Specificity	There is no interference in absorbance from diluent and placebo due to Carvedilol.	There shall be no interference in absorbance from Diluent and placebo due to Carvedilol.
3	Linearity	1. 1.0000 2. 0.00 3. 0.05	1. Correlation coefficient ≥ 0.99 2. y- Intercept to be mentioned in the validation report. 3. Slope to be mentioned in the validation report.
4	Range 50% 100% 150%	0.78% 0.60% 0.37%	RSD ≤ 2.0%
5	Accuracy 50% 100% 150%	0.16% 0.40% 0.13%	Mean recovery of three levels shall bebetween 98.0 % to 102.0%.
6	Precision (Method precision)	0.57%	RSD ≤ 2.0% for six assay determination for individual analyst.
7	Ruggedness Analyst-1 Analyst-2	0.81% 0.51% 0.88%	Cumulative RSD ≤ 2.0%

Change in Extraction Time (± 5 minutes)

Two different sample solutions are prepared as per the procedure given under the sample solution preparation in the tentative assay method calculation and the absorbance of solution was measured at the maximum of 241 nm.The amount of Carvedilol and % RSD was calculated.

Change in Storage Condition

Sample preparation

The standard and sample prepared in initial preparation was preserved at room temperature 25 - 30 °C and freezing temperature at 4 - 8 °C for 24 hours.The absorbance of solution was measured at the maximum of 241 nm.The amount of Carvedilol and % RSD was calculated

Change in Reagent Make

The standard and sample solutions are prepared as per the procedure given under the tentative assay method calculation by changing the make of methanol.

The absorbance of solution was measured at the maximum of 241 nm.The amount of Carvedilol and % RSD was calculated.

The robustness of the analytical method is established through deliberate variation of the change in extraction time, the change in storage condition, the change in reagent make demonstrating that the results obtained under standard condition and variation are compiling within the limit.

Stability of Standard Solution

Stability of the sample, standard and reagents was required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability was desired for solutions and reagents that need to be prepared for each analysis.

The standard solution is prepared and stored for 24hrs. The absorbance of solution was measured at the maximum of 241 nm.The amount of Carvedilol and % RSD was calculated.The result data shows that the results are within the limit at the hold time study of the test solution and standard carried out for 24 hrs

The results of all validation parameters met the acceptance criteria (as per table 21). Hence the analytical method for assay of Carvedilol tablets by UV spectrophotometric method is validated and can be used for routine analysis.

Comparative Study

The assay content of two marketed Carvedilol sample are compared.The standard solution and the sample solutions are prepared for both the marketed sample and the absorbance was found at 241 nm. The amount of Carvedilol and the % RSD was calculated.It is concluded that the method is suitable for the routine analysis since the assay content of both the marketed sample is between 99.11 to 101.20%. The % RSD is also NMT 2%.Hence the method can be used for any brand of Carvedilol sample.

CONCLUSION

In this study a simple, fast and reliable UV spectrophotometric method was developed and validated for the determination of Carvedilol in pharmaceutical formulations. This method was applied directly to the analysis of pharmaceutical dosage forms without the need for separation or complex sample preparation such as extraction steps prior to the drug analysis. Thus the validated method is suitable and can be used for the routine analysis.

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