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Method development and validation of reverse phase high performance liquid chromatography (RP-HPLC) method to determine carvedilol in pharmaceutical formulations

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ABSTRACT

The aspire of the present study was to explain development and consignitive validation of a reverse phase High performance liquid chromatography(RP-HPLC) method for estimation of Carvedilol, an antihypertensive drug in pharmaceutical formulations like tablets and nanoparticles. The chromatographic system consist of a steel plated C_{18} column, an isocratic mobile phase composed of phosphate buffer pH 3.0, acetonitrile and water (75:625:300) and UV detection at 240.0 nm.Carvedilol was eluted at 2.8 minutes without any interfering peak of other excipients used for the preparation of dosage form. The linearity was observed over the range from 1 to 50 µg/mL (R^2 = 0.9999). The intra-day and inter- day precision values were in the range of 0.123-0.276 %.Limit of detection and limit of quantitation were 0.321 µg/mL and 0.721 µg/mL, respectively. All results were validated statistically according to ICH guideline for both tablets and nanoparticles. The release followed the Higuchi kinetic model, indicating diffusion dominated drug release.

Key words: Carvedilol; RP-HPLC; nanoparticles; release kinetics

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INTRODUCTION

Carvedilol (Fig.1.),or (±) –1- (carbazole – 4- yloxy)-3-[[2-(o-methoxyphenoxy) ethyl]amino]-2-prppanol [1] ; is a nonselective β-adrenergic blocking agent with ∞ 1-blocking activity [2] used therapeutically to treat congestive heart failure, cardiac arrhythmias and angina pectoris [3,4].The ratio of ∞_1- to β- adrenergic receptor antagonist potency for Carvedilol is 1:10 [5]. Carvedilol has bioavailability of about 25 to 35% because of extensive first-pass metabolism [6,7].Carvedilol undergoes oxidative metabolism and glucuronidation in the liver; the oxidative metabolism occurs via cytochrome CYP2D6. Carvedilol is eliminated by hepatic metabolism and has a terminal half-life of 7 to 10 hours [8,9].

Nanoparticles (NPs) are considered to be the best drug delivery system, have considerable potential for drug targeting and exhibit several advantages over conventional delivery systems [10,11,12]. These are: high stability conferring long shelf lives, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and possibility of administration through variable routes [13]. Nanoparticles can also be designed to allow controlled drug release from the matrix. All these properties enable improvement of drug bioavailability producing high level of pharmacological action and reduction of the dosing frequency [14].So PLGA-nanoparticles have great potential as sustained drug delivery system for Carvedilol to prolong antihypertensive effects of the molecule.

Oflate Carvedilol is commercially available as tablets, a commertial pharmaceutical formulation. In literature various analytical method are reported found for the analysis of Carvedilol such as High Performance Liquid Chromatography (HPLC) with fluorescence detector [15,16]Mass Spectrometer [17,18]. Determination of

Carvedilol with the help of electrophoresis method has also been reported [19].But HPLC assay for the determination of Carvedilol in any novel drug delivery system has not been reported in any scientific literature.

The objective of the present study was to develop a simple, precise and accurate RP-HPLC method for the estimation of Carvedilol in formulations prepared in the lab as well as commercial tablets were used to validate the method development.

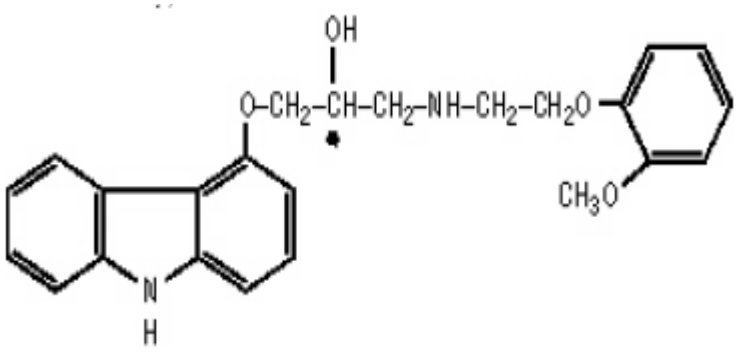


Figure1.Carvedilol (Chemical Abstracts Service No- 72956-09-3)

MATERIALS AND METHODS

1.1. Chemicals and Reagents:

Carvedilol reference sample was obtained from Zydas Cadila Health Care (Ahmedabad, India).Tablets of brand Carvil (Batch No- Zydas Cadila Health care. Ahmedabad, Gujrat. India) containing 3.125 mg of Carvedilol were purchased from local pharmacy. Poly (DL-lactide/glycolide copolymer) (PLGA,75:25 with average molecular weight and inherent viscosity 0.37 dL/g) was procured from Boehringer Ingelheim Co,(Ingelheim, Germany),Poly vinyl alcohol was generously supplied by Torrent Pharmaceuticals, Ahmedabad, Gujarat, India. Acetonitrile and methanol were of HPLC grade and purchased from Merck, India. All other chemicals were of analytical grade and procured from Merck.Milli-Q water was used for mobile phase preparation.

1.2. Preparation and characterization of nanoparticles.

Nanoparticles are prepared by using the method emulsification by sonication-evaporation.[18].Henceforth, this methods will be referred as simple sonication.The method involve preparation of an organic phase consisting of polymer (PLGA) and drug (Carvedilol) dissolved in organic solvent (Dichloromethane).The organic phase is added to an aqueous phase containing surfactant (Polyvinyl alcohol) to form an emulsion. This emulsion is broken down into nanodroplets by applying external energy and these droplets form nanoparticles upon solvent evaporation and was isolated by centrifugation at 10,000xg at 4^{o}C for 45 minutes washed with water and dried under vacuum.

1.3. Particles Size Determination

Particle size distribution was analysed by Master Sizer 2000 (Model: APA 2000, Malvern Instruments, Unighted Kingdom) equipped with a software (Version 1201).So to prevent clumping the dried powdered samples were diluted with dust free water to give the recommended scattering intensity as per Mie theory. Analysis was carried out at least for three times for each batch of sample and mean value were reported.

2.4. Instrumentation and Chromatography

The HPLC system consisted of a LC-20D, (Shimadzu, Kyoto, Japan) pump and a SPD-W-20A, (Shimadzu, Japan) Photo Diode Array (PDA) detector. Separation was achieved using Phenomenex Luna 5 µ C_{18} steel column (150x4.6mm, 250Å, SPD-W-20A, Shimadju, Japan). The isocratic mobile phase was pumped at a flow rate of 0.5 mL/min with 115 kg/f pressure.The mobile phase consisted of Phosphate buffer pH 3.0,acetonitrile and water (75:625:300 v/v/v) was freshly prepared, filtered through a 0.45 µm filter (Millipore, Milford,MA,USA) and degassed for sonication for 15 minutes. The injection volume was 20 µL and the wavelength for the detection was 240.0 nm with sensitivity 0.005 to 0.02 aufs. All the separations were performed by at room temperature.

2.5. Standard and Sample Preparation

Standard preparation

Stock standard solution of Carvedilol was prepared by dissolving 10 mg of drug in 100 mL of methanol to give final concentration of 100µg/mL. Standard solution of carvedilol (1, 2,3,4,5,6,7,8,9,10 and 50 µg/mL) was prepared by

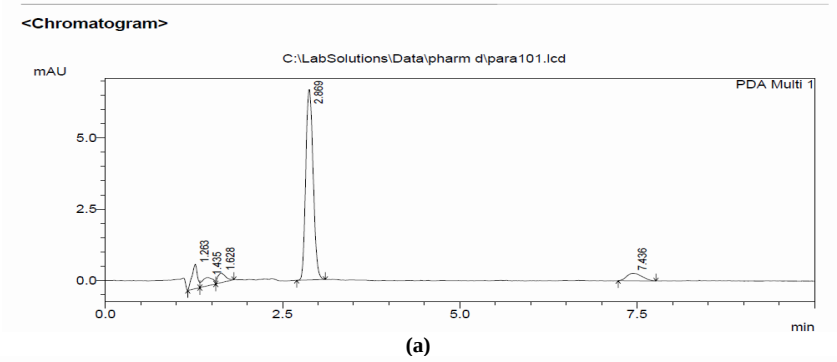
subsequent dilution using the mobile phase. After storing one week at 4^{o}C no significant decrease in responses was observed.

Sample preparation for Assay and recovery study:
For the analysis of dosage form Ten tablets of Carvedilol were accurately weighted and ground to fine powder and mixed thoroughly portion of the powder equivalent to 10 mg of Carvedilol was transferred into a 100 mL volumetric flux and 40 ml of methanol was added and content of the flask was shaken on a thermostatically controlled water batch for 2 hours. Dried nanoparticles equivalent to 10 mg of Carvedilol were dissolved in about 40 mL of methanol in a separate 100 ml volumetric flask and shaken for 2 hours. Both the solution were filtered through Whatman filter paper (No 41).The filter paper was washed with a blank. The washing were added to the filterate and the final volume was made up to 100mL with the blank. After suitable dilution, the drug concentrations with six replicates for tablets and nanoparticles solution were determined by HPLC using the calibration curve. The Data were analysed by linear simple regression by the least square-methods. The recoveries were determined by adding known amount of Carvedilol reference substances (2.5, 5, 7.5, and 10.0 µg) to the sample at the beginning of the process. After then recovery was being performed.

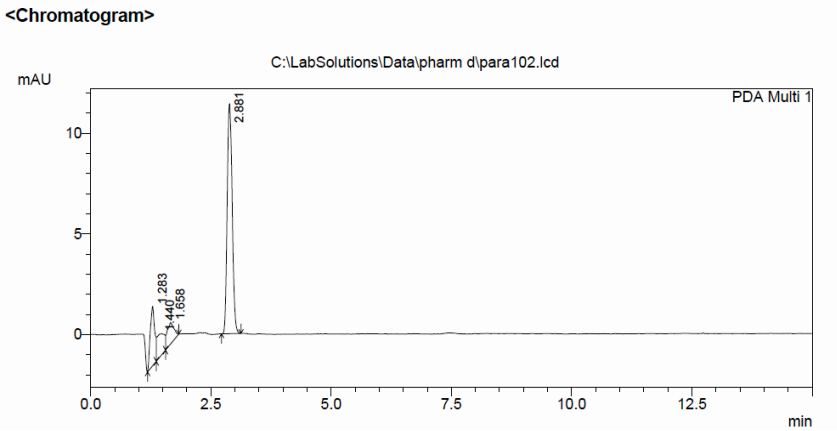
2.6. Method validation
The parameters which were used to validated the method of analysis were linearity, range,accuracy,precision,limit of detection, limit of quantitation, specificity and robustness .The method was validated according to the ICH guideline (International Conference on Harmonization) for the validation of analytical procedures.

2.7.In-vitro drug release studies from nanoparticles
In-vitro release study of Carvedilol was determined by placing 10 mg nanospheres in 25 ml of release medium (PBS buffer,pH6.8).The sample was kept in a orbital shaker (SGM-300,Gallenkamp,Sanyo) maintained at 37^{o}C ± 5 ^{o}C, stirring at 50 rpm.The study was carried out for 24 h with continuous stirring. At specified time intervals, 1 ml aliquot of the release medium was removed and immediately replace with the identical volume of fresh medium. The aliquots were filtered and concentrations of Carvedilol in the release medium were assessed by proposed HPLC method.

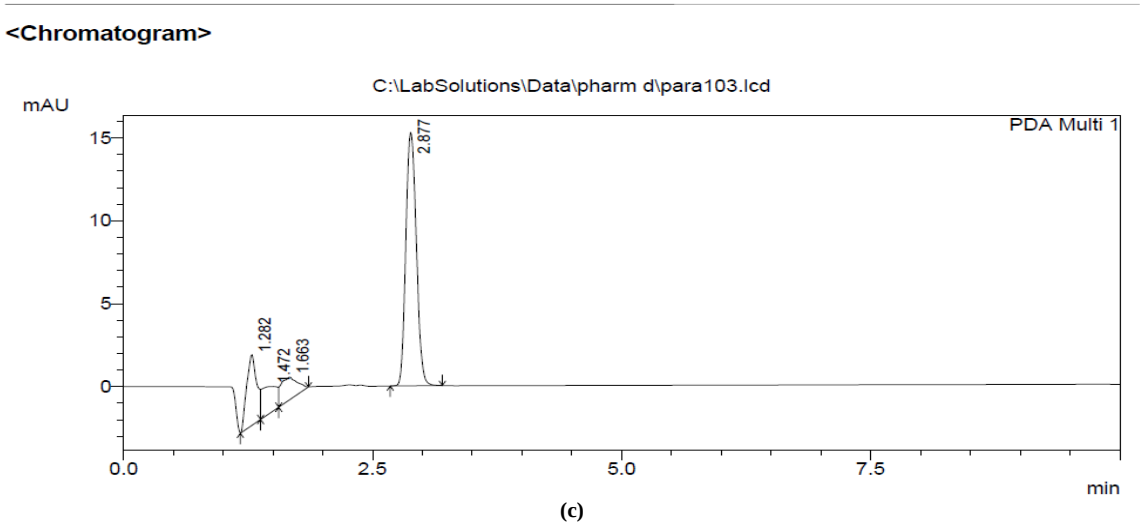
Development and validation of RP-HPLC Method.



(a)



(b)



(c)

Figure 2.Chromatogram of carvedilol standard solution (a), Chromatogram of Carvedilol in sample of tablet solution (b), Chromatogram of Carvedilol in sample of nanoparticles solution(c).

Table 1. Statistical data for calibration curve of Carvedilol.

Parameters	Carvedilol
Linearity	1-50µg/mL
Regression equation	Y=235.15X- 14.009
Correlation coefficient	0.9999
Slope	235.12
Intercept	-14.009
Standard deviation of slope	-12.007
Standard deviation of intercept	0.141
Limit of detection	`0.321 µg/mL
Limit of quantitation	0.721 µg/mL

Table 2. Recovery studies of Carvedilol in tablets and nanoparticles

Dosage form	Level	Amount of sample taken(µg)	Amount of standard spiked(µg)	Percentage of standard recovered	% Recovery± S.D.
Tablets	I	10	10	101.32	100.506±0.8068
	II	10	20	99.85	
	III	10	30	101.56	
	IV	10	40	100.56	
	V	10	50	100.23	
	VI	10	60	99.52	
Nanoparticles	I	10	10	98.25	99.01±1.398
	II	10	20	99.86	
	III	10	30	100.52	
	IV	10	40	98.46	
	V	10	50	100.12	
	VI	10	60	96.85	

Table 3.Assay results of marketed available tablets and prepared nanoparticles

Sample No	**Drug content in Tablets**	**Drug content in Nanoparticles**
1	101.72	99.75
2	100.85	100.35
3	98.25	99.45
4	101.35	99.82
5	101.2	99.76
6	100.25	99.68
	603.62	598.81
Mean	100.6034	99.8017
S.D*	1.2557	0.2979
%RSD**	1.2482	0.2985
SEM***	0.5001	0.1213

S.D. : Standard deviation*
*%RSD** : Percentage relative standard deviation*
*SEM*** : Standard error mean.*

Table 4. ANOVA of intra-inter-day assay of Carvedilol tablets and nanoparticles

Source of variation	SS		df		MS		F_{stat}*		Fat level 1%	Fat level 5%
	Tablet	NP**	Tablet	NP**	Tablet	NP**	Tablet	NP**		
Intraday variation	1.996	0.301	3	3	0.680	0.091	0.998	0.856	9.87	4.89
Interday variation	1.030	0.065	2	2	0.530	0.034	0.882	0.321	11.97	5.47
Error	3.985	0.645	6	6	0.681	0.098				
Total	7.011	1.011	11	11						

Table 5.Kinetic parameters of release data from Carvedilol loaded nanoparticles

[1]Zero order	R^2 value* K**	0.7346 3.0334
[2]First order	R^2 value K	0.8522 -0.0312
[3]Higuchi	R^2 value K	0.9931 2.9105
[4]Korsmeyer- Peppas	R^2 value K N***	0.9231 0.0097 0.6375

[2]First order equation, log c=log c_0 - kt/2.303, [3] Hi-guchi equation, $Q=kt^{½}$,
[4] Korsmeyer-Peppas equation, $M_t/M_\infty=kt^n$

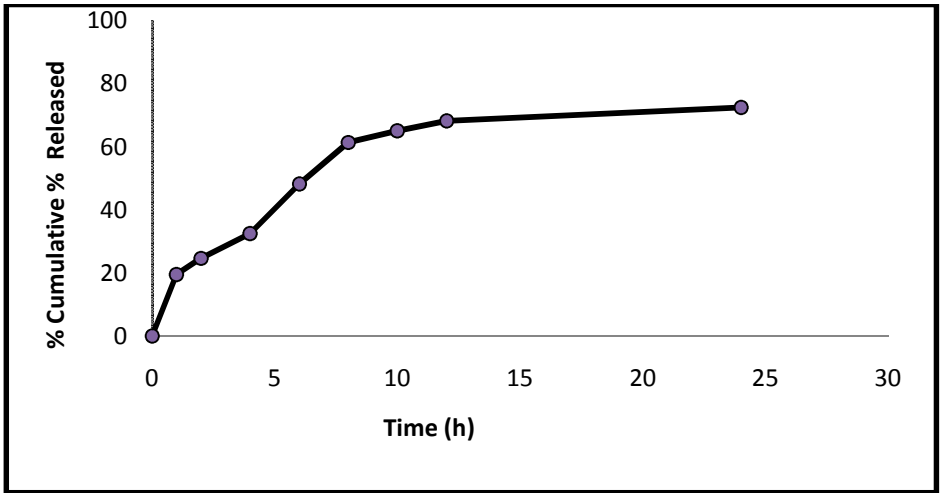


Figure 3: *In vitro* release profile of carvedilol loaded nanoparticles by proposed RP- HPLC Method

RESULTS AND DISCUSSION

Nanoparticles were prepared by emulsification by sonication-evaporation method. The sonication speed was kept constant to get the desired particles size and narrow particle size distribution profile. The nanoparticles used in the proposed RP-HPLC analysis showed an average particles diameter of 220 nm along with a good Poly dispersion index (PDI).These PDI indicated the narrow distribution pattern of the experimental nanoparticles.

The chromatographic conditions were optimized and separation was performed in steel plate C_{18} column having a mobile phosphate buffer phase pH 3.0, acetonitrile and water. This proposed mobile phase composition showed the suitable retention time of Carvedilol and achieved good selectivity. A sharp peak shown a resolution was observed and Carvedilol eluted at about 2.9 minutes. (Figure2 a-c).

Calibration curves were plotted using of standard Carvedilol solution in the range of 1-50 µg.mL.The equation of linear regression and statistical data are presented in Table 1.The linearity of calibration curve was validated by high value of correlation co-efficient(R^2=0.9999).

The limit of quantitation and limit of detection are shown in the Table 1.Low value of LOD and LOQ signifies the method to be sensitive.

The specificity of the proposed method explain that the marketed tablets formulation and the nanoparticles do not interfere with the drug peak. The sharp peak indicates the specificity of the method (Figure 2.).So the proposed method is useful to quantify carvedilol in different pharmaceutical formulation.

The precision was determined by analyzing three different concentration of bulk drug on three different days. Concentrations were determined using calibration curve prepared on each day. The intra and inter day precision were calculated accordingly (Table 4).

The accuracy was evaluated by recovery studies in the table 3.Recovery results are closed to 100 % which level the suitability and the accuracy of the proposed method. The accuracy were further tested by assaying the marketed tablets as well as experimental nanoparticles on the same days and three different days. The results of formulation assay are shown in the Table 3. The study for analysis of variance (ANOVA) was performed on data obtained for intra and inter days assay in Table 4.

Stability of stock solution was determined by comparing the determination of Carvedilol in stock and freshly prepared standard solution. No significant change (<1%) was observed.

The release data were fitted according to the different kinetic models, namely zero order, first order, Higuchi and Peppas. All kinetic parameters of release data from carvedilol loaded nanoparticles are summarized in Table 5.The release profile could be best explained by Higuchi models, the plots showed high linearity, with correlation coefficient (R^2) values of 0.9931.The diffusion mechanism of drug release was indicating that drug release from the selected nanoparticles formulation was diffusion controlled.

CONCLUSION

A consignitive validation of a reverse phase High performance liquid chromatography(RP-HPLC) method was developed for estimation of Carvedilol, in pharmaceutical formulations like tablets and nanoparticles. The proposed RP-HPLC method is simple, sensitive, precise and accurate. It was observed that in-vitro release followed the Higuchi kinetic model, indicating diffusion dominated drug release.

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