TITLE: EVALUATION AND VALIDATION OF CARVEDILOL IN BULK AND PHARMACEUTICAL DOSAGE FORMS.

ABSTRACT

Background: Quality may be described as the character, which determines the degree of excellence. A good quality medication is anything, which will match the specified product requirements, may be safely purchased and confidently used for the purpose for which it is designed. To obtain a high quality medication, the manufacturing for producing a drug should have quality integrated into it. Analytical chemistry is the discipline that seeks ever better methods of assessing the chemical composition of natural and manmade materials. **Aims and objective:** Evaluation and validation of carvedilol in bulk and pharmaceutical dosage forms. **Material and methods:** preparation of standard stock solution and preparation of sample solution as per standard protocol. **Result:** In RP-HPLC method, a wavelength of 242 nm was 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. **Conclusion:** RP-HPLC, HPTLC, UV spectroscopy were found to be sensitive, precise, and accurate. However these three methods can be used for the routine analysis of carvedilolfrom formulation.

Keywords: validation, carvedilol, pharmaceutical, dosage

INTRODUCTION

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[i] . 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

Comment [Admin1]: Quality may be described as the character that determines the degree of excellence. A good quality medication is anything that meets the product specifications, can be purchased safely, and can be used confidently for the purpose for which it was designed.To obtain a high quality medication, the manufacturing process for producing a drug should have quality integrated into it. Analytical chemistry is the discipline that seeks ever better methods of assessing the chemical composition of natural and manmade materials.

Comment [Admin2]: In the RP-HPLC method, a wavelength of 242 nm was retained and the retention time was found to be 2.9 with optimised conditions. Carvedilol showed linearity in the range of 15.62 -93.75µg/ml. where the peak shape was symmetrical and a good correlation coefficient value was obtained.

Comment [Admin3]: To get a good quality drug, the manufacturing process should have quality built into it.

chemical composition of natural and artificial materials. Analytical chemistry is a sub-discipline of chemistry that has the broad mission of understanding the chemical compositionof all matter and developing the tools to elucidate such compositions[ii].

Spectrophotometry is generally preferred by industries as the cost of the equipment is less and the maintenance problems are minimal. The method of analysis based on measuring the absorption of a monochromatic light by colourless compounds in the near ultraviolet path of spectrum (200-380nm).[iii] The photometric methods of analysis are based on the Bouger- Lambert Beer's Law, which establishes that the absorbance of a solution is directly proportional to the concentration of the analyte .The fundamental principle of operation of spectrophotometer covering UV region consists in that light of definite interval of wavelength passes through a cell with solvent and falls on to photoelectric cell that transforms the radiant energy into electrical energy measured by galvanometer. Molecular absorption in the ultraviolet and visible region of the spectrum is dependent on the electronic structure of the molecule. Absorption of energy is quantized, resulting in the elevation of electrons from orbital in the ground state to higher energy orbital in the excited state.[iv]

Comment [Admin4]: The basic concept of functioning of a UV spectrophotometer is that light of a specific wavelength passes through a solvent-filled cell and falls on a photoelectric cell, which converts the radiant energy into electrical energy, which is measured by a galvanometer.

Comment [Admin5]: The quantization of energy absorption causes electrons to be elevated from their ground state orbital to a higher energy orbital in the excited state.

AIM AND OBJECTIVE

Evaluation and validation of carvedilol in bulk and pharmaceutical dosage forms.

MATERIAL AND METHODS

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, and then make up to the volume with methanol. Pipette out 10 ml of this solution into a 100 ml volumetric flask and diluted up tothe 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 thechromatographic 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.

RESULT & OBSERVATION

Table 1 :RP-HPLC profile

Name of Drug	**Retention Time**	**Area**	**Theoretical Plate**	**Tailing Factor**
Carvedilol	2.96	4121905	5350.4	1.40

The analytical method meets the acceptance criteria for accuracy study. Hence the method is accurate for the determination of assay of carvedilol tablets. [Fig 1]

HPTLC profile [Fig 2 and 3]

Linearity:

Aliquots of 0.1-0.5 µg/spot of standard solution of Carvedilol is applied on the plate with the help of micro liter syringe using an automatic sample applicator. The plates were developed, dried and scanned densitometrically at 254 nm. The drug peak-area was calculated for each concentration level and a graph was plotted of drug concentration against the peak area andshown in (Fig.3). Calibration parameters are given in table 2 : 5

Table 2 : Linearity of Carvedilol

SL.No	**Standard concentration µg/spot**	**Peak Area**	**Peak Height**

1	0.1	756.35	35.8
2	0.2	1387.96	72.4
3	0.3	2081.94	123.9
4	0.4	2775.92	168.8
5	0.5	3469.9	203.4

CALIBRATION CURVE OF CARVEDILOL

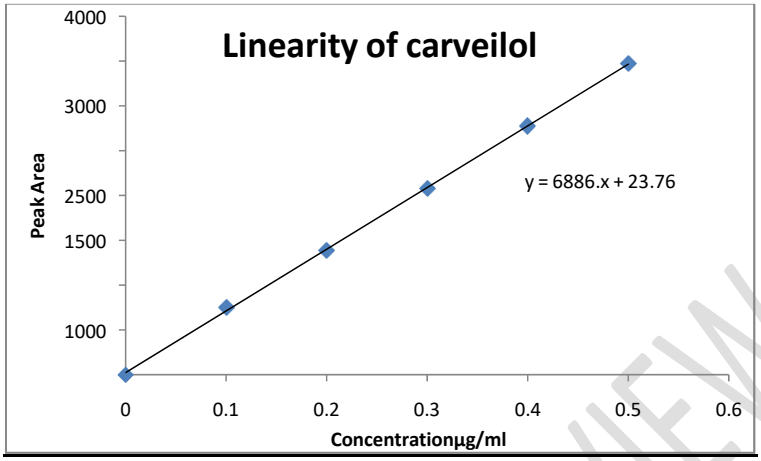


Table 3 : Calibration Parameters for Carvedilol

Parameters	**Carvedilol**
Linearity Range(µg/Spot)	0.1-0.5
Slope	6886
Intercept	23.76
Regression Co-Efficient	0.999

Accuracy:

Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in Table **4**. From the recovery study, it was clear that the method is very accurate for quantitative estimation of Carvedilol in tablet dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

Table 4 :Recovery studies for Carvedilol (n=3)

Label claim (mg / tablet)	**Recovery level (%)**	**Amount added (mg)**	**Amount recovered (mg) ± % RSD**	**% Recovery**

25	80	20	19.96±0.42	99.82
25	100	25	24.65±0.85	98.67
25	120	30	30.45±0.59	101.5

Precision:

The precision of the method (system reproducibility) was assessed by spotting 0.3µl of drug solution six times on a TLC plate, followed by development of plate and recording the peak area for 6 spots. The % RSD for peak area values of carvedilol was found to be 0.58. The results were shown in Table-6.

The method reproducibility (intra-day precision) was determined by analyzing standard solution in the concentration range of 0.1 µg/spot to 0.5 µg/spot of drug for 3 times on the same day and inter-day precision was determined by analysing corresponding standards daily for 3 day over a period of one week. The intra-day and inter-day coefficients of variation (%RSD) are in range of 0.13 to .36 and 0.30 to 0.56, respectively. The results were shown in Table- 6a, 6b.

Table 5: Precision of Carvedilol

S.No	**Concentration (µg/ spot)**	**Peak Area**
1.	0.3	2081.94
2.	0.3	2111.40
3.	0.3	2090.52
4.	0.3	2075.36
5.	0.3	2087.25
6.	0.3	2092.17
Mean	-	2089.77
Percentage Relative Standard	-	0.58

Deviation		

Table 6a: Intra-day Precision of Carvedilol

S.No	Concentration (µg / spot)	Area	Mean	Standard Deviation	% RSD
1. 2. 3.	0.1 0.1 0.1	756.35 761.05 754.50	757.3	3.37	0.44
1. 2. 3.	0.3 0.3 0.3	2080.50 2083.72 2078.25	2080.82	2.74	0.13
1. 2. 3.	0.5 0.5 0.5	3469.50 3465.64 3475.82	3470.32	5.13	0.14

Table 6b: Inter-day Precision of Carvedilol

S.No	Concentration (µg / spot)	Area	Mean	Standard Deviation	% RSD
1. 2. 3.	0.1 0.1 0.1	757.98 754.50 762.35	758.28	3.93	0.52
1. 2. 3.	0.3 0.3 0.3	2081.94 2090.50 2078.25	2083.56	6.28	0.30

UV Profile

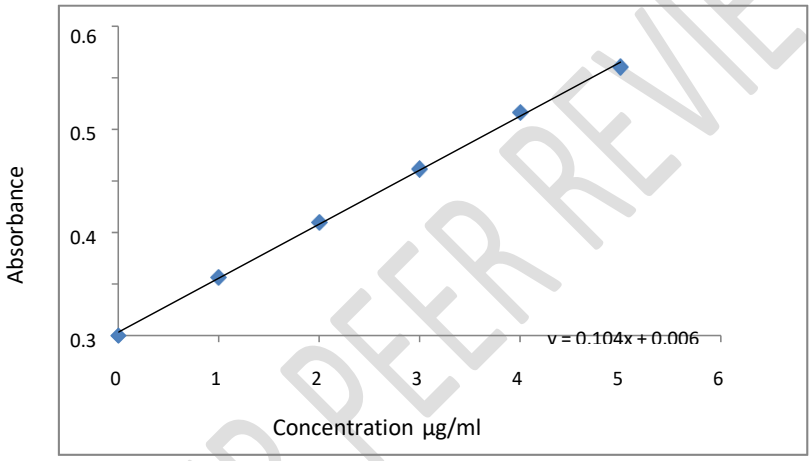
The developed method was validated in terms of linearity, accuracy and stability studies.

Linearity

Carvedilol was found to be linear in a concentration range of 1-5µg/ml. The absorbance of this solution were measured at 242 nm and a calibration graph was plotted using absorbances versus concentration. The correlation co-efficient value was found to be 0.998.

Comment [Admin6]: In a concentration range of 1-5g/ml, carvedilol was shown to be linear. The absorbance of this solution was measured at 242 nm, and the absorbances versus concentration were plotted on a calibration graph. The value of the correlation co-efficient was found to be 0.998.

Fig 4 : CALIBRATION CURVE OF CARVEDILOL



Accuracy

The accuracy,specificity, suitability and validity of the present method were satisfied by conducting percentage recovery studies. A known quantity of the drug was added to the pre- analyzed sample formulation at 50% and 100% levels. The percentage recovery and standarddeviation were calculated.(Table 3).

The % recovery was calculated by using the following formula

$$\%\text{Recovery} = \frac{\text{Amount of drug found after addition of standard drug} - \text{Amount of drug found in sample before addition of standard drug}}{\text{Amount of standard drug added}} \times 100$$

Table 7 : RECOVERY STUDIES

Drug	Level	Amount found in µg	Actual amount Added in µg	%Recovery	% RSD
Carvedilol	50%	12.30	12.22	100.6	0.12
		12.28	12.22	100.4	
		12.30	12.22	100.6	
	100%	24.71	24.64	100.3	0.06
		24.69	24.64	100.2	
		24.70	24.64	100.2	

Stability

The drug solution was found to be stable for about three hours at room temperature. Stabilitydata reported.

Table 8 : STABILITY DATA

Concentration in µg/ml	Time (min)	Absorbance
1	0	0.113
	30	0.110
	60	0.109
	90	0.117
	120	0.112
	150	0.107
	180	0.108

Table 9 : REPEATABILITY STUDIES

Concentration in µg/ml	Absorbance	%RSD
	0.117	
	0.118	

1	0.119	0.93
	0.117	
	0.117	
	0.116	
5	0.516	0.47
	0.513	
	0.511	
	0.510	
	0.515	
	0.511	

Table 10 : INTER-DAY PRECISION

Concentration µg/ml)	**Day**	**Absorbance**	**%RSD**
1	1	0.116	1.02
	2	0.117	
	3	0.115	
	4	0.115	
	5	0.116	
	6	0.114	
5	1	0.512	0.40
	2	0.513	
	3	0.512	
	4	0.516	
	5	0.510	
	6	0.513	

Table 11: INTRA- DAY PRECISION

Concentration µg/ml	6 times in a day	Absorbance	%RSD
1	1	0.115	1.47
	2	0.113	
	3	0.111	
	4	0.114	
	5	0.115	
	6	0.112	
5	1	0.513	0.45
	2	0.515	
	3	0.510	
	4	0.512	
	5	0.515	
	6	0.516	

DISCUSSION

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 following methods[v]. 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. Precision of the method was studied by making repeated injection of the same sample and standard deviation was determined. Inter day and intraday precision was also carried out and % RSD was calculated. In HPTLC during the stage of method development different mobile phase were tried and mobile phase comprising of ethyl chloroform: methanol : toluene in the proportion of 1.5: 3:3.5 v/v/v for carvedilol , were found to be better and produced the R_f value of 0.72 for carvedilol. The linearity of drug was determined by calibration curve and the linearity based on the area observed in the range of 0.1 – 0.5 µg/ ml. The regression coefficient value for carvedilol is 0.999. Interday precision of the drugs was studied. No interference with the additives of the formulation was reported .Recovery studies were carried out for the accuracy parameter and were reported. The validated method was applied for the analysis of tablet containing 25 mg carvedilol drug as the label claim. The method developed was simple. It has showed a good peak and good R_f values. In case of UV-spectroscopic method solubility is the important parameter. Solubility parameter was studied and methanol was selected as the solvent, since it gave a maximum absorbance and a good spectral pattern when compared with other solvents[vi]. The linearity was found to be in the range of 1 -5 µg/ml at the maximum absorbance of 242 nm. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 242 nm. Percentage recovery, linearity, stability studies were also carried out. The above method gave a satisfactory recovery values and found to be stable, linear, hence it can be used for routine analysis of the drug formulation[vii].

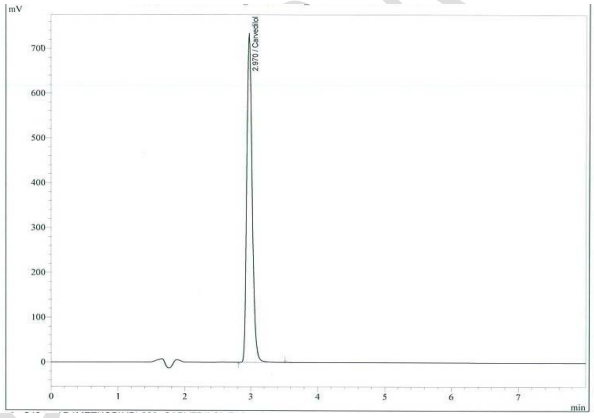
Comment [Admin7]:

These methods, RP-HPLC, HPTLC, UV spectroscopy were found to be sensitive, precise, and accurate. However these three methods can be used for the routine analysis of carvedilolfrom formulation.

CONCLUSION

RP-HPLC, HPTLC should spend most of the impact on the creation and optimization of a technique as this enhances the performance of the last method. It should be simple to verify a properly designed technique. In order to analyse preclinical samples, formulations and commercial samples, a technique should be established quickly. The observations of validation factors like as accuracy, accuracy, speciality and linearity have shown that the techniques devised may be used to routinely analyse carvedillol in bulk and tablets. The results of the validation parameters fulfilled the requirements of ICH and USP and complied with the legislation of BEER.

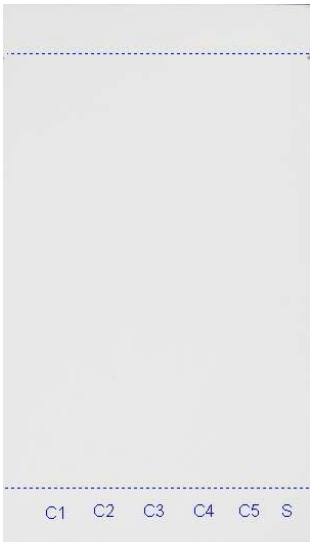
Fig 1: RP-HPLC



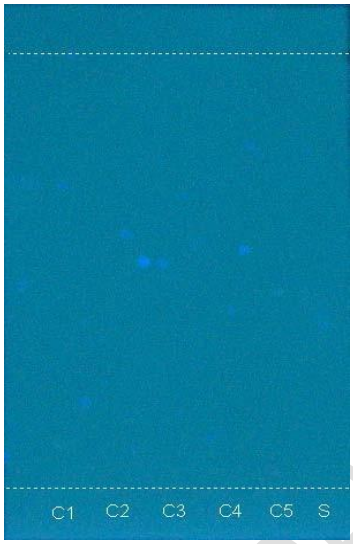
Comment [Admin9]: For the estimate of carvedilol in formulation, validated analytical procedures are used. The following procedures were established for estimating carvedilol in formulation and are simple, precise, quick, and accurate. A wavelength of 242 nm was used for the RP-HPLC technique, and the mobile phase was potassium di hydrogen phosphate buffer: acetonitrile, in the ratio of (60:40). pH 3 was found to be the best condition for analysis when adjusted with formic acid at a flow rate of 1ml/min. With optimal conditions, the retention time was found to be 2.9. Carvedilol's linearity was found to be between 15.62 and 93.75 g/ml. The peak shape was symmetrical, and the correlation coefficient value was high. The percentage label claim and recovery were tested at three distinct levels, 80 percent, 100 percent, and 120 percent. The method's appropriateness was thus established. The precision of the approach was investigated by injecting the same sample many times and calculating the standard deviation. Precision was measured on a daily and intraday basis, and the percent RSD was determined. Various mobile phases were investigated in HPTLC during the technique development stage, and the mobile phase consisting of ethyl chloroform, methanol, and toluene in the proportions of 1.5: 3:3.5 v/v/v for carvedilol was found to be the best and generated an Rf value of 0.72 for carvedilol. The drug's linearity was determined using a calibration curve and the area observed in the range of 0.1 – 0.5 g/ ml. Carvedilol has a regression coefficient of 0.999. The medications' interday precision was investigated. There was no reported interference with the formulation's additives. The accuracy parameter was subjected to recovery studies, which were published. The validated method was used to examine a tablet containing 25 mg of carvedilol, as stated on the label. The procedure that was devised was straightforward. It has a good peak as well as good Rf values. The most essential characteristic in the UV-spectroscopic technique is solubility. The solubility parameter was investigated, a ...

Comment [Admin11]: .
RP-HPLC, HPTLC should spend most of their impact on the creation and optimization of a technique as this enhances the performance of the last method. It should be simple to verify a properly designed technique. A technique should be established quickly in order to analyse preclinical samples, formulations, and commercial samples. The observations of validation factors like accuracy, accuracy, speciality, and linearity have shown that the techniques devised may be used to routinely analyse carvedillol in bulk and tablets. The results of the validation parameters met the requirements of ICH and USP and were in line with the law in BEER.

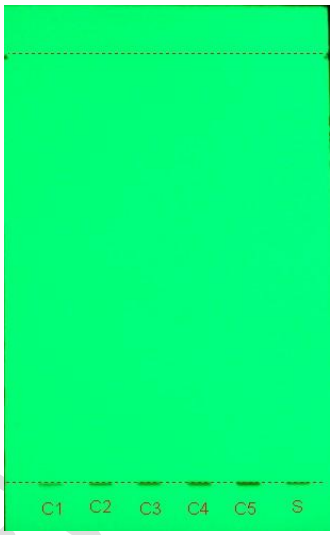
Fig 2: HPTLC profile



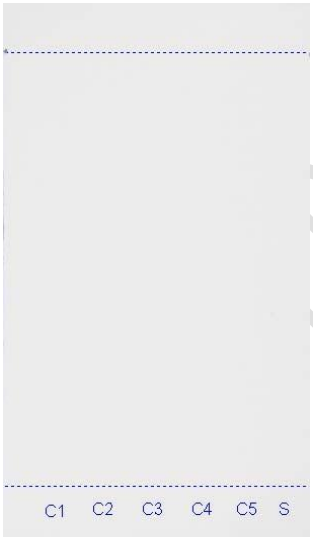
Daylight before mobile phase run



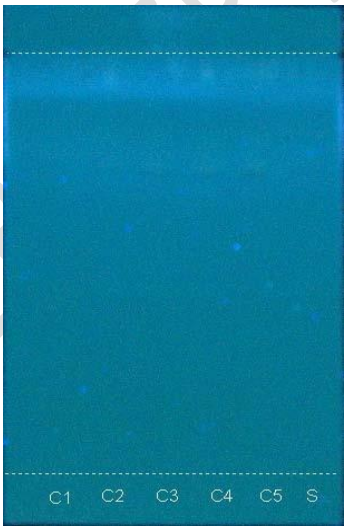
UV 366nm before mobile phase run



UV 254 nm before mobile phase run



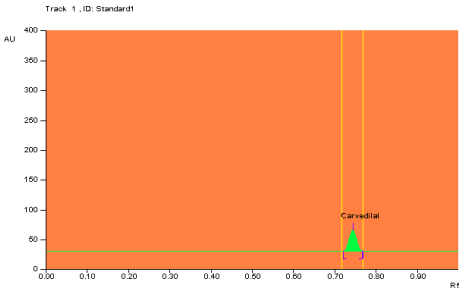
Daylight after mobile phase run



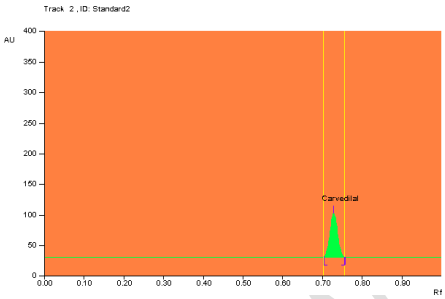
UV 366nm after mobile phase run

UV 254 nm after mobile phase run

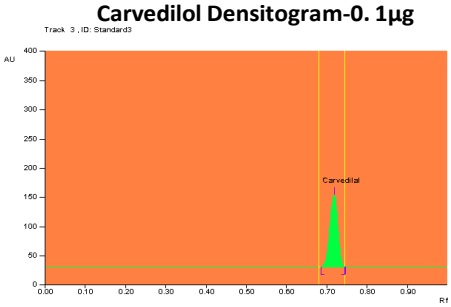
Fig 3: HPTLC Densitogram



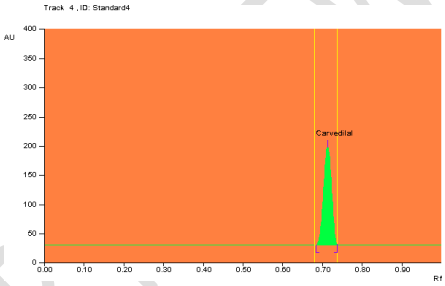
Carvedilol Densitogram-0. 1µg



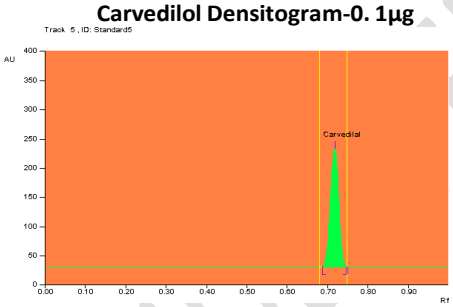
Carvedilol Densitogram-0. 1µg



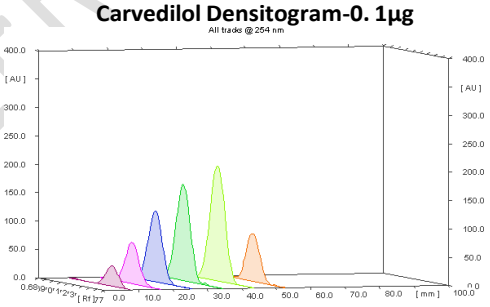
Carvedilol Densitogram-0. 1µg



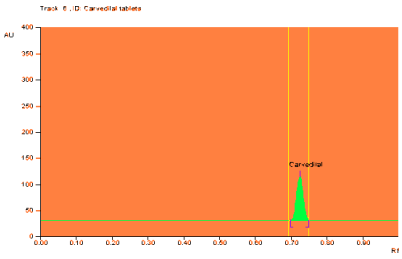
Carvedilol Densitogram-0. 1µg



Carvedilol Densitogram-0. 1µg

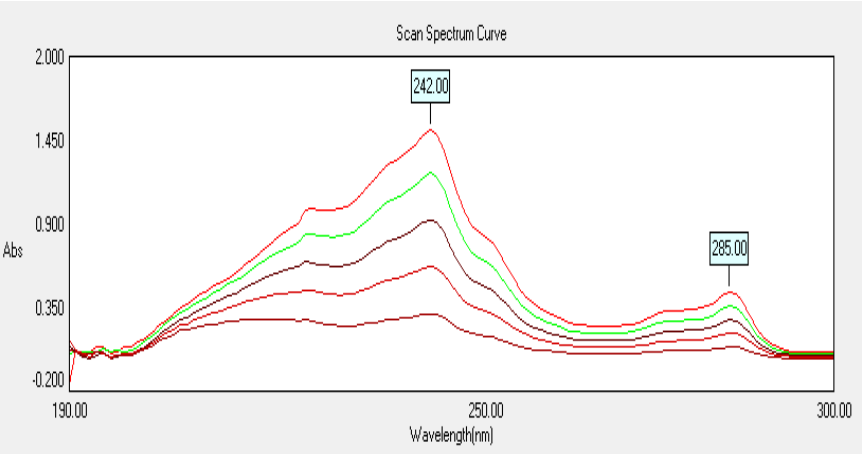


3D display of Peak densitogram – All tracks (Carvedilol standards and sample) @254nm



Carvedilol densitogram -Sample

Fig 4: UV spectrum



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