CHAPTER – 3

VISIBLE SPECTROPHOTOMETRIC AND HPLC METHODS FOR THE ASSAY OF CARVIDILOL IN PURE AND DOSAGE FORMS

3.0. DRUG PROFILE

Carvedilol[1,2]{1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino]-2-propanol} **[Fig.3.01,P.73]** is a nonselective β-adrenergic blocking agent with α1-blocking activity used in the treatment of mild to moderate congestive heart failure (CHF). It is official in European pharmacopoeia [3,4]. Carvedilol is a white to off-white powder with a molecular weight of 406.5 and a molecular formula of $C_{24}H_{26}N_2O_4$. It is freely soluble in dimethylsulfoxide; soluble in methylene chloride and methanol; sparingly soluble in 95% ethanol and isopropanol; slightly soluble in ethyl ether; and practically insoluble in water, gastric fluid (simulated, TS, pH 1.1), and intestinal fluid (simulated, TS without pancreatin, pH 7.5).

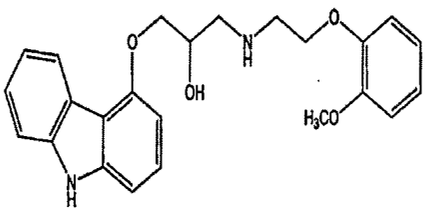


Fig.3.01.Structure of Carvedilol

It is marketed as COREG (carvedilol) 25mg tablet constituting the inactive ingredients such as colloidal silicon dioxide, crospovidone, hypromellose, lactose, magnesium stearate, polyethylene glycol, polysorbate 80, povidone, sucrose, and titanium dioxide.

3.1.1.ANALYTICAL SURVEY

Bera.A[5] et al, Shingbal.DM[6] et al and Sankar.GD[7] et al developed three spectroptometric methods for the determination of of carvedilol in biological samples.

Radi.A and Elmogy.T[8] reported an voltammetric method for the assay of carvedilol in biological samples and only one HPLC method has been reported in biological samples by Placek.P[9] et al.

J.K.Verma[10] et al reported rapid extractive spectrophotometer method for the assay of carvedilol in pharmaceutical formulations. The method is based on the formation of a chloroform soluble ion-pair complex between carvedilol and bromocresol green in an acidic medium. The complex shows absorption maximum at 415nm and the system obeys Beer's law in the concentration range of 5-25mcg/mL

Recently,R.K.Jat[11] et al developed a sensitive and rapid extractive spectrophotometer method for the assay of carvedilol in bulk drug and tablets based on the formation of a chloroform soluble ion-pair complex between carvedilol and bromophenol blue in an acidic medium. This complex showed maximum absorbance at 414nm. Beer's law was obeyed in the concentration range of 5-20mcg/mL.

Based on the above analytical survey the analytically useful functional groups are not completely exploited for the assay of cardivilol in pure and dosage forms and this made the author to choose visible spectrophotomery as an analytical tool for the assay of cardivilol because of its simplicity with reasonable precision and accuracy.

3.1.2.EXPERIMENTAL:

3.1.2.1. APPARATUS:

UV-VISIBLE SPECTROPHOTOMETER: An Elico SL-159 model, 2.0nm high resolution, double beam spectrophtometer (Wavelength range190-1100nm) with 1.0cm matched quartz cells were used for all the absorbance measurements.

3.1.2.2. SOLVENTS & REAGENTS: All the Solvents, materials and reagents used for the assay of carvidilol were of analytical reagent grade and were prepared in double distilled water.

MBTH – Fe(III): Solutions of various reagents such as **MBTH solution** (Fluka; 0.2%), **HCl solution**(Qualigens,1.0M) **Ferric chloride solution** (BDH;0.25%) and **NaOH solution** (Loba, 0.4%, 0.1M)were prepared in the same way as described in **chapter – II (P.34).**

NQS:

NQS solution (Loba; 0.5%, 1.92 x 10^{-2}M)	:	Prepared by dissolving 500mg of NQS in 100mL of distilled water.
NaOH solution (E.Merck; 20%, 5M)	:	Prepared by dissolving 20gm of sodium hydroxide in 100mL of distilled water.

DCQC: Solution of various reagents such as DCQC solution (Fluka; 0.2%) and Buffer solution (pH 9.4) were prepared with double distilled water.

p-CA: Solution of various reagents such as chloranilic acid solution, CA solution (Sd-fine; 0.1%) were prepared in the same way as described in **chapter – II (P.35).**

DDQ: Solution of DDQ,(Fluka; 0.2%) were prepared freshly in the same way as described in **chapter – II (P.35).**

ARS: Solution of various reagents such as ARS solution,(Sd-fine; 0.2%) were prepared in the same way as described under in **chapter – II (P.36).**

TPooo:

Tpooo solution(Fluka; 0.2%)	:	Prepared by dissolving 200mg of Tropaeoline ooo in 100mL of distilled water.
HCl solution (0.1M)	:	8.6mL of conc HCl in 1000mL with distilled water
Chloroform (Qualigens)	:	AR grade of chloroform was used.

3.1.2.2.PREPARATION OF STANDARD STOCK SOLUTION: Standard stock solution of carvidilol was prepared by dissolving 100mg in 100 mL of methanol to get concentration of 1000mcg.mL^{-1}. This solution was diluted appropriately with water to get a

working concentration of **50mcg.mL^{-1}** for **ARS & TPooo; 80mcg.mL^{-1}** for **NQS; 100mcg.mL^{-1}** for **MBTH – Fe(III), 200mcg.mL^{-1}** for **p-CA & DDQ ;250mcg.mL^{-1}** for **DCQC** respectively.

3.1.2.3. PROCEDURE FOR COMMERCIAL TABLETS: Ten tablets were finely powdered and weighed. A portion of the powder equivalent to about 100mg of carvidilol was weighed accurately and transferred into100mL volumetric flask and mixed thoroughly for 20minutes for complete dissolution of carvidilol and then the sample solution was filtered and diluted to 100mL with methanol to get concentration of 1000mcg.mL^{-1} and used for analysis. This solution was diluted appropriately with water to get a working concentration of **50mcg.mL^{-1}** for **ARS & TPooo; 80mcg.mL^{-1}** for **NQS; 100mcg.mL^{-1}** for **MBTH – Fe(III), 200mcg.mL^{-1}** for **p-CA & DDQ ;250mcg.mL^{-1}** for **DCQC** respectively

3.1.2.4.PLACEBO BLANK ANALYSIS: A placebo blank of the composition: starch (10mg), methyl cellulose (10mg), sodium citrate (10mg), magnesium stearate (15mg) and sodium alginate (10mg) was made and then subjected to analysis as described in proposed procedures.

3.1.3.PROPOSED PROCEDURES:

MBTH – Fe(III): Accurately measured aliquots of standard carvidilol solution in the concentration range of (0.5 – 2.5mL; 100mcg.mL^{-1}) were transferred into a series of 10mL volumetric flasks. To each, 0.2mL(0.2%) of MBTH, 0.2mL (0.2%) of ferric chloride were added and allowed to stand for 20 minutes and the volume was made up to the mark with distilled water. The absorbance at 660nm against a reagent blank was measured. The amount of the carvidilol was computed from its calibration graph **(Fig.3.09,P.81).**

NQS: Aliquots of standard carvidilol solution (0.5 – 2.5mL; 80mcg.mL^{-1}) were transferred into a series of 10mL calibrated tubes containing 0.2mL of 0.02N NaOH and 0.2mL of NQS reagent solution was added in each tube and the contents were heated at 50^{0}C for 5min and cooled for 2min in ice water. This operation was performed in the dark. After cooling the contents in the tube with are rinsed 1.0mL of water. Then 0.5mL of con H_2SO_4 was added slowly, mixed and the absorbance were measured after 5min at 512nm against a reagent blank prepared similarly. The amount of carvidilol was calculated from its calibration graph (**Fig.3.10, P.81**).

DCQC: Delivered aliquots of standard carvidilol solution (0.5-2.5mL; 200mcg.mL^{-1}) into a series of 25.0mL calibrated tubes. Then 5.0mL of buffer (pH 9.4) and 2.0mL of DCQC were added successively. The contents were mixed well and kept aside for 10min and diluted upto the mark with distilled water. The absorbance of the colored solution was measured at 465nm against a reagent blank prepared simultaneously. The amount of carvidilol was computed from the appropriate calibration graph (**Fig.3.11, P.81**).

p-CA: Serial volumes of standard solutions of carvidilol ranging from (0.5- 2.5mL; 200mcg.mL^{-1}) were transferred to 20.0mL standard flasks and the volume was brought to 5.0 mL by adding requisite volumes of chloroform. Then, 2.0mL of 0.1% p-CA reagent was added and the volume was brought to 20.0mL with acetonitrile and the absorbance was measured at 528nm against a reagent blank prepared simultaneously. The concentration of the carvidilol was read from the calibration graph (**Fig.3.12,P.81**).

DDQ: Standard carvidilol solution of different aliquots (0.5mL-2.5mL,200mcg.mL^{-1}) were accurately transferred into a series of 20.0mL volumetric flasks and the total volume was adjusted to 3.0mL by adding adequate quantity of acetonitrile to each flask. 2.0mL of 0.2% DDQ solution was added to each flask and the mixture was diluted to the volume with acetonitrile and the absorbance of each solution was measured at 555nm against a reagent

blank. The amount of carvidilol solution present was computed from the calibration graph **(Fig.3.13,P.82).**

ARS & TPooo: Into a series of 125mL separating funnels containing aliquots of standard carvidilol solution (0.5-2.5mL, 50mcg.mL^{-1} for the methods ARS & TPooo), 6.0mL of 0.1M HCI solution and 1.0mL of 0.2% ARS dye solution and 2.0mL 0.2%TPooo dye solution were added successively. The total volume of aqueous phase in each separating funnel was adjusted to 10.0mL with distilled water. To each separating funnel 10mL of chloroform was added and the contents were shaken for 2min. The two phases were allowed to separate and the absorbance of the separated chloroform layer was measured at λ_{max} (440nm for ARS and 490nm for TPooo) against a similar reagent blank. The amount of carvidilol was deduced from the calibration curve of ARS and TPooo **(Figs.3.14 & 3.15, P.82)**

3.1.4.RESULTS AND DISCUSSION:

3.1.4.1.OPTIMIZATION STUDIES OF EXPERIMENTAL PARAMETERS

The spectrophotometric properties of the colored product as well as the different experimental parameters affecting the color development and its stability were carefully studied and optimized for each proposed method. The factors include pH, type of buffer, temperature, time of heating and effect of diluting solvent.

The Optimization studies for the color development for the proposed methods **(MBTH-Fe(III),PCA,DDQ** and **ARS)** for the assay of carvidilol were found to be same as described in **Chapter-II,P.50,54,56.**

NQS: In developing this method a systematic study of the effects of various parameters were under taken by varying one parameter at a time and controlling all other fixed. The effects of

various parameters such as time, volume of NQS & NaOH, solvent for final dilution on the stability and intensity of colored species were studied and the optical condition are incorporated in **TABLE.3.01,P.89.**

DCQC: The effect of various parameters such as the effect of pH, nature and volume of buffer, volume of DCQC, solvent for final dilution and stability of the colored species were studied. The optimum conditions developed and actual conditions chosen for the procedure are recorded in **TABLE.3.02,P.90.**

TPooo: The author performed control experiments by varying one and fixing the other parameters such as type and volume of acid, concentration of dye, organic solvent used for extraction, ratio of organic phase to aqueous phase during extraction, shaking time and temperature in order to establish the optimum conditions for the proposed method **(TABLE.3.03,P.91).**

3.1.4.2.SPECTRAL CHARACTERISTICS:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the each proposed methods **MBTH-Fe(III), NQS, DCQC, PCA, DDQ, ARS** and **TPooo,** specified amounts of carvidilol were taken and colors were developed separately by following the above mentioned procedures individually. The absorption spectra were scanned on a spectrophotometer in the wave length region of 380 to 900nm against similar reagent blank (**Figs.3.02** to **3.08, P.80-81**).

FIG.3.02.ABSORPTION SPECTRA OF CARVEDILOL WITH MBTH – Fe(III)

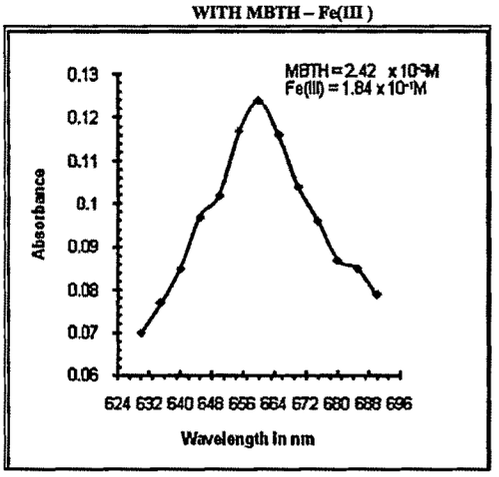


FIG.3.03.ABSORPTION SPECTRA OF CARVEDILOL WITH NQS

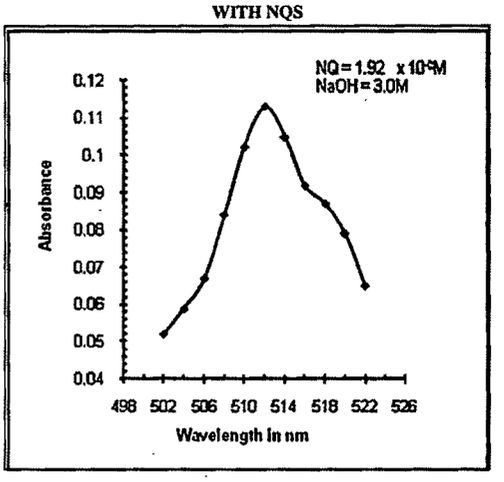


FIG.3.04.ABSORPTION SPECTRA OF CARVEDILOL WITH DCQC

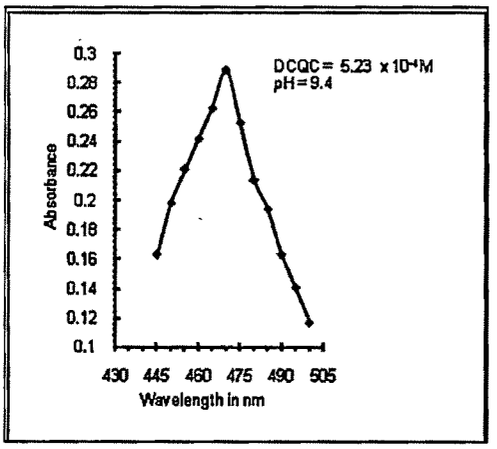


FIG.3.05.ABSORPTION SPECTRA OF CARVEDILOL WITH PCA

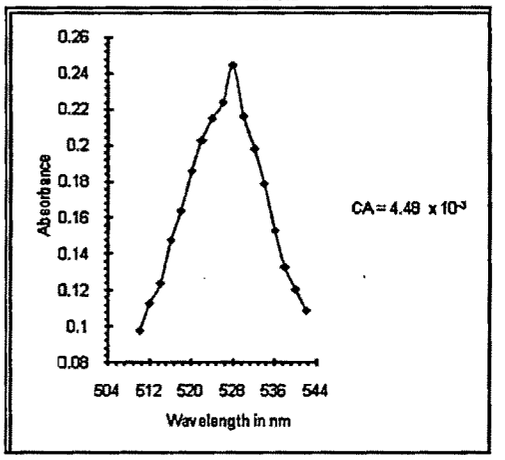


FIG.3.06.ABSORPTION SPECTRA OF CARVEDILOL WITH DDQ

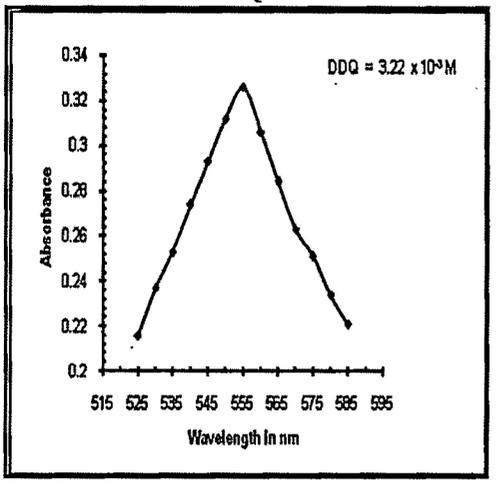


FIG.3.07.ABSORPTION SPECTRA OF CARVEDILOL WITH ARS

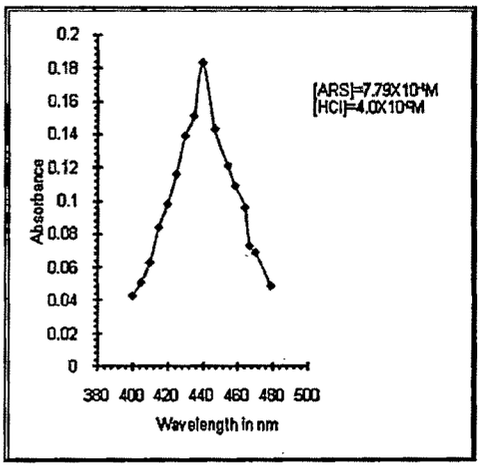


FIG.3.08.ABSORPTION SPECTRA OF CARVEDILOL WITH TPooo

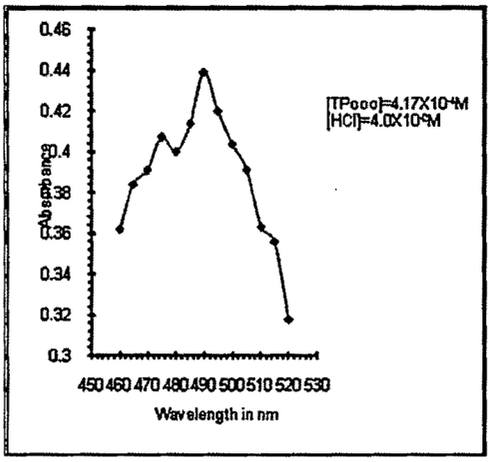


FIG.3.09. BEER'S LAW SPECTRA OF CARVEDILOL WITH MBTH – Fe(III)

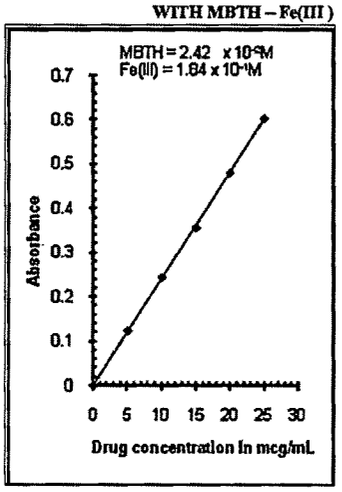


FIG.3.10. BEER'S LAW SPECTRA OF CARVEDILOL WITH NQS

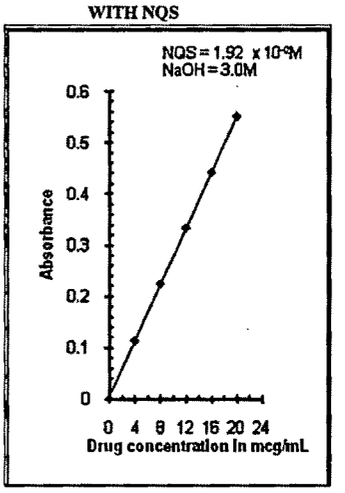


FIG.3.11. BEER'S LAW SPECTRA OF CARVEDILOL WITH DCQC

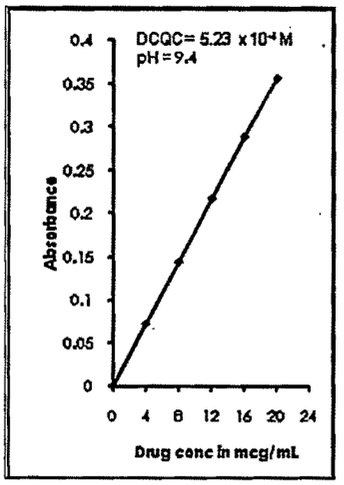


FIG.3.12. BEER'S LAW SPECTRA OF CARVEDILOL WITH PCA

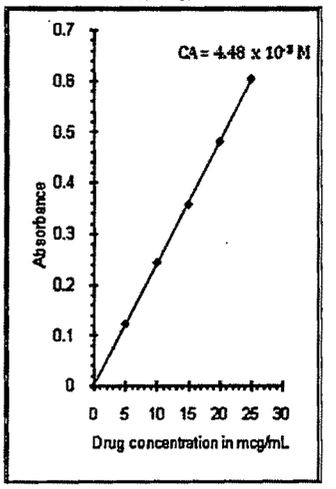


FIG.3.13. BEER'S LAW SPECTRA OF CARVEDILOL WITH DDQ

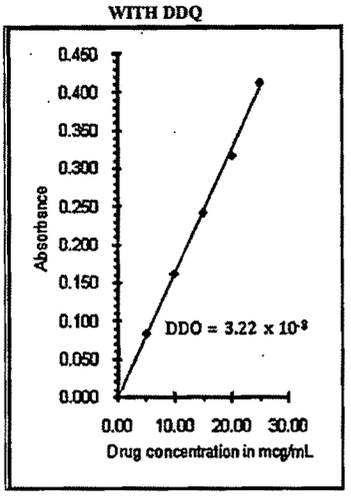


FIG.3.15. BEER'S LAW SPECTRA OF CARVEDILOL WITH ARS

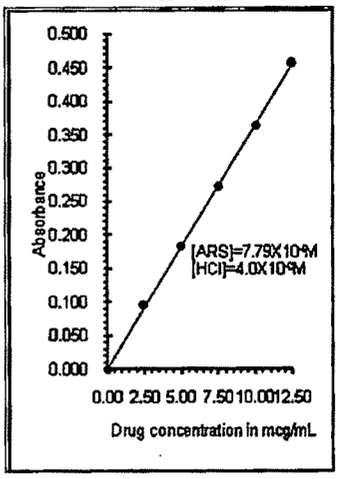


FIG.3.16. BEER'S LAW SPECTRA OF CARVEDILOL WITH Tooo

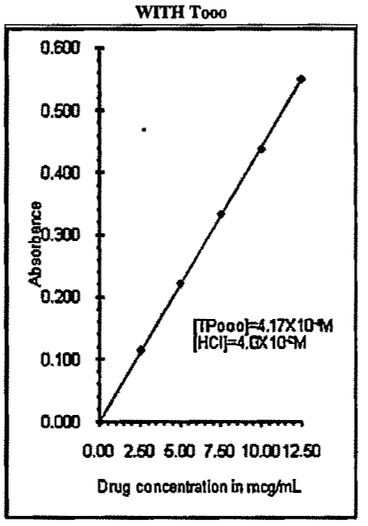


FIG.3.17. RINGBOM SPECTRA OF CARVEDILOL WITH MBTH – Fe(III)

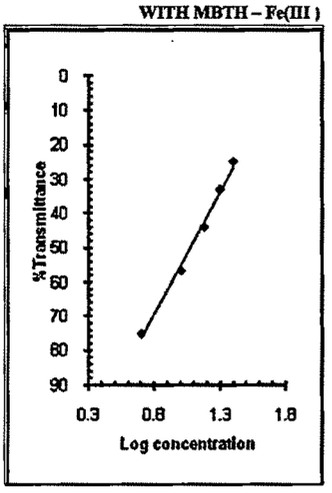


FIG.3.18. RINGBOM SPECTRA OF CARVEDILOL WITH NQS

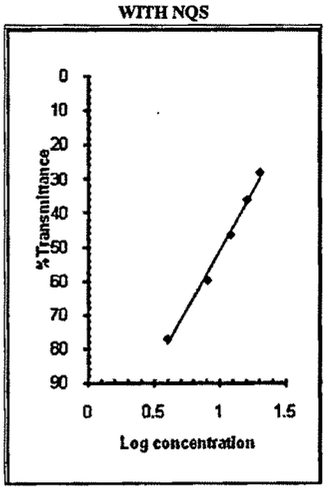


FIG.3.19. RINGBOM SPECTRA OF CARVEDILOL WITH DCQC

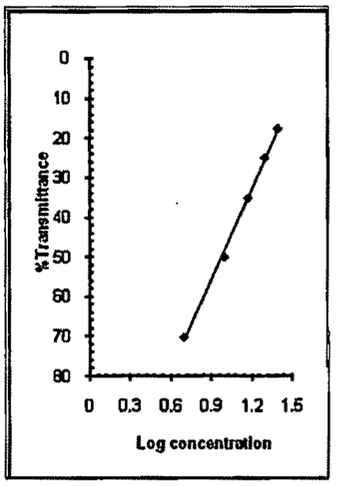


FIG.3.20. RINGBOM SPECTRA OF CARVEDILOL WITH PCA

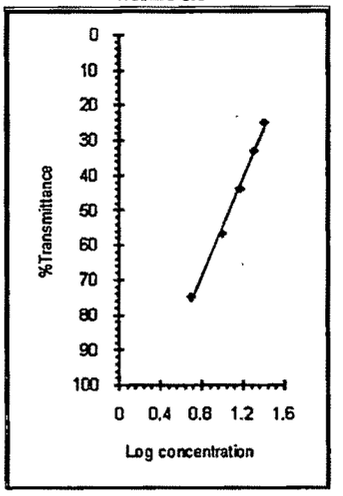


FIG.3.21. RINGBOM SPECTRA OF CARVEDILOL WITH DDQ

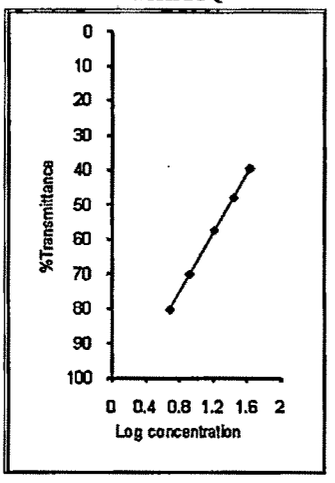


FIG.3.22. RINGBOM SPECTRA OF CARVEDILOL WITH ARS

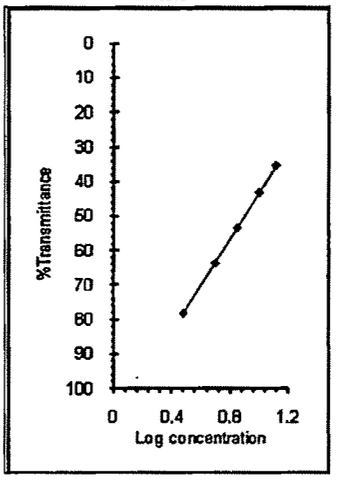
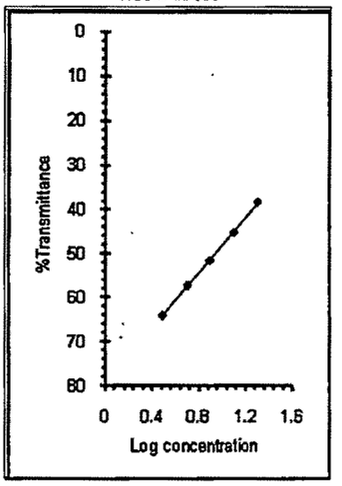


FIG.3.23. RINGBOM SPECTRA OF CARVEDILOL WITH TPooo



3.1.4.3.ANALYTICAL PARAMETERS

3.1.4.3.1.LINEARITY & SENSITIVITY: Under optimum conditions, a linear relation was obtained between absorbance and concentration of carvidilol for the proposed method (**Figs.3.09** to **3.15, P.81-82**).). The calibration graph is described by the equation: Y = a + bX ; Where Y = absorbance, a = intercept, b = slope and X = concentration , obtained by the method of least squares. The optical characteristics such as absorption maxima, Beer's law limits, Molar absorptivity and Sandell's sensitivity standard deviation of slope (S_b), standard deviation of intercept (S_a),standard error of estimation (S_e),% range of error (0.05 and 0.01 confidence limits), LOD and LOQ were calculated for the proposed methods and are summarized in **TABLE-3.04,P.92.**

3.1.4.3.2.PRECISION & ACCURACY: Precision and accuracy of the proposed methods were tested by carrying out the determination of six replicates of pure samples of the carvidilol, whose concentration was within Beer's law range. Values of the standard deviation (SD), relative standard deviation (RSD) and range of error at 95% confidence level were calculated and the results are summarized in **TABLE.3.04,P.92.**

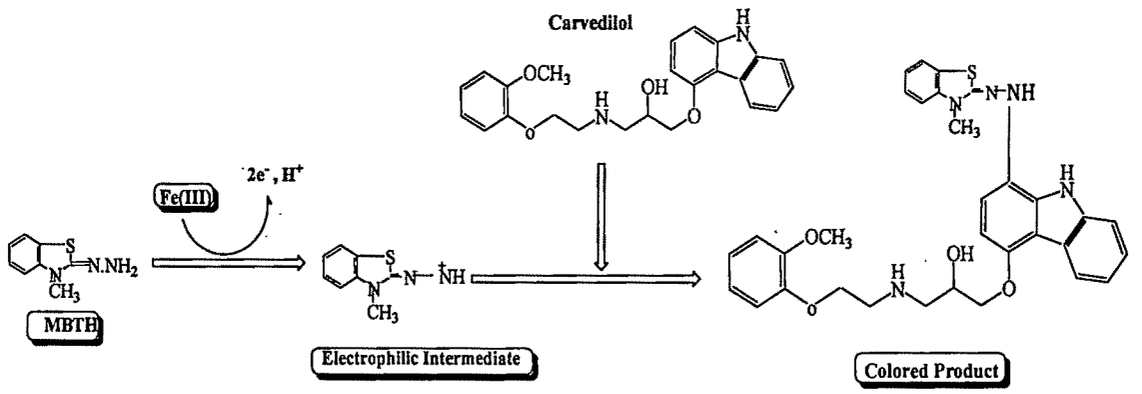
3.1.4.3.3.SELECTIVITY: The proposed methods were tested for selectivity by placebo blank analysIs. A convenient aliquot of the placebo blank solution prepared as described earlier was subjected to analysis according to the recommended procedure. The results of the above analysis indicated that there was no interference by the inactive ingredients for the developed methods (**TABLE not given**).

3.1.4.3.4.APPLICATION TO DOSAGE FORMS: The proposed methods were applied to the analysis of carvidilol in pharmaceutical dosage forms and the results were

statistically compared with reported method by calculating the Student's t- and F-values. The evaluated t- and F-values were less than the tabulated values at the 95% confidence level for eight degrees of freedom, as revealed by the results compiled in **TABLE.3.05,P.93** respectively.

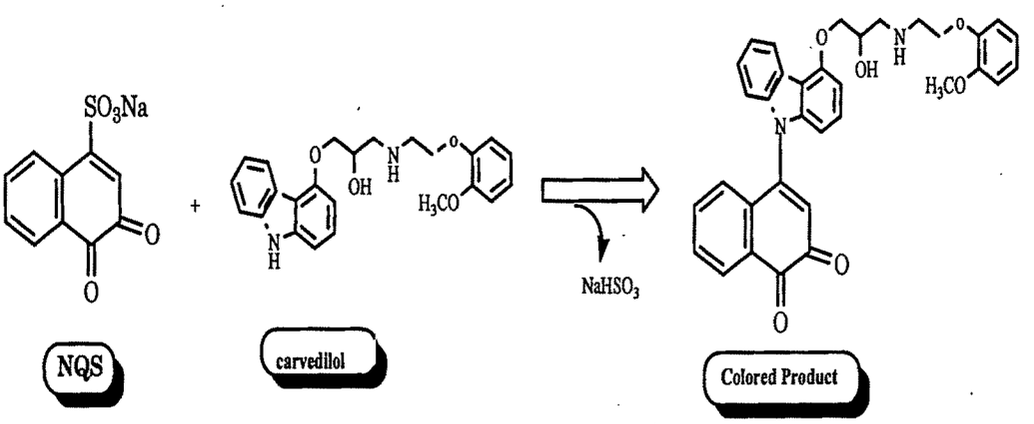
3.1.4.3.5. CHEMISTRY OF COLOR SPECIES:

MBTH -Fe(III): Under the reaction conditions, MBTH on oxidation with Fe(III) loses two electrons and one proton forming and electrophilic intermediate, which is the active coupling species that reacts with the coupler carvidilol by electrophilic attack on the most nucleophilic site on cyclic ring of the coupler para to the nitrogen group. The probable reaction mechanism is based on the analogy which is represented in **Scheme.3.01.**



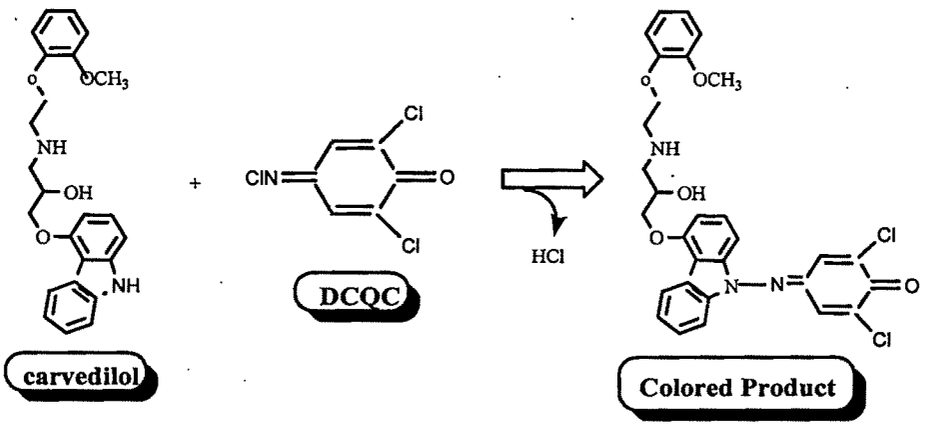
Scheme.3.01: Reaction of carvidilol with MBTH – Fe(III)

NQS: In this method, the presence of secondary amino group of carvidilol permits the development of new spectrophotometric method for its determination through the nucleophilic substitution reaction with NQS. The reaction of carvidilol with NQS is described in **Scheme.3.02.**



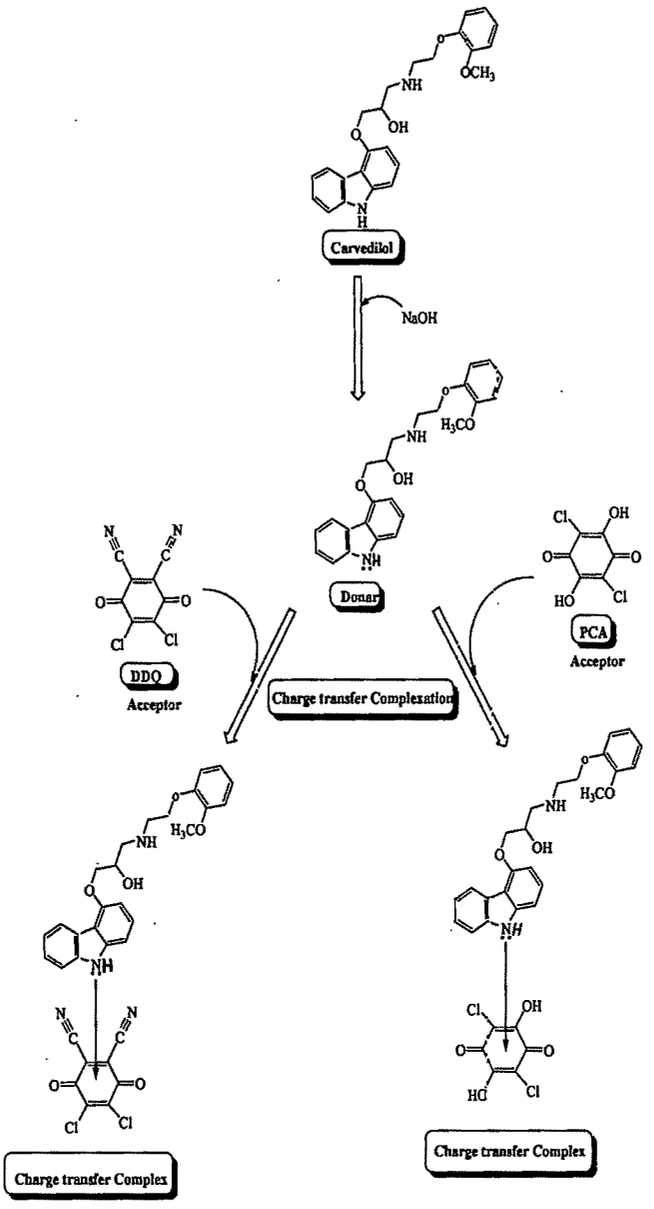
Scheme.3.02: Reaction of carvidilol with NQS

DCQC: The color formation by DCQC with carvidilol (which possesses secondary amino group) was predicted in the **Scheme.3.03.**



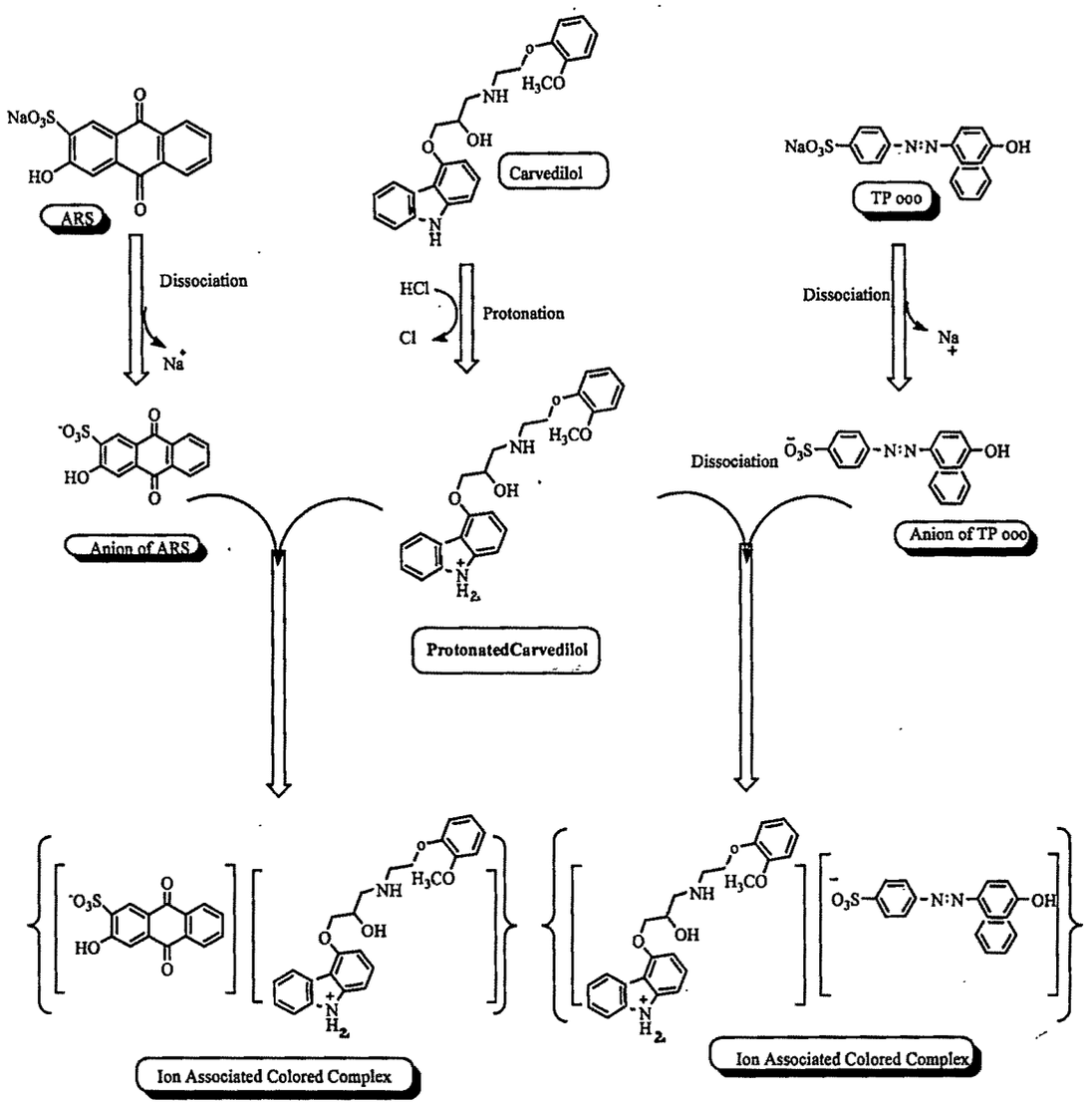
Scheme.3.03: Reaction of carvidilol with DCQC

PCA & DDQ: The present methods are based on charge transfer complexation of carvidilol (n-donor) with π-acceptor chloranilic acid[P-CA] and DDQ rresulting in the formation of original donor-acceptor (DA) complex. The mechanism of reaction is represented in **Scheme.3.04.**



Scheme.3.04: Reaction of carvidilol with Brucine – $NaIO_4$

ARS & TPooo: Carvidilol possesses a secondary amine group which forms extractable ion pair complex with azo-dyes [ARS and TPooo], in acidic medium, which is extractable into chloroform from the aqueous phase. The mechanism of reaction is represented in **Scheme.3.05**.



Scheme.3.05: Reaction of carvidilol with ARS & TPooo

TABLE.3.01

OPTIMUM CONDITIONS ESTABLISHED FOR CARVIDILOL WITH NQS

Parameter	Optimum range	Conditions in proeedure	Remarks
λ_{max} (nm)	480-520	512	--
Effect of ($1.92x10^{-2}$M) NQS on color development	0.25 - 0.75mL	0.5mL	The use of < 0.25mL NQS resulted in a decrease in absorbance, > 0.75mL resulted in cloudiness.
Effect of NaOH (5.0M) on the absorbance of the final colored species	1.4 - 2.8mL	2mL	<1.4mL and>2.8mL was found to disturb Beer's law obeyance in a broad range.
Solvent for final dilution	Water	Water	Other water miscible solvent did not enhance the color of final colored solution.
Stability period after final dilution	immediate- 45 min	8 min	It remains stable upto 45 min.

TABLE.3.02

OPTIMUM CONDITIONS ESTABLISHED FOR CARVIDILOL WITH DCQC

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	455 - 475	465	--------
Effect of buffer pH on color development	Borate buffer 8.5 - 10.5	9.4	Variation of pH beyond the upper and lower limits resulted in low absorbance values
Effect of volume of pH 9.4 buffer.	3.0 - 7.0mL	5.0mL	5.0mL of pH 9.4 buffer solution was necessary for maintaining the pH of the final colored solution.
Effect of volume of (1.9×10^{-3}M) DCQC on the color formation	1.0 - 3.0mL	2.0mL	1mL of DCQC results in low absorbance and >3mL has no advantage.
Solvent for final dilution	Distilled water	Distilled water	Other water miscible solvents such as methanol, ethanol, propanol and acetonitrile were found not to enhance the intensity of the final colored product.
Stability of the final colored species	5 - 30min	5 min	--------

TABLE.3.03

OPTIMUM CONDITIONS ESTABLISHED FOR CARVIDILOL WITH TPooo

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	460-500	490	--
Effect of volume of dye TPooo	0.5-3.0	2.0	2.0mL of TPooo of dye was necessary for covering the broad range of beer's law limits.
Choice of organic solvent for extraction of colored complex	Chloroform	Chloroform	The other water immiscible solvents tested for the extraction of the colored complex into organic phase include chlorobenzene, dichloromethane, CCl_4, C_6H_6 butanol $CHCl_3$ was preferred for its selective extraction of the colored drug-dye complex from the aqueous phase.
Effect of shaking time (min)	1-5	2	Constant absorbance values were obtained for the shaking period of 1-5 min.
Effect of temperature on the colored species (C^0)	Lab-Temp (28±5)	Lab-Temp (28±5)	At low temperature (<20^{0}C) and at high temperature (>35^{0}C) the extraction of the colored species was found to be improper and the stability of the colored species was found to be very less.
Stability of the colored species	Immediate to 60min	7 min	The colored species after separation from organic phase was stable for 60 min, after wards the absorbance gradually decreases.

TABLE.3.04

RESULTS OF OPTICAL AND REGRESSION CHARACTERISTICS OF THE PROPOSED METHODS FOR CARVEDILOL

Parameter	MBTH – Fe(III)	NQS	DCQC	PCA	DDQ	ARS	TPooo
λ_{max} (nm)	660	512	465	528	555	440	490
Beer's law limits (µg/mL)	5.0 - 25.0	4.0 - 20.0	4.0 - 20.0	5.0 - 25.0	5.0 - 25.0	2.5 – 12.5	2.5 – 12.5
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	9.959×10^3	1.138×10^4	7.317×10^3	1.061×10^4	6.585×10^3	1.488×10^4	1.813×10^4
Sandell's sensitivity ($mcg.cm^{-2}$/0.001 AU)	0.0403	0.03579	0.0555	0.0378	0.0617	0.02688	0.02192
Optimum photometric range (mcg/mL)	5.0 - 18.0	5.0 - 17.5	6.5 - 17.5	6.0 – 20.0	5.0 – 20.0	3.0 – 9.5	3.0 – 10.0
Regression equation (Y=a+bc) ;slope (b)	0.0239	0.0274	0.01782	0.02604	0.0159	0.03628	0.04352
Intercept (a)	0.0036	0.0042	0.0017	0.0012	0.0011	0.0017	0.0056
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9999	0.9998	0.9999	0.9999
Standard Deviation on Slope(S_b)	0.000205	0.000163	0.000160	0.000056	0.000166	0.000100	0.000201
Standard Deviation on Intercept(S_a)	0.003404	0.002166	0.02123	0.000938	0.002755	0.000834	0.00166
Standard Error on Estimation(S_e)	0.003246	0.00206	0.00202	0.0008944	0.00262	0.0007958	0.00159
Relative standard deviation (%)*	1.004	1.189	1.159	0.658	1.312	0.657	0.623
% Range of error (confidence limits)							
0.05 level	0.840	0.995	0.969	0.550	1.097	0.532	0.521
0.01 level	1.242	1.471	1.434	0.814	1.023	0.821	0.771
LOD	0.4696	0.2604	0.3931	0.1188	0.5689	0.0759	0.1265
LOQ	1.423	0.7892	1.1914	0.3602	1.7240	0.2300	0.3885

* Average of six determinations considered

TABLE.3.05

ASSAY AND RECOVERY OF CARVEDILOL IN PHARMACEUTICAL FORMULATIONS

Method	Pharmaceutical Formulation	Labelled Amount (mg)	Proposed Method			Found by reference method[11] ±S.D	% recovery by proposed methods**±S.D
			Amount found* (mg) ±S.D	t (value)	F (Value)		
MBTH-Fe(III)	COREG	25	24.95 ± 0.16	0.346	2.25	24.99 ± 0.24	99.83 ± 0.603
NQS		25	24.96 ± 0.18	0.247	1.77		99.87 ± 0.346
DCQC		25	24.94 ± 0.16	0.433	2.25		99.79 ± 0.736
PCA		25	24.97 ± 0.26	0.138	1.17		99.91 ± 0.585
DDQ		25	24.95 ± 0.20	0.314	1.14		99.83 ± 0.436
ARS		25	24.93 ± 0.22	0.527	1.19		99.75 ± 0.126
TPooo		25	24.96 ± 0.24	0.216	1.0		99.87 ± 0.364

*Average ± standard deviation of six determinants the t and F- values refer to comparison of the proposed method. Theoretical values at 95 % confidence limits t = 2.262 and F = 5.05.

** Average of six determinations.

3.1.5.CONCLUSION:

The proposed methods developed by the author were found to be simple, selective and sensitive. The results of statistical parameters and recovery studies clearly indicated the reproducibility and accuracy of the proposed methods. Analysis of the authentic samples containing carvidilol showed no interference from the common excipients. The spectrophotometric methods developed by the author are more sensitive than the existing spectrophtometric and HPLC methods, and are free from experimental variables such as heating or extraction step. The proposed methods rely on the use of simple and cheap chemicals and provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternative methods for rapid and routine determination of pure and tablets as a part of industrial quality control.

3.2.0. DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ANALYSIS OF CARVIDILOL IN PHARMACEUTICAL FORMULATIONS

3.2.1.ANALYTICAL SURVEY

Literature survey revealed that only one HPLC[7] method was developed and applied in the determination of carvidilol in biological fluids. No validated HPLC methods for quantitative determination of carvidilol in bulk drug samples and formulations were reported till date. The aim of this study was to develop a RP-HPLC method, which could be employed for the routine analysis of the drug in pharmaceutical dosage forms using simple mobile phase composition.

3.2.2.EXPERIMENTAL

3.2.2.1. CHEMICALS AND MATERIALS:

The pharmaceutical grade pure sample of carvidilol (99.28%) was procured from CELON Laboratories limited, Andrapradesh. Acetonitrile solvent of analytical grade was obtained from E Merck Ltd, Mumbai, India. Orthophosphoric acid AR grade was procured from Qualigens Fine Chemicals, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system, sonicated and used.

3.2.2.2. INSTRUMENTATION:

The development and validation of the method was performed on a isocratic HPLC system (PEAK) consisting of Isocratic liquid pump, LC 8200 variable wavelength UV detector with Millennium® version 32 software on a Dell computer. The analytical column used to achieve chromatographic separation was a stainless steel YMC Pro C_8 RP column (4.6mmx150mm) purchased from Waters Corporation (Bedford, MA, USA) protected by a guard column of the same material.

3.2.2.3. STANDARD STOCK SOLUTION:

An accurately weighted sample of 10mg of carvidilol was dissolved in methanol to give standard stock solution of 100mcg/mL. A series of working standard solutions (5.0mcg/mL – 25mcg/mL were obtained by diluting the stock solutions with mobile phase (acetonitrile and Potassium dihydrogen phosphate buffer (pH-2.0) in the ratio of 31:69v/v). All the volumetric flasks containing carvidilol were wrapped with aluminium foil and stored in the dark.

3.2.2.4. PREPARATION OF TABLETS SOLUTION:

An average of ten tablets of carvidilol were weighed and ground to fine powder. Accurately weighed powder sample equivalent to 10mg of carvidilol was dissolved in methanol in a 100mL volumetric flask. The flask was placed in an ultrasonic bath at room temperature for 10min. After sonication, the solution was allowed to stand for 5.0min. 1.0mL was transferred into a 100mL volumetric flask and diluted to the mark with mobile phase. A sample of 0.5µLof this solution was directly injected. The average content of the tablets was determined either from the calibration graph or using the corresponding regression equation.

3.2.3.RESULTS AND DISCUSSION

3.2.3.1.CHROMATOGRAPHIC CONDITIONS:

The mobile phase was filtered by passing through a 0.45µm membrane filter (Millipore, Bedford, MA, USA).Chromatographic analysis was carried out at ambient temperature. The compound is separated isocratically with a mobile phase consisting of acetonitrile, water and Potassium dihydrogen phosphate buffer(pH-2.0) in the ratio of 31:69v/v). The flow rate was 1.5mL/min. The eluent was monitored spectrophotometrically

at a wavelength of 240nm. The optimized chromatographic conditions for the determination of carvidilol are represented in **TABLE.3.06,P.97.**

TABLE.3.06

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Chromatographic Parameters	PEAK HPLC
Elution	Isocratic
Mobile phase	Potassium dihydrogen phosphate buffer (pH-2.0) and acetonitrile in the ratio 69:31
Column	YMCPro C_8 RP (4.6 mm i.d x 150 mm)
Flow rate	1.5 mL/ min
Detection	UV at 240 nm
Injection volume	10 micro liters
Temperature	Ambient
Retention time	4.773 minutes
Run time	20 minutes
Area	81812.4 mAU
Concentration	10.0mcg/mL
Pressure	20-25 Mpa

3.2.3.2. METHOD DEVELOPMENT:

Several tests were performed in order to get satisfactory separation-resolution of carvidilol in different mobile phases with various ratios by using C_{18} column. The ideal mobile phase used is acetonitrile and Potassium dihydrogen phosphate buffer(pH-2.0) in the ratio of 31:69v/v to obtain satisfactory and good resolution. The retention of carvidilol on analytical column was evaluated at a flow rate of 1.5mL.min^{-1}. The injection volume was 10µL. The typical chromatogram of sample solution of carvidilol is shown in **Fig.3.24,P.98.** The retention time of standard and sample for carvidilol was satisfactory with good resolution.

Fig.3.24.Typical chromatogram of of carvidilol

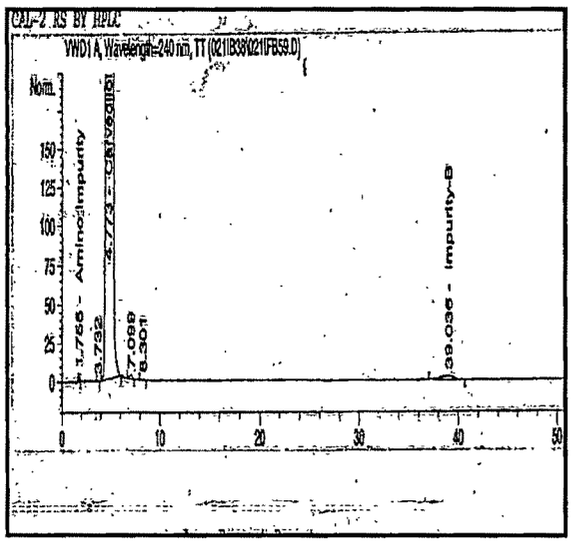
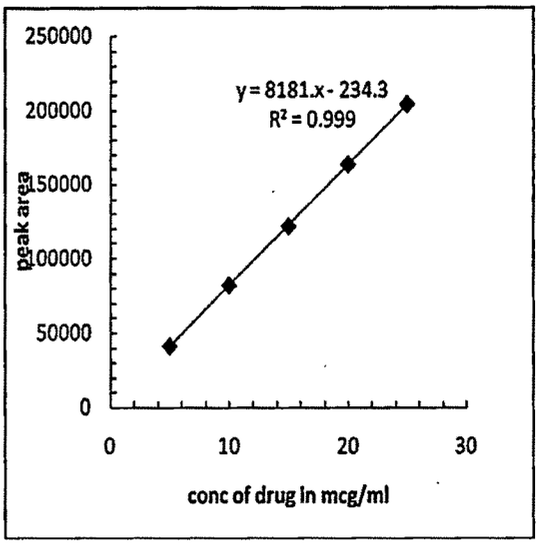


Fig.3.25.Linaerity graph of carvidilol



3.2.3.3.METHOD VALIDATION:

3.2.3.3.1.LINEARITY:

The linearity for HPLC method was determined at five concentration levels ranging from 5.0 - 25.0mcg.mL^{-1} for carvidilol. The calibration curve was constructed by plotting response factor against concentration of carvidilol (**Fig.3.25,P.98**). The slope and intercept value for calibration curve were **Y = 8181X – 234.3 (R^2= 0.9998)** for carvidilol, where **Y** represents the peak area of analyte and **X** represents analyte concentration. The results indicated that significant correlation exists between response factor and concentration of drug within the concentration range indicated on Y-axis (**TABLE.3.07,P.99**).

3.2.3.3.2.SENSITIVITY:

The Limit of Detection (LOD) was determined as lowest concentration giving response and Limit of Quantification (LOQ) was determined as the lowest concentration analyzed with accuracy of the method determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of

Detection (LOD) and the Limit of Quantification (LOQ) for carvidilol were found to be 0.2556mcg.mL^{-1} and 0.7756mcg.mL^{-1} respectively.

TABLE.3.07

CALIBRATION OF THE RP HPLC FOR THE ESTIMATION OF CARVIDILOL

Parameters	Results
Concentration in µg.mL^{-1}	**Peak Area (mAU)**
5.0	40806.2
10.0	81812.4
15.0	121547
20.0	163625
25.0	204531
Regression equation Y = b X + a Slope (b)	8181.25
Intercept (a)	-243
Correlation coefficient	0.9998
Standard deviation on intercept (S_a)	634.56
Standard deviation on slope (S_b)	38.26
Standard error on estimation (S_e)	605.03

3.2.3.3.3.PRECISION:

The precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in **TABLE.3.08,P.100.** From the data obtained, it is found that the developed RP-HPLC method was found to be precise.

TABLE.3.08

PRECISION DATA

Day	Precession Area Mean*
Day-1	81812.4
	81634.9
	80993.7
	82004.6
	81523.2
	81774.3
Average	81624.7
% RSD	0.427

***Average values of six determinations**

TABLE.3.09

RECOVERY STUDIES OF THE PROPOSED HPLC METHOD

Labled amount mcg/mL	Amount added mcg/mL	Total amount mcg/mL	Amount found mcg/mLl	% of Recovery	Mean
10	5	15	14.95	99.66%	99.83%
10	10	20	19.98	99.99%	
10	15	25	24.96	99.88%	

All the values are the averages of three determinations

3.2.3.3.4.ACCURACY [RECOVERY STUDIES]:

Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated .The percentage of individual drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be **99.66 ± 0.120** to **99.99 ± 0.132** reported in **TABLE.3.09,P.100.** The results of analysis **(TABLE.3.10,P.101)** shows that the amounts of drug were in good agreement with the label claim of the formulations.

3.2.3.3.5.RUGGEDNESS AND ROBUSTNESS

Ruggedness test was determined between two instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

TABLE.3.10

RESULTS OF RECOVERY STUDIES OF TABLET CONTAINING CARVIDILOL

Pharmaceutical formulation	Amount of Carvedilol		% of recovery
	Labelled	Found*	
Tablet – 1	25 mg	24.97 mg	99.88 %

*Average of three determinations

3.2.4.CONCLUSION

The RP-HPLC method developed for quantitative determination of carvidilol is linear, accurate, precise, rapid and specific, which make it versatile and valuable in many applications, specifically in pharmacokinetic studies, kinetics studies. The RP-HPLC method was fully validated showing satisfactory data for all method validation parameters tested. The developed method can be conveniently used by quality control department to determine the related substance and assay in regular carvidilol production samples and also stability samples.

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