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Development and validation of RP-HPLC method for determination of carvedilol in bulk and pharmaceutical dosage forms

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for assay of Carvedilol in bulk drug and pharmaceutical dosage forms. Isocratic elution at a flow rate of 1mL/min was employed onHypersil ODS C18, 150 X 4.6, 5µ; Column, mobile phase consisting of Potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate and Acetonitrile in the ratio (50: 50 v/v) adjusted pH 3.0 with diluteorthophosphoric acid solution. The ultraviolet detection wavelength was at 240nm. The method was validated forLinearity,Precision,Accuracy, Ruggedness and Robustness as per ICH Guidelines. The LOD and LOQ have also been established and found to be 0.8346µg/mL and 2.5292 µg/mL. The validated method can be successfully applied for the estimation of Carvedilol inpharmaceutical dosage forms.

Keywords: Carvedilol, RP-HPLC, Validation, Assay, 240 nm

INTRODUCTION

Carvedilol (Fig.1.),or (±) –1- (carbazole – 4- yloxy)-3-[[2-(o-methoxyphenoxy) ethyl]amino]-2-propanol, is an antihypertensive agent with beta – and alpha -1-adrenergic receptor blocking activities [1-3].Carvedilol has much greater antioxidant activity than other commonly used beta-blockers [4-5]. It hasbeen prescribed as an antihypertensive agent and an angina agent [6-7] and for treatment of congestiveheart failure [8].However, to date this theoretical benefit has not been

established in clinical trials, and the current version of the ACC/AHA guidelines on congestive heart failure management does not give preference to Carvedilol over other beta-blockers. The most common side effects include dizziness, fatigue, hypotension, diarrhea, asthenia, bradycardia, and weight gain [9]. A case report of a patient with panic disorder associated sleep disturbances and nightmares with the improper usage of Carvedilol [10].Carvedilol has enantiomers with distinct pharmacodynamics [11]. The term "racemic Carvedilol" is sometimes used to explicitly denote that both enantiomers are applied.[12].

Carvedilol is indicated in the management of congestive heart failure (CHF), as an adjunct to conventionaltreatments (ACE inhibitors and diuretics). The use of Carvedilol has been shown to provide additional morbidityand mortality benefits in CHF[13].Carvedilol (Carvil) is available at the following doses 3.125 mg (smallest), followedby 6.25 mg,12.5 mg, and 25 mg white tablets. OnJanuary 10, 2006 Carvedilol supply became limited in the UnitedStates, due to changes in documentation procedures at a plant. This was lifted on April 27, 2006

in a Dear Pharmacist letter[14].On October 20, 2006, the FDA approved a controlled release formulation of Carvedilol; it is marketed as Coreg CR.

Several analytical methods such as High-performance liquid chromatography (HPLC) with fluorescence detector [15-20], massspectrometer [21-22] or electrochemical detection [23] has been used for the analysis of Carvedilol and its enantiomers in biological samples. Determination of Carvedilol by capillary electrophoresis has also been reported [20,24]. There have been few published articles on the evaluation of Carvedilol in pharmaceutical formulations using HPLC with UV detector [25-27] and differential pulse voltammetric determination [28] have been presented. Hence an attempt has been made to develop new HPLC method which is simple, rapid, reproducible and economical method for estimation of Carvedilolin tablet dosage form. This method has been successfully used for quality-control analysis of drugs and for other analytical purposes.

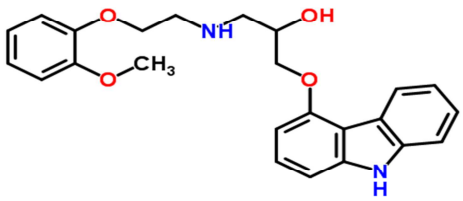
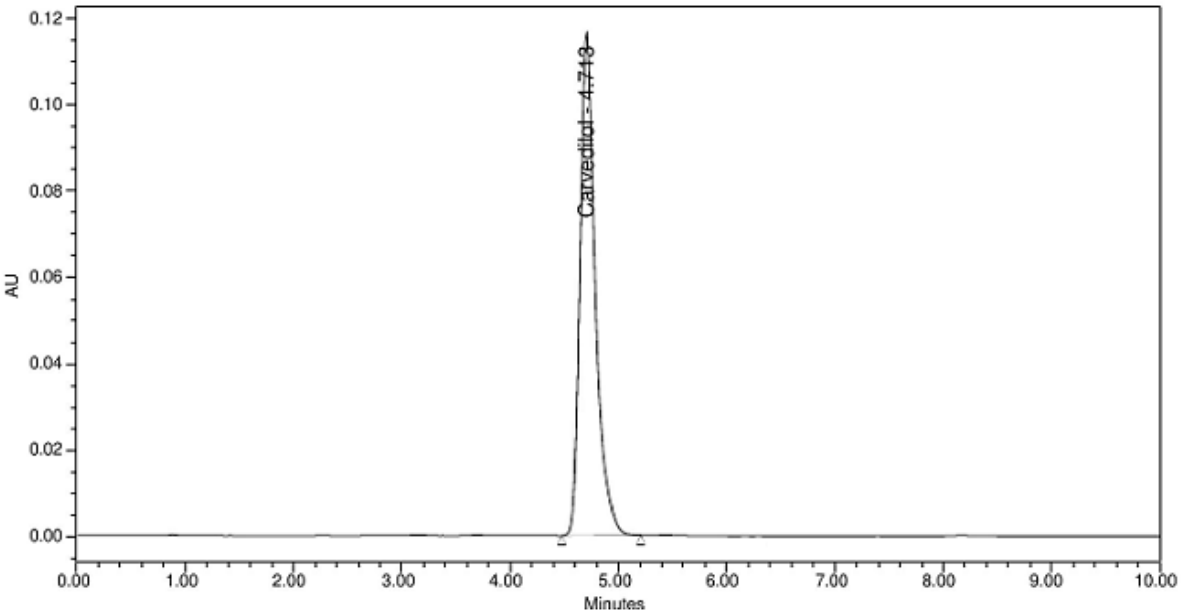


Figure-1: Structure of Carvedilol



	Peak Name	RT	Area	% Area	USP Plate Count	Symmetry Factor	Height
1	Carvedilol	4.713	1151965	100.00	5789.7	1.40	116731

Figure-2: Chromatogramof Carvedilol

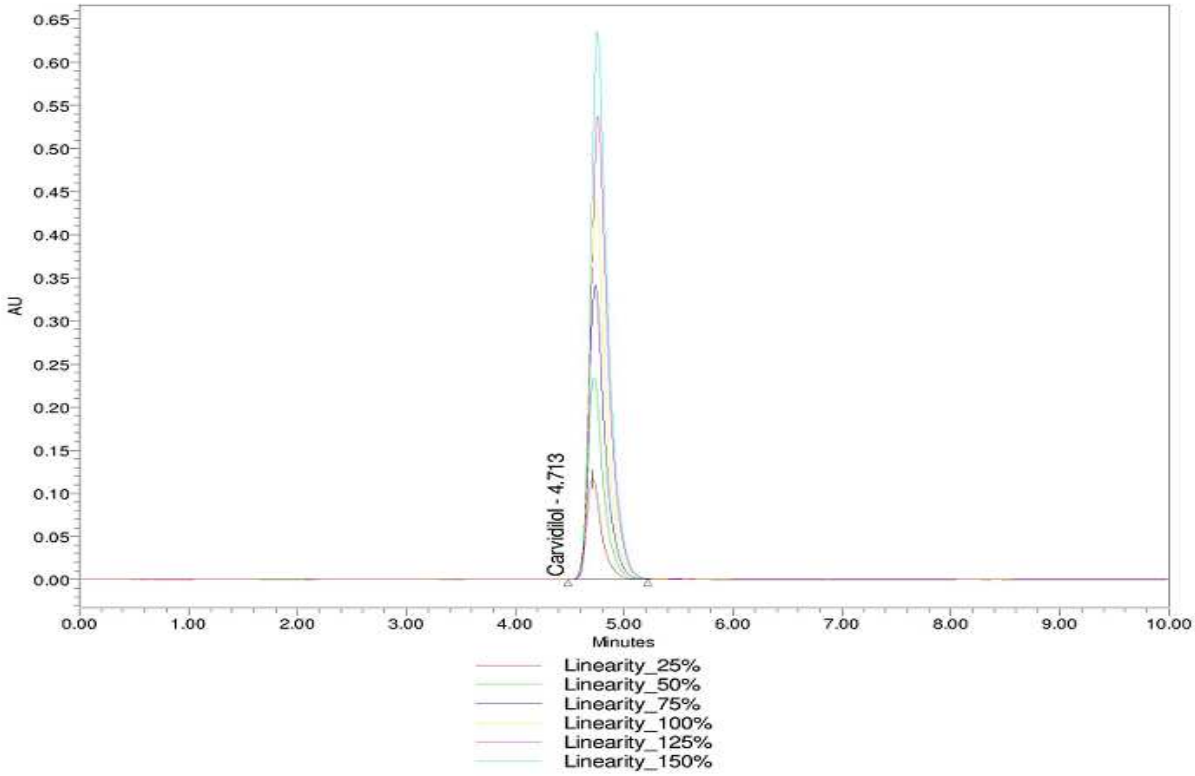


Figure-3: Overlay Report of Carvedilol

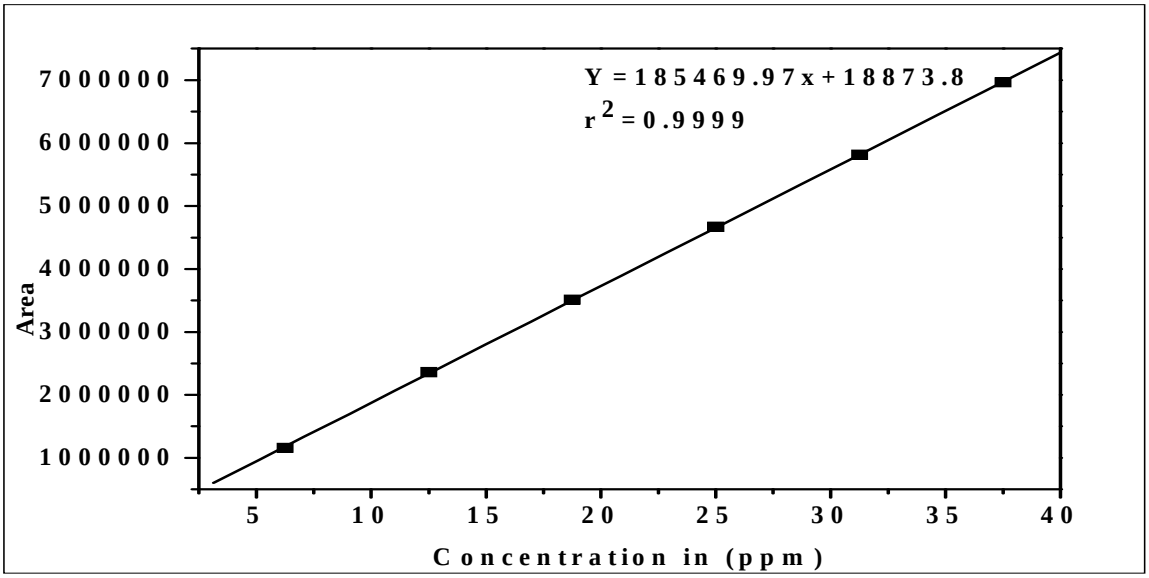


Figure -4: Linearity of Carvedilol

Drugs and chemicals used

Carvedilol pure sample of Car 250 mg were obtained as a gift sample from Hetero drugs limited, Hyderabad, Andhra Pradesh, India. The solvent Acetonitrile (HPLC grade) purchased from SR Scientifics Private Limited (Tirupati, India). Other chemicals and reagents such as potassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and phosphoric acid, were of AR grade obtained from Bros Scientifics. Tirupati, Andhra Pradesh,

India. Purified HPLC grade water prepared by using 0.45 Millipore Milli-Q waterPurification system was used throughout the experiment.

PREPARATION OF SOLUTIONS AND REAGENTS

Mobile phase

Based on the solubility and chemical properties of the drugs, a mobile phase consists of mixed buffer, acetonitrile in the ratio of 50:50 v/v. Mixed buffer was prepared by dissolving accurately weighed 2.72 g Potassium dihydrogen orthophosphate and 0.5 gms of dipotassium hydrogen phosphate in 1000ml water, adjusted to p^H 3.0 with dilute orthophosphoric acid solution of HPLC grade water. Sonicate the resulting solution and degas it using vacuum filtration through 0.4 micron membrane filter.

Standard stock preparation

Weigh and transfer **25** mg of Carvedilolworking standard into 100 mL volumetric flask, add 100 mL of diluent and sonicate to dissolve and dilute to volume with diluent. Transfer10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Preparation of Sample solution

Weigh and transfer **25** mg of Carvedilol sample into 100 mL volumetric flask, add 100 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

SPECIFICITY

Standard Solution Preparation

Weigh and transfer **25** mg of Carvedilolworking standard into 100 mL volumetric flask, add 100 mL of diluent and sonicate to dissolve and dilute to volume with diluent. Further transfer10 mL of above solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Solution: Use assay solution as sample preparation.

Blank Preparation: Use diluent as Blank solution.

Procedure: Inject Blank, Individual standards, mixed standard and sample Solution.

PRECISION

System Precision

Preparation of solution

Dilute 10 ml of standard stock solution with 100 mL of diluent.Inject the above solution six times.

Method Precision

Preparation of solution

Dilute 10 ml of standard stock solution, with 100 mL of diluent.Prepare six solutions and inject each solution.

Acceptance criteria

The % of RSD for Area and RT from Repeated injections should not be more than 2.0**.**

LINEARITY

The Linear detector response for CAR drug is demonstrated by concentration verses Area obtained by linear sample preparations. Over the range of 25 to 150% with respect to the target concentration (Dosage).

ACCURACY

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard at three concentration levels and each concentration is injected three times (Like 50% 100% and 150%).

Acceptance criteria

The % of recovery should be between 98 to 102%.

RUGGEDNESS: The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days.

Acceptance criteria
The % RSD of areas from six injections should not be more than 2.0**%**

ROBUSTNES: The robustness of test method is demonstrated by carrying out method variations like mobile phase flow changes, mobile phase compositions and column oven temperature variations etc…

The result should show some variation from standard results.

Acceptance criteria
The % of RSD of areas & RTs from repeated injections should not be more than 2.0 **%**

ASSAY
Standard préparation
Transfer 10 ml of standard stock solution in to 100 mLvolumetricflask and make up to volume with diluent.

Sample Preparation
Transfer sample equ. to 25 mg of CAR in to 100 mL volumetric flask add 100 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure
Inject 20 µL of blank solution, standard solution, and sample solution finally record the chromatogram and calculate percentage of assay.

CHROMATOGRAPHIC CONDITIONS
Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature. The data was recorded using Waters (alliance) HPLC System is equipped with Empower software-2 software Separation was performed on a 150 X 4.6, 5µ particle size Hypersil ODS C_{18} column. Mobile phase consisting of a mixed buffer, acetonitrile (50:50 v/v), pH3.0 adjusted with orthophosphoric acid. Flow rate was kept at 1.0 mL/min. Wavelength was set at 240 nm.

METHOD VALIDATION
The method was validated as per ICH guidelines for specificity, linearity, quantification limit, precision, accuracy, recovery and stability. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the excipients at the retention times of CAR. The accuracy of the method was determined by recovery experiment. The precision of the method was demonstrated by interday and intraday variation studies, six repeated injections of standard and sample were made and percentage RSD was calculated. In the intraday variation studies six repeated injections of standard and sample solutionwas carried out by injecting on the same day at different intervals and percentage RSD was calculated. In the inter day variation studies six repeated injections of standard and sample solution were made for three consecutive days and percentage RSD was calculated. The linearity of the method was demonstrated at six concentration levels of the mixed standards of CAR.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions
In order to develop an isocratic reverse phase HPLC method for the determination of CAR in single dosage form the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that potassium dihydrogen orthophosphate and dipotassium hydrogen phosphate buffer, pH 3.0 adjusted with orthophosphoric acid give better peak shape than other buffers. Hypersil ODS C_{18}, 150 mm x 4.6 mm, 5 µm column was used. The analyte gave better response at 240 nm wavelength using UV detector. The flow rate

was kept 1.0 mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention time of CARwas found to be 4.713min. Chromatogram of Carvedilol is as shown in fig-2.

VALIDATION

The proposed method was showed short elution time. The system suitability test was performed as per the USP and international conference of harmonization (ICH) guidelines to confirm the suitability and the reproducibility of the method. Six consecutive injections of the standard solution were performed and evaluated for repeatability, tailing factor, theoretical plates and resolution. 0.25 %RSD value was found to be CAR. The tailing factor and theoretical plates were found to be perfectly within the limits. The method was linear over the range 25-150 µg/mL of Carvedilol the calibration curve was constructed by plotting response factor against concentration of drug. The slope and intercept value for calibration curve was Y= 1854769.97x+18873.8 (r^2=0.9999) (the linearity curve as shown in fig-4) shows that an excellent correlation between response factor and concentration of drug. The limit of detection (LOD) and limit ofquantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solution. The LOD was found to be 0.8346µg/mL and LOQ is 2.5292µg/mL.

The system precision study was performed to determine the repeatability of the method. Six samples of standard were prepared at 50%, 100% & 150% levels and assayed according to the procedure. The method precision study was performed to determine the reproducibility of the method. Six samples of tablets were prepared at 50%, 100% &150% levels and assayed according to the procedure. The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level, 10 and 20 of the standard drug of analyte was added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for Carvedilol from the combination formulation are shown in Table 4. The results are well within the acceptance limit and hence the method isaccurate. The tailing factor and theoretical plates were found to be perfectly within the limits. The system precision and method precision results are shown in tables -2 & 3.Ruggedness, Assay and Robustness results are shown in tables-5, 6, 7, 8&9.

Table: 1. Linearity of Carvedilol

%	Conc(mcg)	Area
25	6.2500	1151965
50	12.5000	2355430
75	18.7500	3509530
100	25.0000	4665089
125	31.2500	5814351
150	37.5000	6959812

Table: 2. System Precession ofCarvedilol

S.No	Name	RT	Area
1	Injection-1	4.735	4688244
2	Injection-2	4.732	4719780
3	Injection-3	4.726	4717149
4	Injection-4	4.723	4714980
5	Injection-5	4.727	4718854
6	Injection-6	4.727	4712065
StdDev(±)		0.004	11892.1
%RSD		0.092	0.25

Table: 3. Method precession of Carvedilol

S.No	Name	RT	Area
1	Injection-1	4.733	4699456
2	Injection-2	4.736	4701756
3	Injection-3	4.731	4709588
4	Injection-4	4.73	4703346
5	Injection-5	4.729	4713254
6	Injection-6	4.723	4706584
StdDev(±)		0.004	5158.2
%RSD		0.092	0.11

Table: 4. Accuracy of Carvedilol

Accuracy -50%		Accuracy-100%		Accuracy-150%	
S.No	**Area**	**S.No**	**Area**	**S.No**	**Area**
Injection-1	2352098	Injection-1	4666176	Injection-1	6960164
Injection-2	2348905	Injection-2	4664024	Injection-2	6963299
Injection-3	2347094	Injection-3	4667855	Injection-3	6956075
Avg	2349366	Avg	4666018	Avg	6959846
Amt Recovered	**50.31**	Amt Recovered	**99.97**	Amt Recovered	**149.05**
%Recovery	**100.63**	%Recovery	**99.97**	%Recovery	**99.37**

Table: 5. Ruggedness of CarvedilolDay-1

S.No	Name	RT	Area
1	Injection-1	4.733	4699456
2	Injection-2	4.736	4701756
3	Injection-3	4.731	4709588
4	Injection-4	4.73	4703346
5	Injection-5	4.729	4713254
6	Injection-6	4.723	4706584
Avg		4.730	4705664
StdDev(±)		0.004	5158.2
%RSD		0.092	0.11

Table: 6. Ruggedness of Carvedilol Day-2

S.No	Name	RT	Area
1	Injection-1	4.733	4689021
2	Injection-2	4.735	4690145
3	Injection-3	4.731	4661754
4	Injection-4	4.735	4701321
5	Injection-5	4.73	4699545
6	Injection-6	4.731	4697685
Avg		4.733	4689912
StdDev(±)		0.002	14677.2
%RSD		0.046	0.313

Table: 7. Ruggedness ofCarvedilol Day-1&Day-2

S.No	Name	RT	Area
1	Injection-1	4.733	4699456
2	Injection-2	4.736	4701756
3	Injection-3	4.731	4709588
4	Injection-4	4.73	4703346
5	Injection-5	4.729	4713254
6	Injection-6	4.723	4706584
7	Injection-7	4.733	4689021
8	Injection-8	4.735	4690145
9	Injection-9	4.731	4661754
10	Injection-10	4.735	4701321
11	Injection-11	4.73	4699545
12	Injection-12	4.731	4697685
AVG		4.731	4697787.92
StdDev(±)		0.00348	13329.837
%RSD		0.07	0.28

Table: 8. Assay Results of Carvedilol

4722607	25	10	100	100	99.6	200.4	100.000	Result
4737648	100	100	200.3	10	100	25		99.73

Table: 9. Robustness of Carvedilol

S.N	Peak Name	RT	Area	% Area	Height	USP Plate Count	USP Tailing
1	**Carvedilol**	4.735	5855729	100	469682	5392.8	1.49
2	**F1&F2**	3.965	3914939	100	409966	4183.1	1.41
1	**Carvedilol**	4.688	4664074	100	458556	5031.8	1.41
2	**T1&T2**	4.835	4700670	100	432832	4780.1	1.48

CONCLUSION

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and suitable for quantification of Carvedilol. The proposed method gives good resolution among the analyte. Themethod is very simple, rapid and no complicated sample preparation is needed. High percent of recovery shows the method is free from interference of excipients present in the formulation and the method is accurate.

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