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Full Paper

Development of carvedilol assay in tablet dosage form using HPLC with fluorescence detection

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Abstract: A simple HPLC method was developed and validated for quantitation of carvedilol in dissolution medium and tablet dosage form. Chromatographic separation was achieved on a Alltima® C18 (250 mm×4.6 mm) column using a mobile phase containing 0.01 M Na_2HPO_4 in water and acetonitrile (30:70 v/v) adjusted to pH 3.0 by ortho-phosphoric acid at a flow rate of 1.0 ml/min and employing fluorescence detection with 300- nm excitation and 343-nm emission wavelengths. The method was validated for specificity, linearity, accuracy, precision and stability. Dissolution test parameters were also investigated. Moreover, the proposed analytical method was applied to monitor the formulation content uniformity and labelled amount of commercially available carvedilol drugs.

Keywords: carvedilol, HPLC, validation, dissolution test, quality control

Introduction

Carvedilol, or (±)-1-9H-(carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl]amino]-2-propanol (Figure 1), is an antihypertensive agent with β- and α_1-adrenergic receptor blocking activities [1-3]. Carvedilol has much greater antioxidant activity than other commonly-used β-blockers [4-5]. It has been prescribed as an antihypertensive agent and an angina agent [6-7] and for treatment of congestive heart failure [8].

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

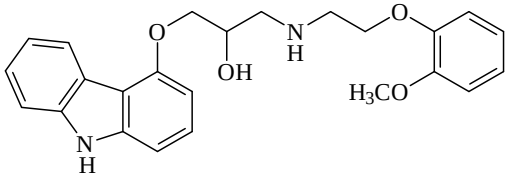


Figure 1. Chemical structure of carvedilol

High-performance liquid chromatography (HPLC) with fluorescence detector [9-14], mass spectrometer [15-16] or electrochemical detection [17] has been used for the analysis of carvedilol and its enantiomers in biological samples. Determination of cavedilol by capillary electrophoresis has also been reported [14,18]. There have been few published articles on the evaluation of carvedilol in pharmaceutical formulations. That using HPLC with UV detector [19-21] and differential pulse voltammetric determination [22] have been presented.

The dissolution test and quantitative assay are very important features of the quality control of drugs in the pharmaceutical industry. The dissolution test is currently used as an in vitro bioequivalence test and generally for obtaining dissolution profile and profile comparison to establish the similarity of pharmaceutical dosage forms [23-24]. To the best of our knowledge, there is no official assay guideline for carvedilol in dosage forms and dissolution samples in any pharmacopoeia, nor any dissolution test for this pharmaceutical in dosage forms reported in the literature. Thus, in this paper an attempt is made to develop and validate a simple, efficient and reliable method for the determination of carvedilol intended for pharmaceutical applications by HPLC using fluorescence detection. Carvedilol assay in tablet formulation and dissolution samples is described and the optimisation of a dissolution protocol for carvedilol-containing tablets is presented. Evaluation of the dissolution profiles of two marketed carvedilol products by the optimised method is also reported.

Materials and Methods

Chemicals and reagents

Standard carvedilol (99.91%) was obtained from Salutas Pharma GmbH (Barleben, Germany) and was used as certified reference compound for quantitative analysis. Other chemicals were of analytical reagent grade purchased from various sources. All solvents were of HPLC grade obtained from VWR Prolabo (Leuven, Belgium). All experiments were performed with purified water obtained from TKA ROS 300 (Niederelbert, Germany).

Chromatographic conditions

The HPLC system used for the assay consisted of a dual plunger pump (LC-10ATVP, Shimadzu, Kyoto, Japan), a fluorescence detector (RF-10AXL, Shimadzu), a system controller (SCL-10AVP, Shimadzu) and a Rheodyne (7725) sample injector (Rohnert Park, CA) fitted with a 20-µl sample loop. The separation was performed at ambient temperature on an Alltima® C18 (250 mm×4.6 mm i.d., 5 µm, 250 Å) column purchased from Alltech (Deerfield, IL). The column was fitted with a guard column packed with C18 (4.0 mm×3.0 mm i.d.; Phenomenex Torrance, CA). The mobile phase was a

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

mixture of 0.01 M Na_2HPO_4 in water and acetonitrile (30:70 v/v) adjusted to pH 3.0 by ortho-phosphoric acid and had a flow rate of 1.0 ml/min. The mobile phase was degassed by ultrasonication prior to use and was allowed to recirculate during the analysis. The peak areas were determined using a fluorescence detector with excitation wavelength and emission wavelength set at 300 nm and 343 nm respectively [25].

Preparation of standard solutions

A stock solution of carvedilol (1 mg/ml) was prepared with the mobile phase as solvent. Calibration standard solutions were prepared by diluting the stock solution to 1, 5, 10, 20, 30 and 40 μg/ml with the dissolution medium.

Analytical method validation

Specificity: Specificity was assessed by examining peak interferences from dissolution medium. This was done by inspecting chromatograms of blank and spiked medium samples.

Linearity: Six-point standard calibration curves were prepared over a concentration range of 1-40 μg/ml for carvedilol. The data of peak area versus drug concentration were constructed by unweighted least-square linear regression analysis.

Accuracy and precision: Accuracy and precision were determined from six replicates of each carvedilol concentration (1, 5, 10, 20, 30 and 40 μg/ml) within the range of the calibration curve. Accuracy and precision were expressed as % accuracy and % coefficient of variation (CV) respectively.

Stability: The dissolution medium containing standard carvedilol was kept at 37±0.5°C for 2 hr under light shaking and then left at room temperature for 24 hr. The response of the 24-hr aged solutions was evaluated against a freshly-prepared standard solution.

Dissolution

Dissolution of carvedilol tablets was optimised using Dilatrend® (carvedilol tablets: 6.25, 12.5 and 25 mg, manufactured by Roche S.p.A., Segrate, Italy). In each experiment, twelve tablets were randomly selected. Dissolution testing was performed in accordance with the USP <711> [26] using apparatus II (VK 10-1500, Vankel Industries Inc., Cary, NC). The dissolution apparatus was used with paddles at 50 rpm and a bath temperature of 37±0.5°C. The dissolution media were evaluated using 0.1 N HCl solution (pH 1.2), acetate buffer (pH 4.5) and phosphate buffer (pH 6.8) [26]. Dissolution was carried out according to the drug release guidelines [27]; 900 ml of the freshly prepared medium was used in a rotating vessel. At each sampling time point, the dissolution sample (5 ml) was collected from each vessel and filtered through a 0.45-μm porosity nitrocellulose membrane (Millipore, Bedford, MA). Fresh medium (5 ml) weas replaced in each vessel after sampling. A 20-μl aliquot of each sample was injected into the HPLC system for analysis. The quantity of carvedilol in the dissolution medium was calculated from a calibration curve. The results were estimated as % labelled amount of the dissolved active ingredient.

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

Application to drug quality controls: dissolution profile comparison

Dilatrend® (6.25, 12.5 and 25 mg) as reference product and the same does of Brand A (generic carvedilol tablets) as test product were studied. The procedure for dissolution as previously described above was followed. According to the US FDA guideline [28] for dissolution profile comparisons, the difference factor (f_1) and similarity factor (f_2) were calculated as follows:

$$f_1 = \{(\Sigma^n_{t=1} | R_t - T_t |) / (\Sigma^n_{t=1} R_t)\} \times 100$$

$$f_2 = 50 \times \log \{(1 + (1/n) \Sigma^n_{t=1} (R_t - T_t)^2)^{-0.5} \times 100\}$$

in which R_t and T_t are the percentages of Dilatrend® and Brand A respectively that were dissolved at each time point, and n is the number of sampling time points.

Assay in tablet formulation

Standard preparation: An accurately weighed quantity of carvedilol working standard was dissolved in the mobile phase to afford a solution having a concentration of 0.025 mg/ml.

Assay preparation: Twenty tablets of the test or reference product were weighed and then finely powdered. An accurately weighed portion of the powder, equivalent to about 12.5 mg of carvedilol, was transferred to a 50-ml volumetric flask and 20 ml of mobile phase was added. The volumetric flask was shaken mechanically for 5 min, sonicated for 10 min and diluted to volume. One ml of this solution was transferred to a 10-ml volumetric flask and diluted with mobile phase to volume. A portion of this solution was filtered through a 0.45-μm-porosity nylon filter membrane (Millipore).

Procedure: A 20-μl aliquot of standard or sample preparation (test and reference products) was injected into the HPLC system described above. The quantity (in mg) of carvedilol in the portion of tablets was obtained by the formula: 500 C (r_U / r_S), in which 500 is the dilution factor, C is the concentration (in mg/ml) of carvedilol in the standard preparation, and r_U and r_S are the carvedilol peak areas obtained from the assay and standard preparations respectively. The results were then estimated as % labelled amount.

For the determination of dosage-unit uniformity by assay of individual units [26], 10 units each of the test and reference products were selected. Each tablet was finely powdered, transferred to a 50-ml volumetric flask and diluted to volume. A portion of this solution was transferred to a 10-ml volumetric flask and diluted with mobile phase to volume, to afford a final concentration of 0.025 μg/ml. A portion of this solution was filtered through a 0.45-μm-porosity nylon filter membrane and the filtrate (20μl) was analysed by HPLC in the same manner as above. The content of carvedilol in each tablet was calculated by comparison with the standard solution at 0.025 μg/ml.

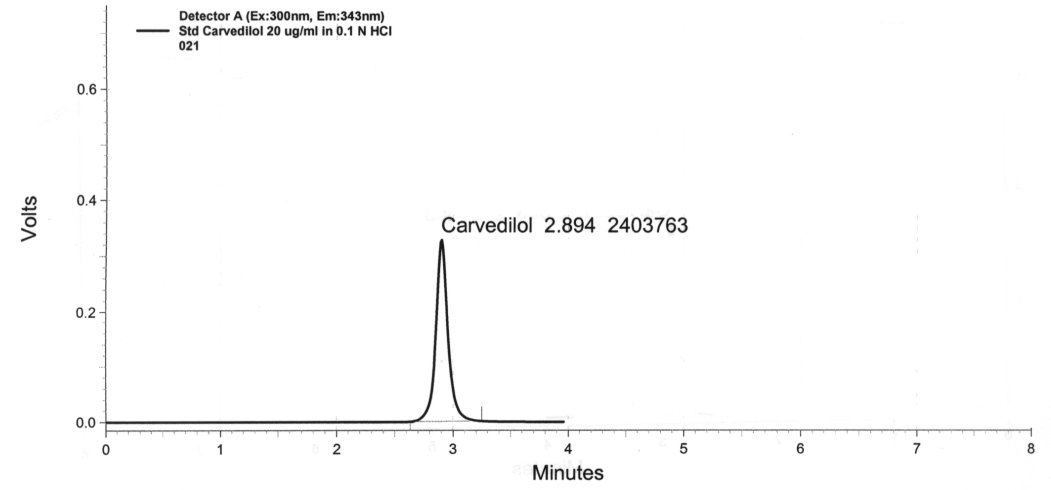
Results and Discussion

Specificity and optimisation of chromatographic conditions

The method demonstrates excellent chromatographic specificity with no interference from tablet excipients, mobile phase or dissolution medium at the retention time of carvedilol. Representative chromatograms of carvedilol in the three dissolution media are shown in Figure 2. At equal concentration, a smaller peak area of carvedilol is observed in the phosphate buffer pH 6.8 as compared with those in the HCl solution pH 1.2 and the acetate buffer pH 4.5. Apparently, the pH of

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

the medium has certain effect on the fluorescence intensity of carvedilol. The retention time of carvedilol is 2.8 min and each analysis can be done within 4 min under specified conditions.



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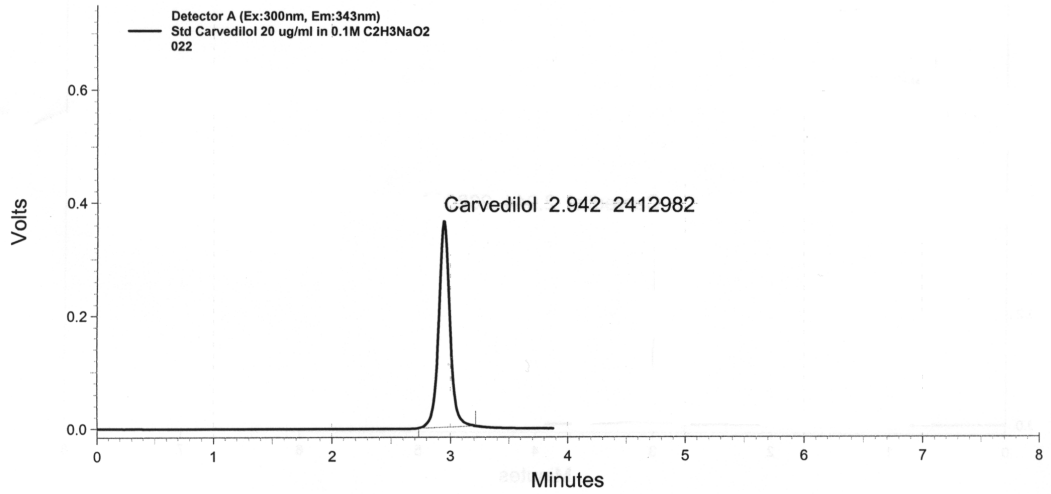

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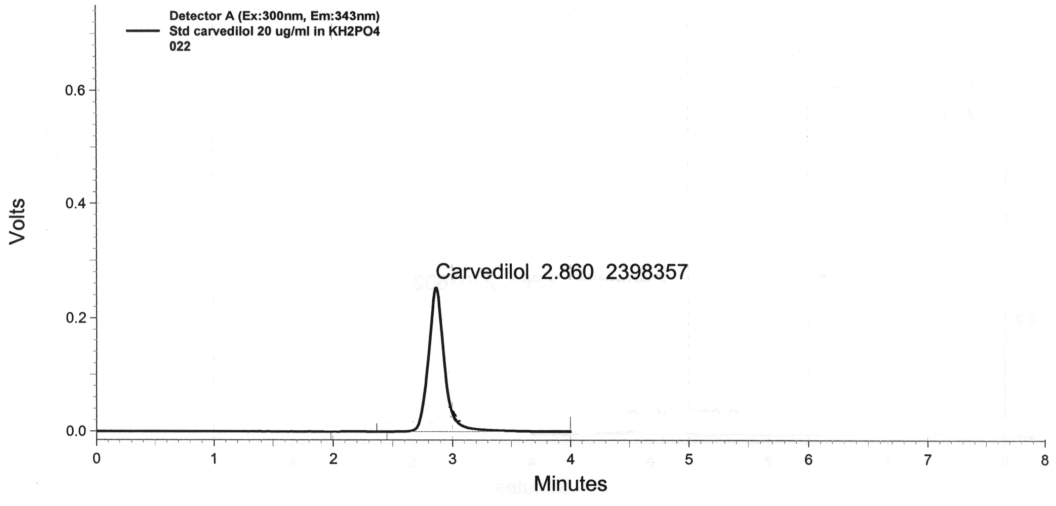


(C)

Figure 2. Representative HPLC chromatograms of carvedilol (equal concentration) in: (A) 0.1 N HCl solution pH 1.2; (B) acetate buffer pH 4.5; (C) phosphate buffer pH 6.8

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

Method validation

According to Category III of the compendial assay procedures [26], a minimal assessment is required in terms of linearity, accuracy, precision and stability in three different media. The calibration curves for carvedilol in all dissolution media show good linearity with regression coefficient greater than 0.99 in the concentration range of 1-40 μg/ml. This means that there is a good correlation between peak area and drug concentration. The equation of linear regression and regression coefficient of the calibration curve for each medium is presented in Table 1. The results of the accuracy and precision determinations are shown in Table 2. The accuracy is between 95-105% and the intra-day precision expressed as % CV is less than 6.67% for the three dissolution media. The solutions remained stable in all dissolution media tested for the time period specified and no degradation products were observed in any chromatogram.

Table 1. Slope, intercept and regression coefficient of calibration curves obtained from three different dissolution media (*n*=6)

Medium	Slope	Intercept	Regression coefficient
0.1 N HCl solution pH 1.2	124367.00	-25017.39	0.9994
Acetate buffer pH 4.5	117218.42	4996.24	0.9989
Phosphate buffer pH 6.8	122211.38	-22638.78	0.9981

In vitro dissolution study

A dissolution test is normally employed for lot-to-lot quality control of pharmaceuticals in solid dosage form. Since carvedilol is not officially available in the pharmacopoeia, we have developed the dissolution testing condition for this drug. Drug release was carried out in accordance with the US pharmacopoeia general methods (Apparatus II) [26]. The temperature was kept constant at 37±0.5°C and the volume in each vessel kept at 900 ml. The dissolution parameters such as pH of medium, stirring speed and sampling time interval were optimised in terms of dissolution rate and precision. The most suitable dissolution method is shown in Table 3. In all three dosage strengths, Dilatrend® exhibits delayed dissolution in phosphate buffer pH 6.8 (Figure 3C) compared with dissolution in HCl solution pH 1.2 (Figure 3A) and that in acetate buffer pH 4.5 (Figure 3B). From the dissolution profile (Figure 3), 12.5 mg of Dilatrend® in HCl solution pH 1.2 and acetate buffer pH 4.5 show fastest dissolution. The different dissolution rates might stem from the difference in solubility in different pH media. For routine quality control test using a single-point specification [26], the acceptance criteria of tolerance should be at least 80% (Q) dissolution within 30 min in acetate buffer pH 4.5.

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

Table 2. Accuracy and precision of the method for determining the concentration of carvedilol in three dissolution media (*n*=6)

Medium	Actual concentration (μg/ml)	Detected concentration (Mean ± SD; μg/ml)	Accuracy (%Accuracy)	Precision (%CV)
0.1 N HCl solution pH 1.2	1	1.04 ± 0.03	104.49	2.42
	5	4.82 ± 0.16	96.46	3.29
	10	9.89 ± 0.26	98.89	2.64
	20	20.19 ± 0.33	100.96	1.62
	30	30.39 ± 0.09	101.31	0.29
	40	39.66 ± 0.36	99.15	0.92
Acetate buffer pH 4.5	1	0.98 ± 0.07	98.42	6.67
	5	4.77 ± 0.17	95.40	3.58
	10	10.00 ± 0.53	99.95	5.32
	20	20.42 ± 0.31	102.10	1.53
	30	30.04 ± 1.96	100.14	0.59
	40	39.79 ± 0.71	99.47	1.78
Phosphate buffer pH 6.8	1	1.04 ± 0.05	103.91	5.02
	5	4.97 ± 0.22	99.37	4.42
	10	10.08 ± 0.14	100.85	1.39
	20	19.78 ± 0.66	98.91	3.31
	30	30.14 ± 0.59	100.46	1.95
	40	39.99 ± 1.31	99.97	3.28

Table 3. Optimal conditions for dissolution test of carvedilol tablets

Condition	Data / Unit
Apparatus	Apparatus II (Paddle) [26]
Dissolution medium	Acetate buffer pH 4.5
Volume of dissolution medium	900 ml
Temperature of dissolution medium	37±0.5°C
Revolution of stirrer	50 rpm
Number of tablet in vessel	1 tablet
Sampling time	5, 10, 15, 20, 30 and 45 min
Sampling volume	5.0 ml
Medium replacement	Yes

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

Application to drug quality controls: comparison of dissolution profiles

Approval of multi-source formulations using comparative in vitro dissolution studies should be based on generation of comparative dissolution profiles rather than a single-point dissolution test [27]. When comparing the test and reference products, dissolution profile can be compared using f_1 and f_2. Two dissolution product profiles are declared similar if f_1 is between 0-15 and f_2 is between 50-100 [28]. The results of dissolution efficiency in the three dissolution media, with Dilatrend® and Brand A as reference and test products respectively, are presented in Table 4. The dissolution profiles of 6.25-mg and 12.5-mg formulations in all dissolution media show f_1 and f_2 within acceptable ranges. The results of the two formulations therefore reflect sameness of the two curves and thus equivalence of the in vitro performance of the two products. On the contrary, the 25-mg formulation shows disagreement with the above guideline. This difference might be due to the excipient in the formula and the size of tablet, both of which can vary among brands. It is then necessary to carry out an in vivo study to guarantee the bioequivalence between the products.

Table 4. The difference and similarity factors between Dilatrend® (reference product) and Brand A (test product) in three different dissolution media

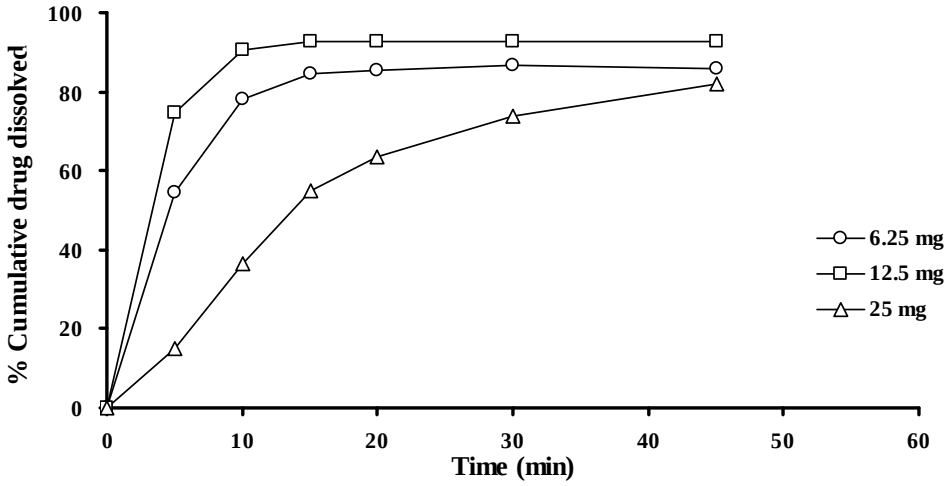
Dissolution medium	6.25 mg		12.5 mg		25 mg	
	f_1	f_2	f_1	f_2	f_1	f_2
pH 1.2 0.1 N HCl solution	4.20	69.41	7.71	56.36	41.30	28.52
pH 4.5 acetate buffer	5.75	61.59	0.89	88.24	10.36	38.46
pH 6.8 phosphate buffer	6.15	68.96	7.56	68.02	17.59	55.74

f_1 = difference factor (0-15), f_2 = similarity factor (50-100)

Assay in tablet formulation

The validated HPLC assay was applied to the quality control of two products. The % labelled amount and content uniformity are presented in Table 5. None of the formulation tested contains less than 95% of the labelled amount. Results of content uniformity experiment show that carvedilol content in each tablet from every product examined is in the range of 85.0-115.0 % and the RSD values are less than 6%. According to the acceptance limit of pharmacopoeia [24], this indicates a uniform distribution of drug in the tablets without any significant variation.

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19



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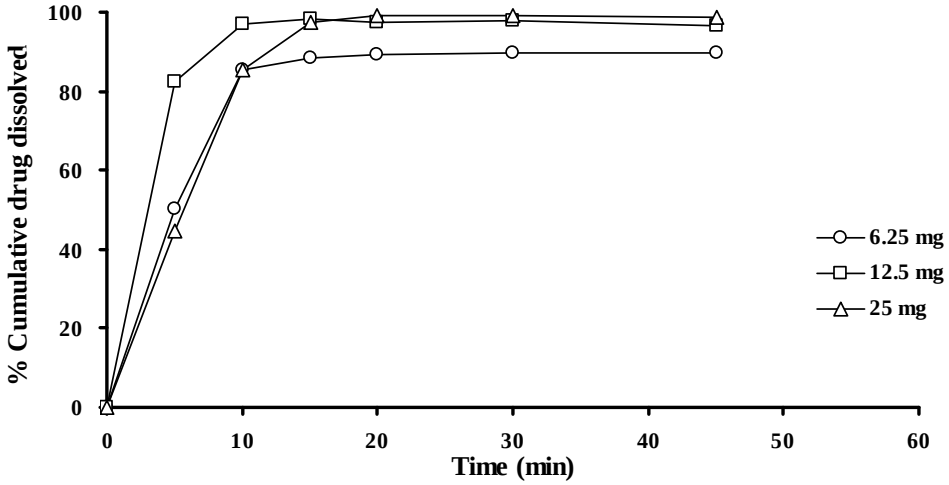

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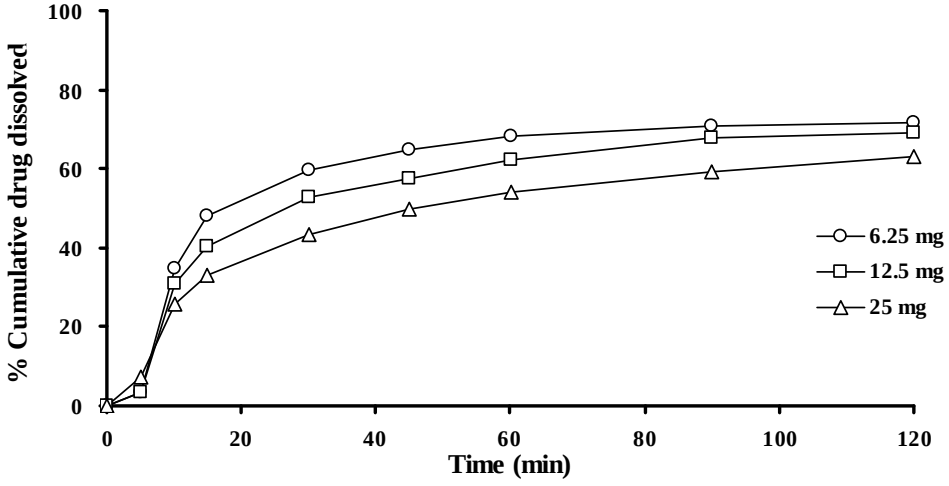

(C)

Figure 3. Dissolution profiles of carvedilol tablets (Dilatrend®) in: (A) 0.1 N HCl solution pH 1.2; (B) acetate buffer pH 4.5; (C) phosphate buffer pH 6.8

Maejo Int. J. Sci. Technol. **2010**, *4*(01), 8-19

Table 5. Content of carvedilol in Dilatrend® and Brand A tablets

	Dose	Dilatrend®	Brand A
% Labelled	6.25 mg	95.82±0.45	97.74±1.27
amount	12.5 mg	98.07±0.93	95.71±0.38
(Mean ± SD)	25 mg	97.78±0.80	96.76±0.90
Content	6.25 mg	95.76-99.18 (1.38)	96.02-102.73 (1.89)
uniformity	12.5 mg	94.44-97.51 (1.02)	93.55-97.60 (1.67)
(Range in %)	25 mg	95.01-99.30 (1.36)	97.01-100.65 (1.45)

Note: Numbers in parentheses represent % RSD.

Conclusions

A method of quantitative determination of carvedilol using HPLC with fluorescence detector has been developed for the dissolution test and the quality control of the tablet formulation. The validation results have demonstrated that this method is accurate, precise, linear and specific. The dissolution test developed for carvedilol tablets is considered satisfactory. The optimal conditions for the dissolution profile determination are: 900 ml of acetate buffer (pH 4.5) medium at 37±0.5°C and paddle apparatus with 50-rpm stirring speed. The drug delivery requirement should be at least 80% dissolved in 30 min. The method can also be applied for quality control of drug content in pharmaceutical preparations.

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