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METHOD DEVELOPMENT AND VALIDATION OF FORCED DEGRADATION STUDIES OF CARVEDILOL BY USING UV SPECTROSCOPY

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ABSTRACT

Aim: The main objective of this work describes simple, rapid UV spectroscopy method. It was developed for the forced degradation method of carvedilol in bulk and pharmaceutical dosage form.

Methods: The estimation was carried out on UV spectroscopy PERKIN ELEMER with LAMDA25 methanol used as a solvent. UV detection was carried out at 243 nm. The developed method was validated for linearity, accuracy, precision, limit of detection and quantification as per ICH guidelines.

Results: The LOD and LOQ were found to be 10.30 µg ml, 31.23µg ml for carvedilol. The high percentage recovery and low percentage recovery of coefficient of variance confirms the suitability of the method and followed by forced degradation studies, the amount of degradation is within 5-20% as per the ICH guidelines. proposed method was successfully used for the qualitative analysis of commercially available bulk and pharmaceutical dosage form of carvedilol.

Conclusion: A new simple analytical method has been developed to apply for the evaluation of the stability of CARVEDILOL to quantify and its degradation products in a solid premix dosage forms.

Keywords: Carvedilol, UV- Spectroscopy, forced degradation method.

INTRODUCTION:

3-(9H-carbazol-4-yloxy)-2-hydroxypropyl [2-(2-methoxyphenoxy) ethyl] amine (Figure1) Carvedilol is used to treat heart failure and hypertension (high blood pressure). It is also used after a heart attack that has caused your heart not to pump as well. Mechanism of action Carvedilol is a racemic mixture in which nonselective beta-adrenoreceptor blocking activity is present in the S (-) enantiomer and alpha-adrenergic blocking activity is present in both R (+) and S (-) enantiomers at equal potency. Carvedilol's beta-adrenergic receptor blocking ability decreases the heart rate, myocardial contractility, and myocardial oxygen demand [1-4]. Carvedilol also decreases

systemic vascular resistance via its alpha adrenergic receptor blocking properties. Carvedilol and its metabolite BM-910228 (a less potent beta blocker, but more potent antioxidant) have been shown to restore the inotropic responsiveness to Ca^{2+} in OH free radical-treated myocardium. Carvedilol and its metabolites also prevent OH radical-induced decrease in sarcoplasmic reticulum Ca^{2+} -ATPase activity. Therefore, carvedilol and its metabolites may be beneficial in chronic heart failure by preventing free radical damage [4-7]. The main object of the work is to develop a new method development and validation of forced degradation Studies of carvedilol by using UV spectroscopy [7-10].

Fig No: 1 Chemical structure of carvedilol

MATERIALS AND METHODS:

Chemicals:

Working standards of pharmaceutical grade carvedilol (<99%) was obtained reference sample, a tablet containing carvedilol is equivalent to cardivas 250mg was purchased from local market. Methanol was purchased from Merck chemicals, Mumbai, India.

Instruments:

UV/VIS spectrophotometer (Perkin Elmer lambda 25) used with 1cm path length quartz cell, analytical balance (shimadzu) were used.

Selection of wavelength:

Weigh accurately 100mg of carvedilol dissolved in 100ml of methanol in a 100ml of standard volumetric flask and the volume was made upto 100ml with methanol to obtain the concentration 1000µg/ml. From the above stock solution 10ml was pipetted out and transferred into 100ml standard volumetric flask and the volume was made upto 100ml with methanol to obtain the concentration 100µg/ml. Further diluted with methanol to obtain a 50µg/ml. The above solution was scanned between 200 – 400nm against the reagent blank was shown in the figure no: 2

Figure No: 2 Wavelength spectrum of carvedilol

Preparation of standard stock solution:

Weigh accurately 100mg of standard carvedilol dissolved in 100ml of methanol in a 100ml of standard volumetric flask and the volume was made upto 100ml with methanol to obtain the concentration 1000µg/ml. From the above stock solution 10ml was pipetted out and transferred into 100ml standard volumetric flask and the volume was made upto 100ml with methanol to obtain the concentration 100µg/ml of carvedilol. Further diluted with methanol to obtain a 8µg/ml of carvedilol. Measure the absorbance of prepared standard solution at 243nm using reagent as blank. The amount and percentage purity was shown in Table No: 1.

Sample preparation:

Weigh accurately about 20 tablets. A powder equivalent to 250mg of carvedilol dissolved in 250ml of methanol in a 250ml standard volumetric flask and the volume was made upto the mark to obtain the concentration of 1000µg/ml. From the above stock solution 10ml was pipetted out and transferred into 100ml standard volumetric flask and the volume was made upto 100ml with methanol to obtain the concentration 100µg/ml of carvedilol. Further diluted with methanol to obtain a 8µg/ml of carvedilol. Measure the absorbance of prepared standard solution at 243nm using reagent as blank. The amount and percentage purity was shown in Table No 2.

Method validation:

The forced degradation method validated by evaluating linearity, accuracy method and limit of detection (LOD) and limit of quantification (LOQ) were performed according with ICH guidelines.

Linearity:

From the standard stock solution of concentration 0µg/ml,0.2,0.4,0.6,0.8,1ml was transferred to 5 10ml standard flask and make up the volume with solvent. The concentration carvedilol was found to be 2µg-10 µg/ml. The calibration curve was shown in the Figure No: 3 and Table No: 1

Table No: 1 Regression data for carvedilol

Concentration (ppm)	Absorbance analysis	statistical
2	0.254	slope=0.134
4	0.562	correlation
6	0.822	coefficient
8	1.122	=0.996
10	1.302	

Table No: 2 Accuracy Result for Carvedilol

Drug	label claim (%w/w)	level	Amount added (mg)	Amount recovered (mg)	% recovery	Average % recovery
Carvedilol	6.25	50%	3	9.734	97.34%	97.43%
	6.25	100%	6	12.35	97.34%	
	6.25	150%	9	15.61	97.62%

Figure No: 3 Calibration curve of Carvedilol

Accuracy:

Accuracy of the method was carried out by standard addition method at three level of concentration (50%, 100%, 150%) the result of recovery (%) and %RSD shown in Table No: 2

Precision:

The precision of the method was determined by the sample assayed for 5 times. The inter day and intraday precision were carried out and all the % RSD of the values were found within limit (Table No: 3)

Table No: 3 precision results for carvedilol

Drug	Intraday	Interday
	Precision (%RSD)	Precision % (RSD)
Carvedilol	1.76%	1.83%

Limit of detection and quantification:

According to ICH guidelines(ICH,2005),the approach based on the standard deviation of intercept value and the slope of the calibration graph was used for determining the limits of detection($3.3 \times \text{slope} / \text{S.D.}$) and limits of quantification ($10 \times \text{slope} / \text{S.D.}$). The LOD and LOQ values for the Carvedilol are presented in (Table No; 4)

Table No: 4 LOD&LOQ results for Carvedilol

Concentration (µg/ml)	Absorbance
L.O.D.($3.3 \times \text{SD} / \text{Slope}$)	10.30 µg/ml
L.O.Q.($10 \times \text{SD} / \text{Slope}$)	31.23 µg/ml

Stress degradation studies:**Stress degradation by hydrolysis under acidic condition:**

To 3ml of stock solution (1000 µg/ml) of Carvedilol, 1ml of 3N HCl was added in 10ml volumetric flask and the volume were made upto the mark with methanol. Kept at normal condition for 90mins, 60mins, time interval, 1ml of solution was pipette out from this flask, and diluted with methanol the volume up to 10ml the appropriate concentration (30 µg/ml). This solution was taken in cuvette. For the blank, 0.5ml solution of 3N HCl and 0.5ml solution of 3N NaOH were diluted with methanol in 10ml of volumetric flask.

After 90mins, again 1ml of the solution was pipette out from the flask and the above procedure was repeated. Values are presented in Table No: 5

Stress degradation by hydrolysis under alkaline condition:

To 3ml of stock solution (1000 µg/ml) of Carvedilol, 1ml of 0.1N NaOH was added in 10ml volumetric flask methanol. Then, at normal condition for 90mins. After 60mins, time interval, 1ml of solution was pipette out from this flask, diluted with methanol in order to make the volume up to 10ml (30 µg/ml). This solution was taken in cuvette. For the blank, 0.5ml solution of 0.1N HCl and 0.5ml solution of 0.1N NaOH were diluted with methanol in 10ml of volumetric flask. After 90mins, again 1ml of the solution was pipette out from the flask and the above procedure was repeated. Values are presented in Table No: 5

Dry heat induced degradation:

Carvedilol sample was taken in a petriplate and exposed to a temperature of 70°C for 48hours in an oven. After 48hours, 10mg of the sample was diluted with methanol in order to make the volume up to 10ml. From this solution, dilutions were carried out to achieve the concentration (30 µg/ml) and the solution was taken in a cuvette for the UV-VIS analysis. Values are presented in Table No: 5

Oxidative degradation:

To 1.5ml of the stock solution of Carvedilol (1000 µg/ml), 1ml of 30%w/v of hydrogen peroxide added in 10ml of volumetric flask by using methanol.

The volumetric flask kept at room temperature for 15min. For the blank, 1ml of the 30% w/v of hydrogen peroxide was kept at normal condition for overnight in 10ml of volumetric flask. Both the solutions were heated on boiling water bath to remove excess of hydrogen peroxide. Finally, after 15mins dilutions were made from the stock solution to achieve the concentration (30µg/ml). The solution was then taken in a cuvette and analyzed in UV. Values are presented in Table No: 5

TABLE No: 5 Stress degradation studies for the determination of Carvedilol

Condition	Time	% Degradation
0.1N NaOH (1ml)	60 min	2.75
3N HCl (1ml)	90 min	7.73
	60 min	5.19
30% Hydrogen peroxide (1ml)	90 min	14.65
	15 min	3.75
Dry heat 70°C	48 hr	10.69
Photolytic	3hr	17.62
	6hr	19.36

Photolytic degradation:

Sample of Carvedilol was exposed to near ultraviolet lamp in photo stability chamber providing illumination of not less than 1.2 million lux hours. 10mg sample was dissolved in methanol and volume made up to 10ml (30µg/ml) and taken in cuvette for the UV analysis. Table No: 5

RESULTS AND DISCUSSION:

The proposed method for carvedilol showed the maximum absorbance at wavelength 243 nm. Linearity was observed in the concentration range of 2-10 µg/ml. The concentration of the drug present in the tablet was determined by the single component analysis at 243 nm. The drug content was found to be 95.51% for carvedilol. The correlation coefficient (r^2) value of the drug was found to be 0.996. The percentage RSD for the three replicates was found to be less than 2.0%. The LOD&LOQ of carvedilol were found to be 10.3µg/ml and 31.23µg/ml respectively and the stress degradation study value It indicates that there was no interference show that good accurate and precise.

CONCLUSION:

The proposed method is specific in estimating the commercial formulation without interference of excipients and the other additives. Hence, this method can be used for routine determination of

CARVEDILOL in the bulk sample and pharmaceutical formulation. The proposed method for stability study shows that there is appreciable degradation of CARVEDILOL found in stress conditions. A new simple analytical method has been developed to apply for the evaluation of the stability of CARVEDILOL to quantify and its degradation products in a solid premix dosage forms.

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