ISSN: 2321-3132

Research Article

International Journal of Chemistry and Pharmaceutical Sciences
IJCPS, 2013: Vol. 1(4): 257-262
(Online at www.pharmaresearchlibrary.com/ijcps)

Isocratic Reverse Phase HPLC Method-Determination and Validation of Cilostazol

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Abstract
A simple isocratic HPLC method has been developed and subsequently validated for determination of Cilostazol in pharmaceutical dosage forms. The method employs an Agilent Zorbax Eclipse XDB C18 column (250mm x 4.60mm id, 5 µm Particle size) with flow rate of 1.0ml/min using UV detection at wave length 257nm. The separation was carried out using a mobile phase consisting of a mixture of 500ml of water, 350ml of Acetonitrile and 150ml of methanol. The retention time for Cilostazol was found to be 7.03minutes. A linear response was observed over the concentration range of 25-150µg/mL for the assay of Cilostazol. The limit of detection and the limit of quantification for Cilostazol were found to be 0.584 ppm and 1.020 ppm respectively. The results of analysis were validated statically and by recovery studies. Hence the proposed method was found to be accurate, precise, reproducible and specific and can be used for analysis of the drug in tablet formulation.
Key words: Cilostazol, Isocratic HPLC method, UV detection, Validation

1. Introduction
Cilostazol is a phosphodiesterase inhibitor with therapeutic focus on cAMP. It inhibits platelet aggregation and is a direct arterial vasodilator. Its main effects are dilation of the arteries supplying blood to the legs and decreasing platelet coagulation [(1)]. It is slightly soluble in methanol and ethanol, and is practically insoluble in water, 0.1 N HCl, and 0.1 N NaOH. Cilostazol is chemically called as 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1H)-quinolinone.

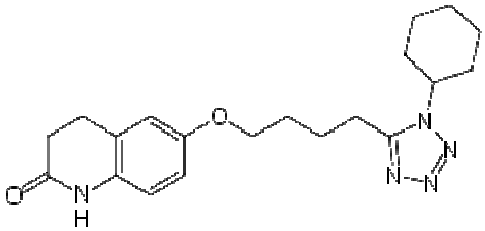


Fig: 1 Structure of Cilostazol

There are very few [(2-5)] analytical methods available for the quantization of Cilostazol individually. The purpose of this study was the development of a simple isocratic HPLC method with Ultraviolet detection for Cilostazol assay in tablets and validates the same as per the ICH guidelines.

1.1 Materials and Methods
HPLC grade Acetonitrile, Methanol was obtained from Merck (Darmstadt, Germany). Water is of Milli- Q grade is used. Cilostazol working standard is obtained from AIZANT Drug Research Solutions Hyderabad, The analytical sample of Cilostazol (50 mg) tablets procured from market.

2. HPLC Method and Chromatographic Conditions

The development and validation [6,7] of the assay was performed using the following conditions, Agilent Zorbax Eclipse XDB, C18 (150 x 4.6mm) 5µm particle size. The peak purity was determined on 2996 photodiode array detector (PDA)The mobile phase containing a mixture of Water, Acetonitrile, and Methanol in the ratio of 500:350:150 % v/v. The flow rate is monitored at 1.0ml/ min. Detection was performed at 257nm and identification of Cilostazol was made comparing its retention time and their UV spectra using the PDA detector.

Appropriately weigh and transfer about 25mg of Cilostazol reference standard into 250ml volumetric flask. Add about 50ml of methanol and Sonicate for about 3mins to dissolve the material. Make up to the volume with diluent and inject in to HPLC. The solutions were injected in triplicates for each concentration using 20µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak area Vs respective concentration of Cilostazol was found to be linear in the range of 25-150µg/ml with correlation coefficient 0.995.

2.1 Assay Method for Formulations:

Transfer 5 tablets into 250ml volumetric flask and add about 50ml of methanol and Sonicate for 10min with intermittent shaking. Add about 100ml of diluent and Sonicate for 20mins. Maintain the sonication bath temperature below 25ºc throughout the sonication. Takeout the flask and make upto the volume with diluent.

Pipette out 1ml of above solution to 100ml of volumetric flask make up to the volume with diluent. Filter a portion of sample solution through 0.45µm nylon membrane filter and separately inject 20µl of the blank, standard (five injections) and sample solution in duplicate into the HPLC, record the chromatographs and data is observed statistically (Table-1).

Table: 1 Assay of Cilostazol tablets (50mg)

Drug	Labeled Amount (µg/ml)	Amount taken(µg/ml) Mean(± S.D)	% Label Claim	%RSD
50 mg (Pletal)	25	25.003 ± 0.0115	100.012	0.011499
	50	49.993 ± 0.0151	99.986	0.015102
	100	99.983 ± 0.0057	99.983	0.005701

3. Validation

3.1 Specificity

The specificity of the method was evaluated for interference due to presence of excipients. If the excipients used in formulation did not interfere with the drug peaks and thus the method is specific. The HPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention time ranges. Chromsatograms were represented as figures 2 & 3 Blank and Placebo chromatograms respectively. The representative chromatograms for standard and the formulation were shown in figures 4 & 5. The figures show that the selected drug was clearly eluted. Thus the proposed HPLC method is selective.

Fig: 2 Typical Chromatogram of Cilostazol Blank

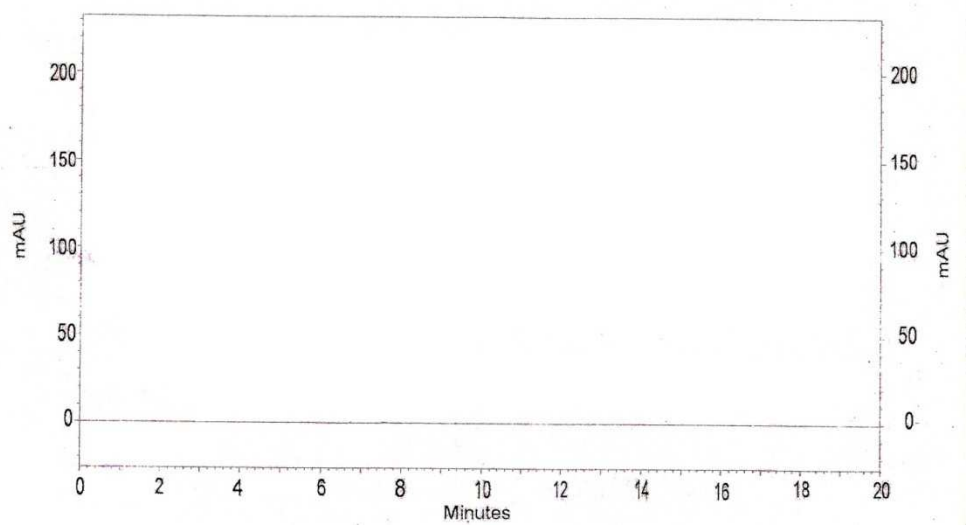


Fig: 3 Typical Chromatogram of Cilostazol Placebo

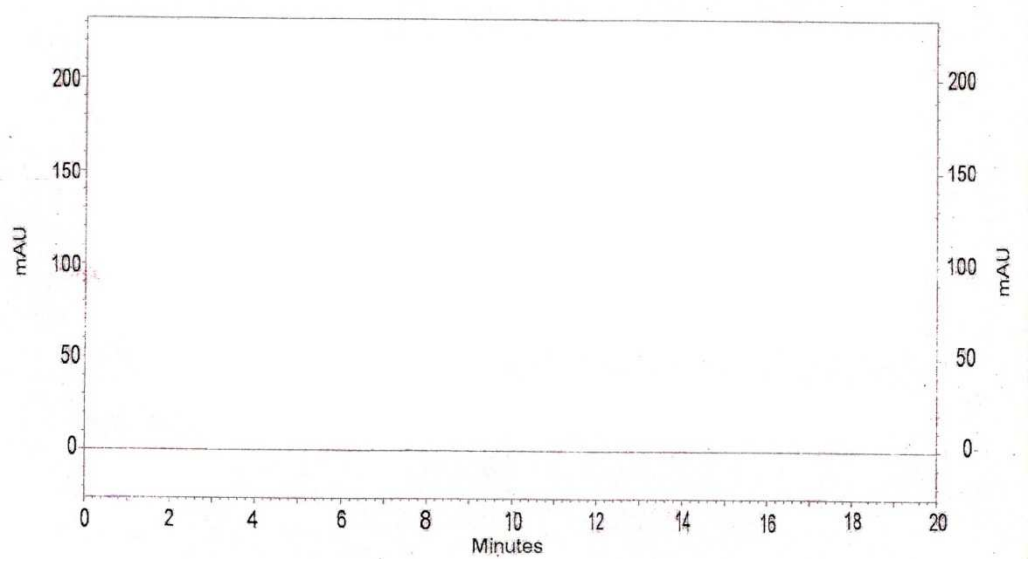


Fig: 4 Typical Chromatogram of Cilostazol Standard

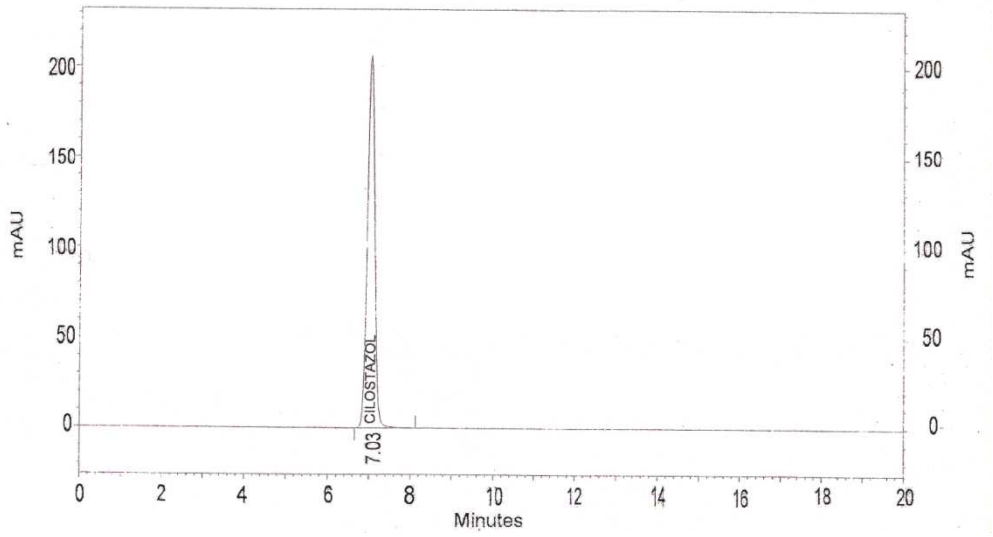
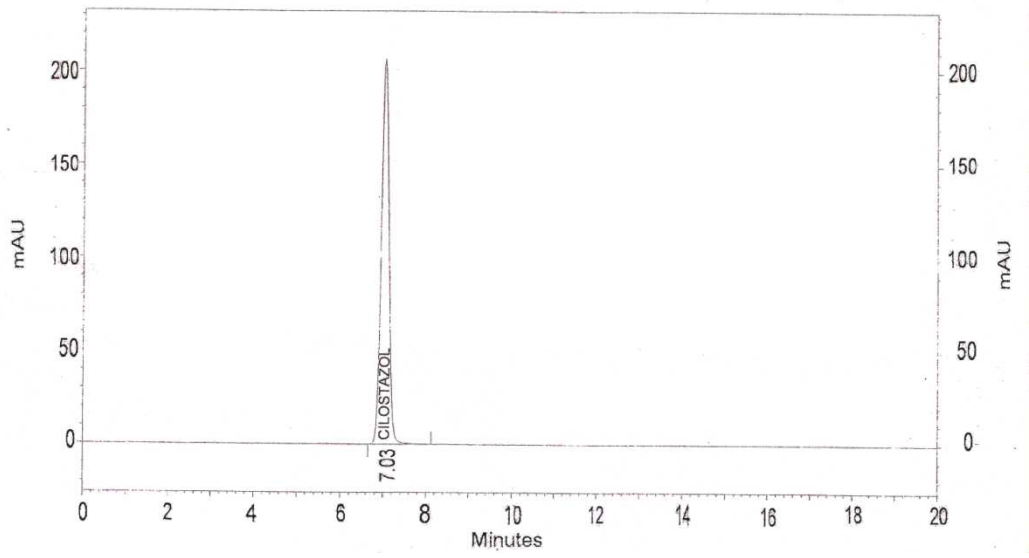


Fig: 5 Typical Chromatogram of Cilostazol Sample



3.2 System Suitability

Having optimized the efficacy of a chromatographic separation the quality of the chromatography was monitored by applying following system suitability tests: Capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all the analyte peak area for two consecutive injections was < 2.0%. For system suitability 6 replicates of working standard samples were injected and the parameters like Retention time, Plate number(N), Peak area and peak asymmetry of sample were calculated these results are presented in the table: 2

Table: 2 System suitability of Cilostazol (n=6)

Retention Time RT	Peak area of Cilostazol	plate count	Tailing
7.032	2573871	7389	1.023

3.3 Range of linearity

Standard curves were constructed using six standard concentrations in a range of 25-150 µg/ml for Cilostazol. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. A good linear relationship (R^2 = 0.995) was observed between the concentrations of Cilostazol and respective peak areas. The calibration graph was found to be y = 21539x + 36210 where Y is the peak area and X is the concentration of Cilostazol in the range of 25-150µg/ml. Statistical data and Linearity graph were shown in the table :3 and Figure.6

Table: 3 Linearity Data

Linearity Concentration	Peak Area	Average area	SD	%RSD
25	803204	803419	195.169	0.0242
	803468			
	803585			
50	1506982	1506808	213.176	0.0141
	1506871			
	1506570			
75	2008375	2008468	86.633	0.0043
	2008545			
	2008489			
100	2573871	2575060	2030.612	0.0788
	2573905			
	2577405			
125	3058193	3058390	1014.497	0.0331
	3059489			
	3057489			
150	3527898	3528475	9828.695	0.2785
	3518947			
	3538579			

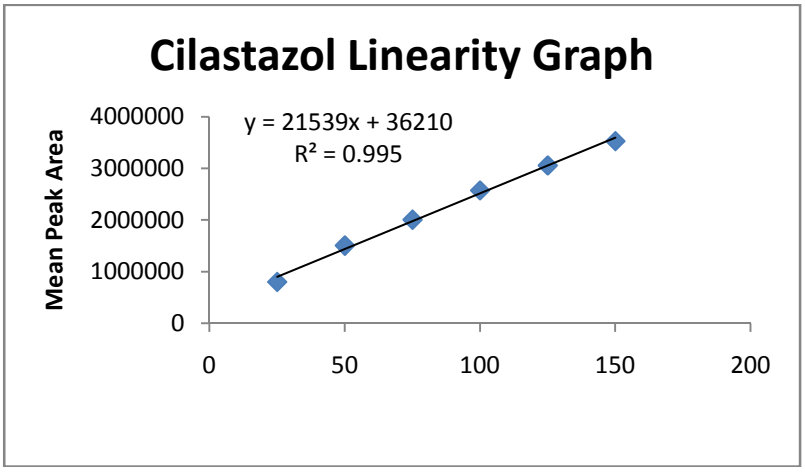


Fig: 6 Calibration curve of Cilostazol

3.4 Precision

The ICH documents[8] recommended that repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e., three concentrations and three replicates of each concentration) precision was studied to find out intra and interday variations of the proposed method at three different levels (50,100,150µg/ml for Cilostazol) on the same and on three different days respectively. The results were interpreted by statistical analysis by calculating % RSD values and all the results were within the acceptance criteria of not more than 2 % and the results are tabulated in the table: 4.

Table: 4 Summary of Inter-Day and Intra-day precision

Drug	Conc. (µg/ml)	Intraday Precision			Interday Precision		
		Mean amount Found (µg/ml)	±SD	%RSD	Mean amount Found (µg/ml)	±SD	%RSD
Cilostazol	50	49.98	0.0100	0.0200	49.99	0.0152	0.0305
	100	100.01	0.0173	0.0170	100.01	0.0057	0.0057
	150	149.99	0.0152	0.0101	149.99	0.0208	0.0138

3.5 Accuracy

The accuracy of the method was evaluated by determination of the recovery of Cilostazol at three levels concentration. Tablets sample solutions were spiked with Cilostazol standard solution, corresponding to 50% to 150% of the nominal analytical concentration (50-150 µg/ml). Their results showed good recoveries ranging from 99.99% to 101.01% for Cilostazol. The %RSD (0.0305) of mean recovery data obtained for each level is within R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study. (Table: 5)

Table: 5 Statistical data for Accuracy

Amount added (µg/ml)	Amount found (µg/ml)	% Mean Recovery	Statistical Analysis of % recovery	
			SD	%RSD
50	50.0066	100.0133	0.0305	0.0305
100	100.0167	100.01	0.0057	0.0057
150	149.9933	99.99	0.0101	0.0101

3.6 Robustness

Typical variations in high performance liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. The effect of variations in percent Water, Acetonitrile (ACN) and Methanol from 500:350:150 to 460:370:170 and 540:330:130 ratio mobile phases was evaluated. While the tailing factor, number of theoretical plates and resolution showed a little change with Acetonitrile and Methanol ratio variations, the retention time and, consequently no subsequent change was observed (Table6). Agilent Zorbax Eclipse XDB, C_{18} (250x4.6 mm, 5µm particle size) was used with the original mobile phase and the temperature variations tested were higher and lower values than the value set this method because temperature can cause changes on the elution of components and The effect temperature variation above and below the value of assay method showed a little change on the peak area The chromatographic parameters of Cilostazol showed only minor fluctuations with temperature changes.

Table: 6 Robustness Studies

Parameter		%RSD of peak area	Theoretical Plates	Asymmetry
Flow rate ± 20% (1.0ml/min)	0.8 ml/min	0.032	2573884	1.2
	1.2 min/min	0.073	2573910	1.3
Organic phase Variation ± 2% (500:350:150)	(Water:ACN:MeoH) 460:370:170	0.059	2573936	1.1
	Water:ACN:MeoH) 540:330:130	0.047	2573962	1.2
Temperature variations ±5 °c	30	0.061	2573988	1.2
	20	0.086	2573884	1.2
Column variation	Symmetry C_{18} (150x 4.6mm, 5µ)	0.038	2573910	1.3
	Inertsil ODS-3V (150X 4.6mm,5µ)	0.056	2573981	1.2

4. Results and Discussion

The present study was carried out to develop a sensitive, precise and accurate HPLC method for the analysis of Cilostazol in bulk samples and its pharmaceutical dosage form. The retention time for Cilostazol is 7.032mins. Each of the samples was injected six times and the same retention times were observed in all cases. The peak area of different concentration set up as above was calculated. A good linear relationship (R^2 = 0.995) was observed between the concentrations of Cilostazol and respective peak areas. The calibration graph was found to be y = 21539x + 36210 where Y is the peak area and X is the concentration of Cilostazol in the range of 25-150µg/ml when we analyzed the proposed RPHPLC method for finding out intra and inter-day variations, a low coefficient of variation was observed (Table 4). This shows that the present HPLC method is highly precise. The drug content in the tablets was quantized using the proposed analytical method. The mean content of Cilostazol in tablets is shown in (Table: 1).The amount of Cilostazol from the preanalyzed sample containing known amounts of the drug is shown in (Table: 5). About 99.99 -100.01% Cilostazol could be recovered from the preanalyzed sample indicating the high accuracy of the proposed HPLC method. The proposed method validated according to ICH guidelines [8]. The proposed RP-HPLC method was found to be simple, precise, highly accurate, specific and less time consuming. It can be concluded that the proposed method was suitable for the estimation of Cilostazol in tablets for routine quality control analysis.

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