ASSAY METHOD AND VALIDATION FOR THE CARVEDIDOL TABLET FORMULATION BY USING RP-HPLC.

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A simple, efficient, and precise stability indicating RP-HPLC method has been developed and validated to measure Carvedilol at wavelength (242 nm) in order to assay. Carvedilol is used to treat chronic heart failure. Methanol was used as a solvent with λmax of drug was found to be 242 nm. The samples were eluted in an isocratic method using YMC pack pro c18(100×4.6mm,5µ) column at ambient temperature,for 8min run time with 1.0ml/min flow rate was used with a mobile phase consisting of pH 3.0 Buffer Dipotassium Hydrogen Phosphate: ACN (60:40) using as diluents through ambient temperature delivered at a flow rate 1.0mL/min. Linearity was observed in the range of 25-150% with a regression coefficient of 0.99. The method was quantitatively evaluated in terms of accuracy (recovery), linearity, in accordance with standard ICH validation guidelines. The method is simple and suitable for analyzing Carvedilol in bulk and in pharmaceutical formulations.

Keywords-,RP-HPLC, carvdilol,wavelength ,ICH validation guideline.

INTRODUCTION:

Carvedilol tablets are indicated for the treatment of mild to severe chronic heart failure of ischemic or cardio myopathic origin .usually in addition to diuretics ACE inhibitors and digitalis . They can be used alone or in combination with other antihypertensive agents especially thiazide type diuretics should not be given to patients with severe hepatic impairment .It is a non selective β-adrenergic blocking agent with α-1 blocking activity .Carvedilol has much greater antioxidant activity than other commonly used β blockers .Tablet containing inactive ingredients as colloidal silicon dioxide, crospovidone, hypromellose, lactose monohydrate, magnesium stearate, polyethylene glycol, polysorbate, povidone, and titanium dioxide.[10,20]

Chemical Structure:

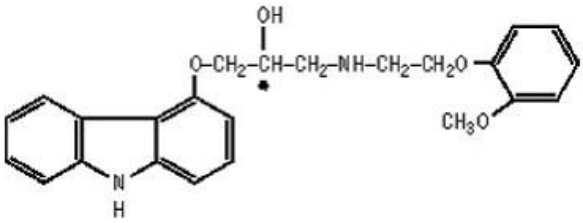

Fig 1: Chemical Structure Carvedilol

Chemical Name :

(±)-[3-(9H-carbazol-4-yloxy)-2 hydroxy propyl] [2-(2-methoxy phenoxy) ethyl] amine.

Molecular Formula:

C24H26N2O4

Charecterestics:

White or off –white crystalline powder.

Solubility:

Insoluble in water, sparingly soluble in 95% ethanol and isopropanol, slightly soluble in ethyl ether soluble in methanol, methylene chloride, freely soluble in dimethyl sulfoxide[2,3,6,8].

Mechanism Of Action:

Carvedilol is a racemic mixture in which non-selective β- adreno receptor blocking activity is present in the S(-) enantiomers and α_1 adrenergic blocking activity is present in both R(+) and S(-) enantiomers at equal potency. Carvedilol has no intrinsic sympathomimetic activity.

Therapeutic Uses:

Antihypertensive agents. Adrenergic β- antagonist. Adrenergic α-antagonist. Vasodilator agents.

Dosage:

Patients weighing < 85 kg (187 lb) - 25mg twice daily.

Patient weighing > 85kg (187lb) -50mg twice daily.

How To Use:

25 mg taken twice daily for two weeks .This dosage is same regardless of the age of weight of the patient. It should be swallowed as a whole tablet and should not crushed, and chewed .Carvedilol should be taken with food to slow the rate of absorption and reduce the incidence of orthostatic effects .Patient should be observed in the office for one hour after initial dose is given.

Storage:

Store in a close, cool and light resistant container.

Brand Names:

Cardivas, Coreg, Carvil, Karvileks.

High Performance Liquid Chromatography:

Quality can be defined as the character, which defines the grade of excellence. A good quality drug is something, which will meet the established product specifications, can be safely bought and confidently used for the purpose for which it is intended.[11-14] To get a good quality drug ,the manufacturing for making a drug should have quality built into it.

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Analytical chemistry is a subdiscipline of chemistry that has the broad mission of understanding the chemical composition of all matter and developing the tools to elucidate such compositions.[2-9]

HPLC is able to separate macromolecules and ionic species labile natural products, polymeric materials, and a wide variety of other high –molecular weight poly functional group. HPLC is the fastest growing analytical technique for the analysis of the drugs. It's simplicity, high specificity, and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids .In this ,the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 310µm.Modern LC uses very small particles for packing. The small particle size results in more rapid approach to the distribution equilibrium and consequently smaller plate height, so that a given length of column includes large number of plates which makes the column efficient and the peak narrow. But close packing of these small particles reduces the flow rate of the mobile phase through the packed bed (the packing said to develop high back pressure) and in order to achieve a reasonable flow rate it is necessary to apply pressure to the mobile phase. So the designation, put forth as high pressure liquid chromatography. Thus HPLC is having advantages of improved resolution, faster separation, improved accuracy, precision and sensitivity. [15-16,19]

MATERIAL AND METHOD:

Material:

Carvediol, Acetonitrile, Methanol, Potassium dihydrogen phosphate, toluene, HPLC water,Chloroform.

Chromatographic conditions:

YMC pack pro c18(100×4.6mm,5µ) column at ambient temperature,for 8min run time with 1.0ml/min flow rate was used. Phosphate Buffer : ACN (60:40) used as mobile phase with pH 3.Detection done at 242nm.

Solution Preparations :

Preparation Of Buffer Solution:

Dissolve about 6.80g of potassium di hydrogen phosphate in1000 ml of HPLC/ Milli – Q water.

Adjusts the pH to 3.0 ± 0.05 with formic acid.

Preparation Of Mobile Phase:

Prepare a mixture of buffer and acetonitrile in the ratio of 60:40 Filter through 0.45µ

Membrane filter and degas.

Preparation Of Standard Solution:

Weigh accurately about 62.5mg of carvedilol reference /working standard in to a 100ml

Volumetric flask, add 30ml of methanol sonicate to dissolve . Then make up to the volume With methanol . Pipette out 10ml of this solution into a100ml volumetric flask and dilute up to the mark with mobile phase. Filter through 0.45µ membrane filter.

Prepare the standard solution in duplicate calculate the similarity factor for standard-I and Standard -II solutions by using the following formula.

Area of standard solution-1 × weight of STD (in mg) solution-2

Area of standard solution-2 (1st injection) weight of STD (in mg) solution-1

Note: similarity factor for both the standard solutions should be between 0.98 to 1.02.

Preparation Of Sample Solutions:

For 25 mg tablets:

Weigh and transfer 5 tablets into a100ml volumetric flask. Add 50ml of methanol and sonicate to dissolve then make up to the volume with methanol, pipette out 5ml of this solution into a 100ml volumetric flask and dilute up to the mark with mobile phase. Filter through 0.45µ membrane filter.

Procedure:

Inject 10µl portions of blank, standard solution and sample solutions into the chromatograph and record the chromatograms. Record the peak responses for the major peaks[21-24]. Fig 1,fig2.table 1,table 2.

Evaluation Of System Suitability:

1. The relative standard deviation for five replicate injections should not be more than 2.0%
2. Tailing factor for carvedilol peak should be not more than 2.5
3. Theoretical plate for carvedilol peak should be not less than 1500.

Validation Program For Assay:

1.system suitability

2.specificity

3.linearity range.

System Suitability:

Five replicate injections of the standard solutions were injected the percentage RSD for the peak area and tailing factor for carvedilol were calculated. Table3.

Acceptance Criteria:

a) % RSD for standard injections should be not more than 2.0%
b) Tailing factor for carvedilol peak should be not more 2.

Specificity:

Blank, placebo, standard, sample solution injected into HPLC system. There was no interference from the blank and placebo at the retention time of carvedilol peak. Peak purity reveals that carvedilol peak was homogeneous and there were no co-eluting peaks at the retention time of carvedilol peak.

Placebo Preparation:

Weighed and transferred 1.812 gm of placebo into 100ml volumetric flask, 50 ml of methanol added and sonicated to dissolve. Then made up to the volume with methanol. Pipette out 5 ml of this solution into a 100 ml volumetric flask and diluted up to the mark with mobile phase. Filtered through 0.45µ membrane filter and injected into the chromatogram.

Acceptance Criteria*:*

i) All individual peaks should be well separated.
ii) The interference of carvedilol peak from the other peaks should be nil.
iii) The purity of carvedilol peak should be NLT 0.99.

Accuracy/Recovery:

Known amount of carvedilol spiked with placebo at about 80%,100%, and 120%of working concentration in triplicate and analysed as per testing procedure .The percentage recovery was calculated from the amount found and actual amount added. Fig3,fig4,fig5.table4.

Acceptance Criteria:

a) The % recovery should be in between 95% and 105%.
b) The %RSD for all recovery values should be not more than 2%.

Linearity And Range :

The linearity of an analytical procedure is its ability (within a given range)to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of the analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including the concentrations). For which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.

Procedure:

Preparation Of Standard Stock Solution:

Weigh accurately and transfer about 62.52 mg of carvedilol into a 100 ml volumetric flask. 50 ml methanol added and sonicated to dissolve, then make upto the volume with methanol. Pipette out 10 ml of this solution into a 100 ml volumetric flask and diluted upto the mark with mobile phase. Filtered through 0.45µ membrane filter.

Preparation Of Sample Solution:

Transfer the accurately weighed samples 15.62,31.25, 46.88, 62.50, 78.13, 93.75 mg respectively into individual 100 ml flask . 50 ml methanol added and sonicated to dissolve, then make up to the volume with methanol. Pipette out 10 ml of this solution into a 100 ml volumetric flask and diluted up to the mark with mobile phase. Filtered through 0.45 µ membrane filter.

Inject 10µl of blank solution and each linearity level standard solutions into the chromatographic system and measure the peak area .

The linearity of carvedilol was performed in the range of 15.62µg/ml to 93.75µg/ml (25% - 150 % of working concentration). A graph was plotted with concentration in µg/ml on x axis and peak area on y axis. Slope, y intercept, correlation coefficient (r value), were determined.fig6,table5.

Acceptance Criteria:

The correlation coefficient should be not less than 0.99.

Result:

The Result of the above study is tabulated as table 6. The test parameters meet the acceptance criteria .The method employed in this analysis of carvedilol tablets –Assay is validated.

Validated analytical methods are aimed for the estimation of carvedilol in formulation. Simple, precise, rapid, accurate methods were developed for the estimation of carvedilol in formulation by Estimation of carvedilol by RP-HPLC.

In RP-HPLC method, a wavelength of 242 nm was selected and the mobile phase which consist potassium di hydrogen phosphate buffer : acetonitrile, in the ratio of (60:40). pH 3 adjusted with formic acid at a flow rate

of 1ml/min were found to be optimum condition for analysis. The retention time was found to be 2.9 with optimized conditions.

Carvedilol showed the linearity in the range of 15.62 -93.75µg/ml. Where the peak shape was symmetrical and a good correlation coefficient value was obtained.

The percentage label claim and recovery at three different levels, 80%, 100%, 120%, level was carried out. The suitability of the method was thus proved.

Conclusion:

The analytical method meets the acceptance criteria for accuracy study. Hence the method is accurate for the determination of assay of carvedilol tablets.

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Figures and Tables-

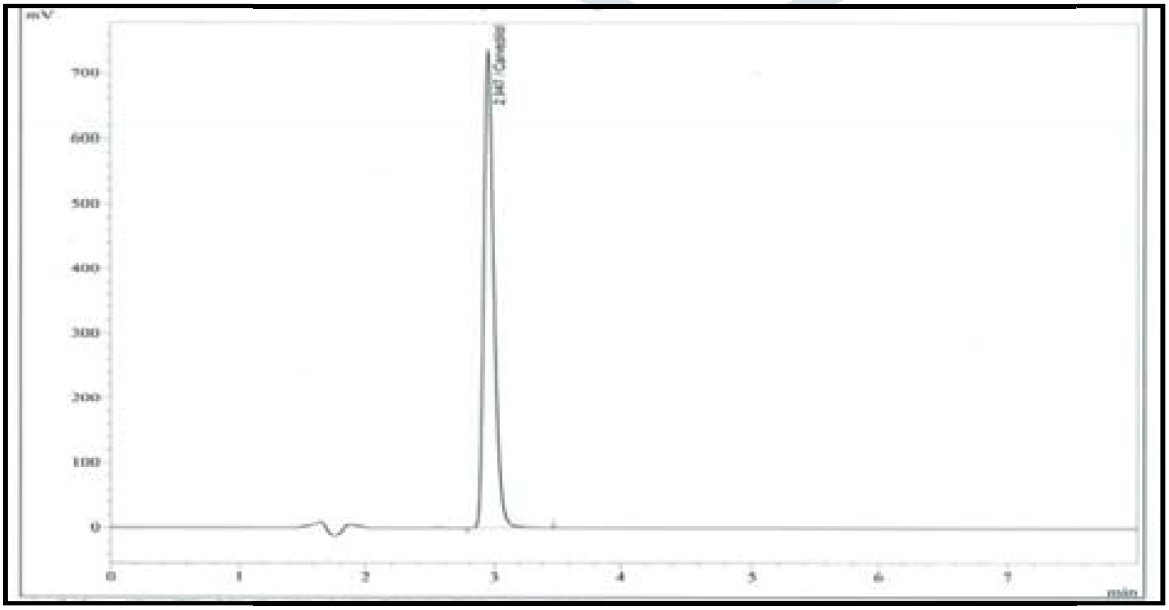

Fig 1:Chromatogram for Sample Solution

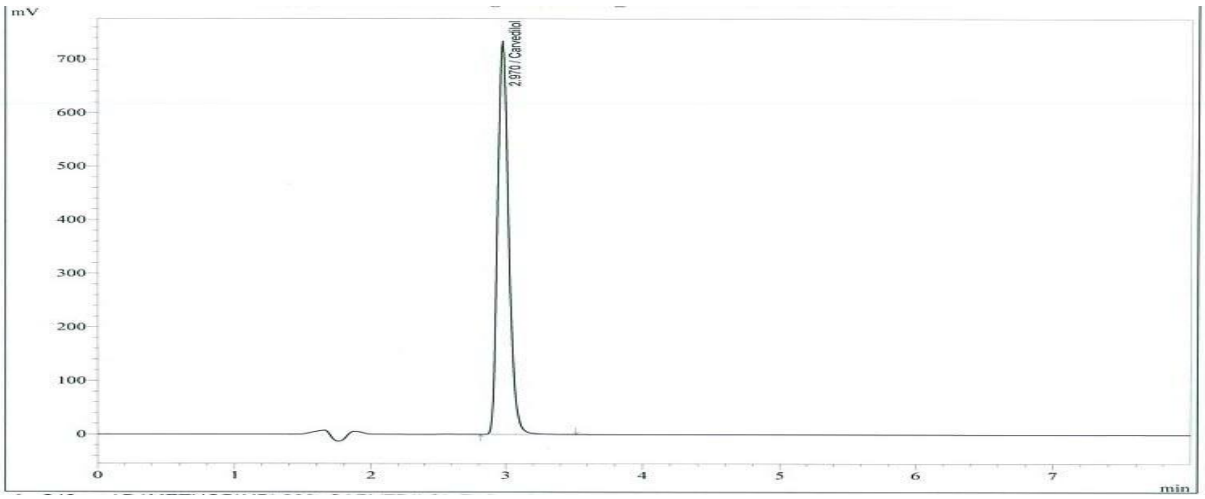

Fig 2:Chromatogram of standard solution

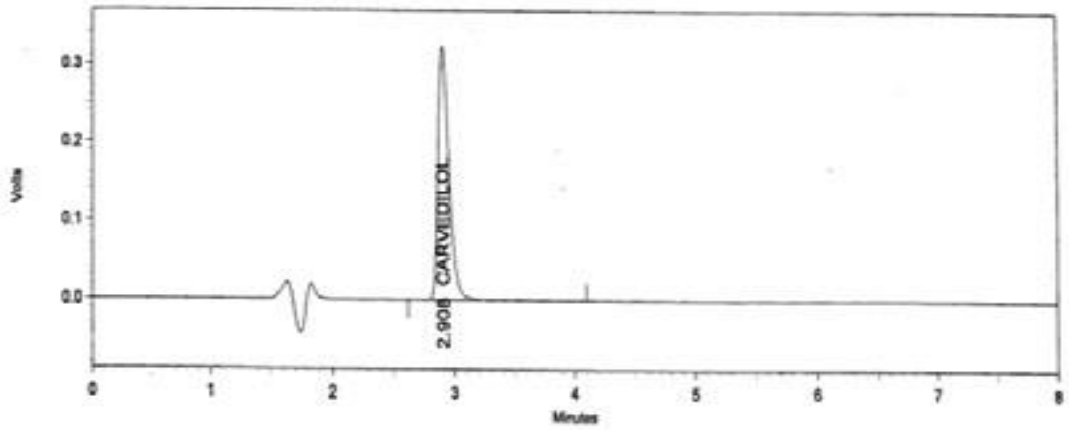

Fig 3:accuracy at 80%

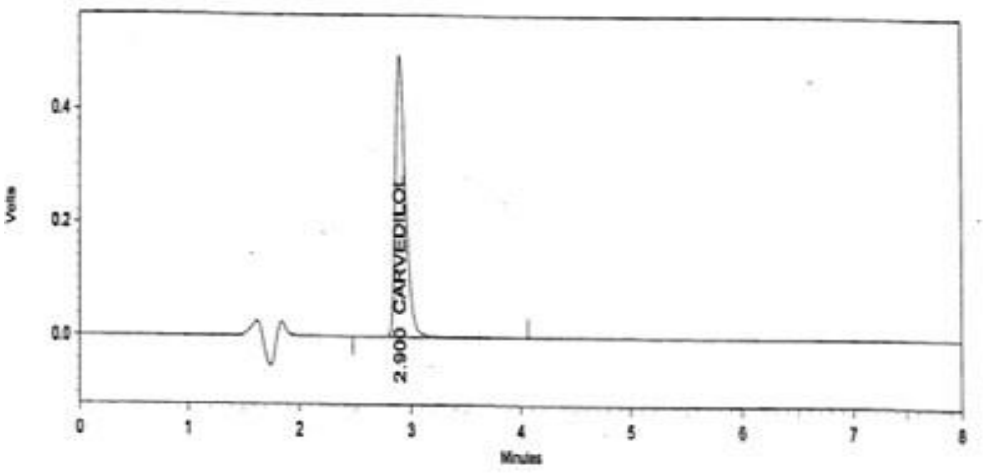

Fig 4:accuracy at 100%

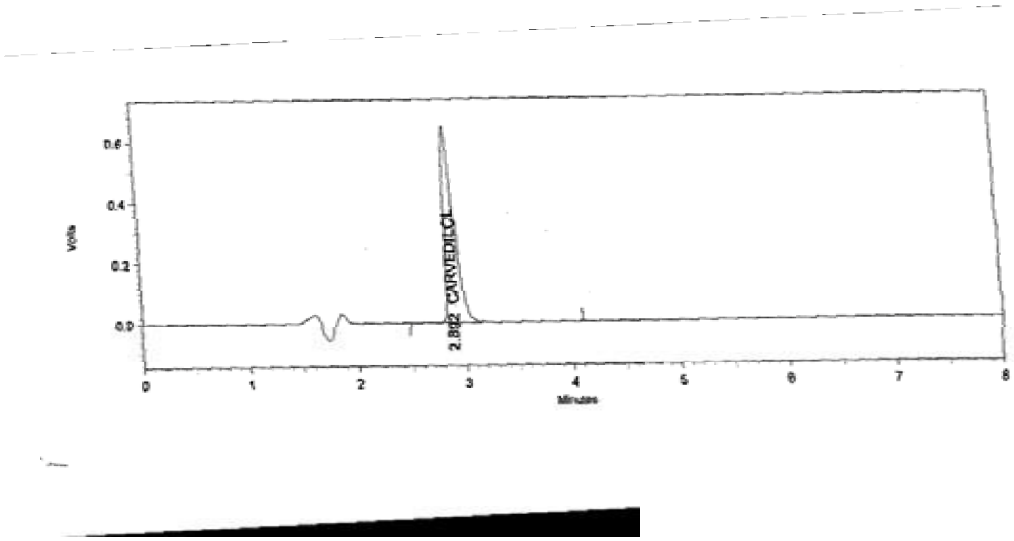

Fig 5:Accuracy at 120%

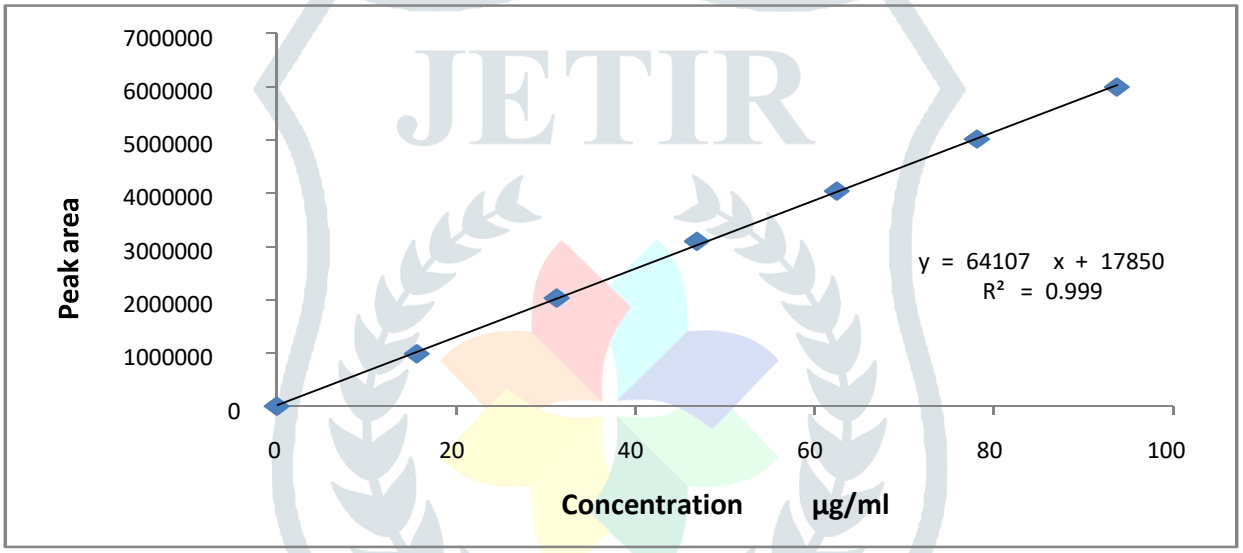

Fig 6 :Calibration Curve for Carvedilol

Sr.no	description	No.of Injections
1	blank	1
2	Standard solution-I	1
3	Standard solution-I	1
4	Standard solution-I	4
5	Sample solution	2
6	Standard solution-I	1

Table 1: Injection Sequence

Drug	Amount mg/tab	%Label claim	%RSD
	Label	Found	
Carvedilol	25 mg	25.43 25.4 25.38 25.45 25.43 25.4	0.051

Table 2:Analysis of carvedilol formulation

No of injection	Area	Tailing factor
1	4121144	1.514
2	4111957	1.521
3	4112953	1.520
4	4135077	1.525
5	4118577	1.516
Average	4119942	1.519
SD	9286.85	
%RSD	0.23	

Table 3: System Suitability

LEVEL	Amount found in µg	Actual amount added in µg	%recovery	Mean	%RSD
Level-1 80%	19.80 19.80 19.78	19.72 19.72 19.72	100.4 100.4 100.3	100.4	0.07
Level-2 100%	24.71 24.69 24.70	24.64 24.64 24.64	100.3 100.2 100.2	100.2	0.07

Level-3 120%	29.76 29.77 29.81	29.58 29.58 29.58	100.6 100.6 100.8	101.7	0.12

Table 4: -Accuracy

LEVEL	CONCENTRATION IN µg/ml	PEAK AREA
25%	15.62	985504
50%	31.25	2030094
75%	46.88	3096294
100%	62.50	4039967
125%	78.13	5016528
150%	93.75	5992094

Table 5: Linearity.

S.NO	TEST	ACCEPTANCE CRITERIA	RESULT
1	System suitability	a. %RSD for std injection is NMT 2.0% b. Tailing factor for carvedilol peak is NMT 2.5	0.23 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% and 105%. b. The %RSD for all recovery values should be NMT 2%.	98.1% and 102.7% 0.64%
4	Linearity	The correlation co efficient should be NLT 0.99	0.99

Table 6: Summary &Conclusion For Assay