

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF CILOSTAZOL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Cilostazol is a quinolinone-derivative medication used in the alleviation of the symptoms of intermittent claudication in individuals with peripheral vascular disease. A simple, selective, precise, accurate and cost effective reverse phase HPLC method has been developed and validated for estimation of Cilostazol in tablet dosage form. In the chromatographic conditions, phenomenex synergi polar RP 80A (150 X 4.6 mm, 4 μ m) stationary phase with mobile phase consisting of potassium phosphate buffer (pH 3.0 \pm 0.05): acetonitrile (60: 40 v/v) was used at a flow rate of 1.2 mL/min. and column temperature was maintained at 25°C. Cilostazol was detected at 259 nm. The chromatographic procedure separated Cilostazol and potential interfering peaks in an analysis time of 6 min. with Cilostazol eluting at about 3 min. The assay method was found linear in the concentration range of 50% to 150% of assay working concentration (0.1 mg/mL) with a correlation coefficient of 0.9998. The percentage recovery of assay was found between 100.1 and 101.2. The developed method was validated with respect to specificity, linearity, accuracy, precision, sensitivity, robustness and solution stability as per ICH guidelines. The proposed method can be used for routine analysis of Cilostazol formulations in quality control laboratories.

KEY WORDS

Cilostazol, HPLC, Validation, Dissolution, Extended Release.

INTRODUCTION:

Cilostazol is a quinolinone derivative and cellular phosphodiesterase inhibitor, more specific for phosphodiesterase III (PDE III). Although the exact mechanism of action of is unknown, cilostazol and its metabolites appears to inhibit PDE III activity, thereby suppressing cyclic adenosine monophosphate (cAMP) degradation. This results in an increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. Cilostazol is a selective inhibitor of phosphodiesterase type 3

(PDE₃) with therapeutic focus on increasing cAMP¹. An increase in cAMP results in an increase in the active form of protein kinase A (PKA), which is directly related with an inhibition in platelet aggregation. PKA also prevents the activation of an enzyme (myosin light-chain kinase) that is important in the contraction of smooth muscle cells, thereby exerting its vasodilatory effect².

Reverse Phase HPLC:

In this chromatographic technique, the stationary phase is non-polar, and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first.³

Steps involved in development of RP-HPLC method:

Selection of chromatographic method:

The proper selection of methods depends upon the nature of the sample (ionic or ionisable or neutral molecule) its molecular weight and stability. The drug selected is polar and ionic hence reversed phase chromatography was used because of its simplicity and suitability.⁴

Selection of stationary phase:

Matching the polarity of sample and stationary phase and using a mobile phase of different polarity achieve a successful separation.⁵

Selection of mobile phase:

Reverse phase bonded packing, when used in conjunction with highly polar solvents; approach is ideal and is a universal system for liquid chromatography. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.⁶

Selection of suitable detector:

Detector is the eye of HPLC system that measures the compounds after their separation on the column. There are basically two types of detectors- the bulk property detectors and solute

property detectors. Detectors, in order of their popularity are UV, fluorescent, conductivity, polarimeter and refractive index detectors. UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest versions of equipment's are available with photo diode- array detectors (PAD or DAD).

Method optimization:

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and shape, plate count, asymmetry, capacity, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.

A Literature survey reveals that various analytical methods have been reported for the estimation of Cilostazol based on different technique, such as; LC-MS, LC-TMS, HPLC with UV detection assay for its quantification in plasma and serum. The aim of this study was to develop a stability indicating assay method for estimation of Cilostazol in a pharmaceutical dosage form. The method uses UV detection with a run time of 6 min. The method has several advantages like simple mobile phase, low injection volume, less run time over the reported methods. The developed method was validated as per international conference on harmonization (ICH) Q₂ (R₂) guidelines.⁹⁻¹²

MATERIALS AND METHODS

Drug profile of Cilostazol

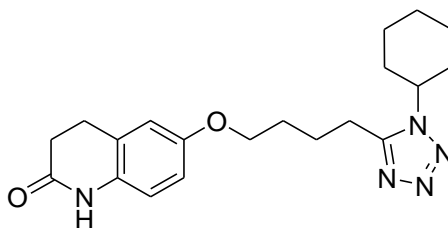


Fig.1. Structure of Cilostazol

IUPAC Name : 6-[4-(1-cyclohexyltetrazol-5-yl) butoxy]-3,4-dihydro-1H-quinolin-2-one
Chemical formula : C₂₀H₂₇N₅O₂

Molecular weight : 369.469
Description : Whiteto off-white powder.
Solubility : Freely soluble in acetic acid, chloroform, n-methyl-2-pyrrolidone, DMSO; slightly soluble in methanol, ethanol. Practically insoluble in ether. 0.1NHCl, 0.1N NaOH
Category : Phosphodiesterase inhibitor-platelet aggregation and vasodilation
 λ_{\max} : 259 nm

Drugs Used:

Table 1: List of Drugs Used

S. No.	Drugs	Manufacturer
1.	Cilostazol	Cipla Pharma
2.	Pletoz 100 mg tablets Commercial Tablets	Cipla Pharma

Reagents Used:

Table 2: List of Reagents Used

S. No.	Chemicals	Manufacturer Name	Grade
1	Water	Merck	HPLC
2	Methanol	Merck	HPLC
3	Acetonitrile	Merck	HPLC
4	Potassium phosphate, (Monobasic)	Merck	G.R
5	O-Phosphoric acid	Merck	HPLC

Equipment and Apparatus Used:

Table 3: Equipment and Apparatus Used

S. No.	Instrument Name	Model Number	Software	Manufactures Name
1	HPLC	Alliance UV-Visible detector-2487	Empower	Waters
2	U.V Double beam spectrophotometer	SL 210	-	ELICO
3	Digital weighing balance (Sensitivity 5 mg)	BL-200H	-	SHIMADZU
4	P ^H -meter	LI-120	-	ELICO
5	Sonicator	3305013	-	SISCO

Preparation of mobile phase:

A combination of mobile phase containing potassium phosphate buffer (pH 3.0 \pm 0.05): acetonitrile (60:40 v/v) was mixed and degassed in ultrasonic water bath for 5 minutes finally filtered through 0.45 μ membrane filter. This prepared solution was used as mobile phase.

Diluent:

Potassium phosphate buffer pH 3.0 and methanol in the ratio of 50:50 (v/v) was used as diluent.

Preparation of standard solution: (0.1mg/ml)

Weighed accurately 20 mg of Cilostazol working standard into 200 ml volumetric flask, added 80 mL of diluent made to dissolve and made up the volume with diluent.

Preparation of sample solution: (0.1mg/ml)

Crushed to powder (20 tablets), weighed and transferred the tablet powder equivalent to 20 mg of Cilostazol into 200 mL volumetric flask added 80 mL of diluent, sonicated for 10 min. and diluted to volume with diluent. Filter the solution through nylon 66-membrane filter. A small portion of the extract (say 10 ml) was withdrawn and filtered to ensure the absence of particulate matter. The filtered solution was appropriately diluted with the diluent.

Wavelength selection:

About 0.25 mg/mL of Cilostazol solution was accurately prepared by dissolving the standard in water. The Cilostazol solution was scanned in the 200-400 nm UV region. The wavelength maximum (λ_{max}) was observed at 259 nm and this wavelength was adopted for absorbance measurement.

Calculation:

$$\text{Assay \%} = \frac{\text{sample area}}{\text{Standard area}} \times \frac{\text{dilution sample}}{\text{dilution of standard}} \times \frac{P}{100} \times \frac{\text{Avg.wt}}{Lc} \times 100$$

Where:

Avg.wt = average weight of tablets

P = percentage purity of working standard

LC = label claim of Cilostazol mg/ml

RESULTS AND DISCUSSION:

Optimized method:

It was performed on Phenomenex Synergi polar RP 80A (150 X 4.6mm, 4 μ) with a mobile phase

Optimized chromatographic conditions:

Column:

Phenomenex Synergi polar RP 80A (150 X 4.6 mm, 4 μ m)

Column temperature: Ambient

Wave length: 259 nm

Mobile phase ratio:

Potassium phosphate buffer: Acetonitrile (60: 40 v/v)

Flow rate : 1.2 min/ml

Injection volume : 20 μ l

Run time : 6 minutes

Validation of developed RP-HPLC method:

As per the International conference on harmonization (ICH) guidelines the method validation parameters such as linearity, precision, accuracy, system suitability, limit of detection and limit of quantitation were optimized.

Assay

Sample and standard solutions were injected into the chromatographic system and measured the area for Cilostazol and calculated the % assay by using the above formula.

composition of potassium phosphate buffer: acetonitrile (60: 40 v/v) at a flow rate of 1.2 ml/min. 20 μ l of sample was injected and the run time was 6 minutes.

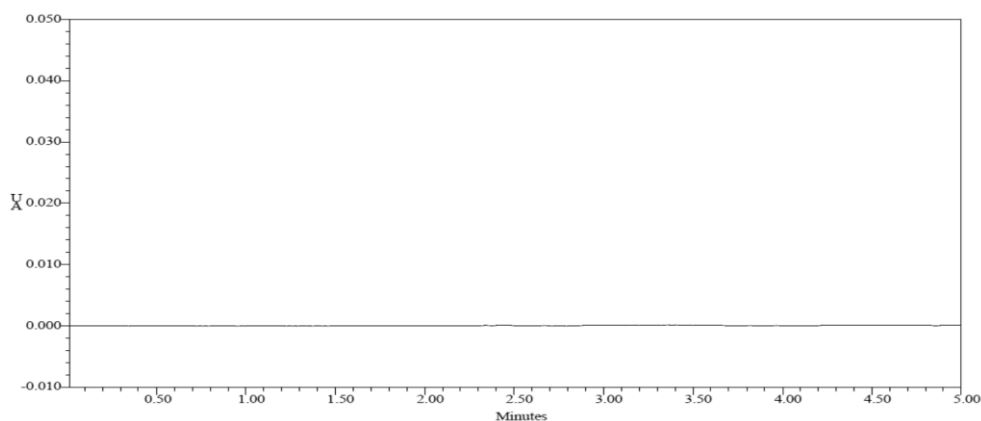


Fig. 2: Chromatogram showing blank preparation (mobile phase)

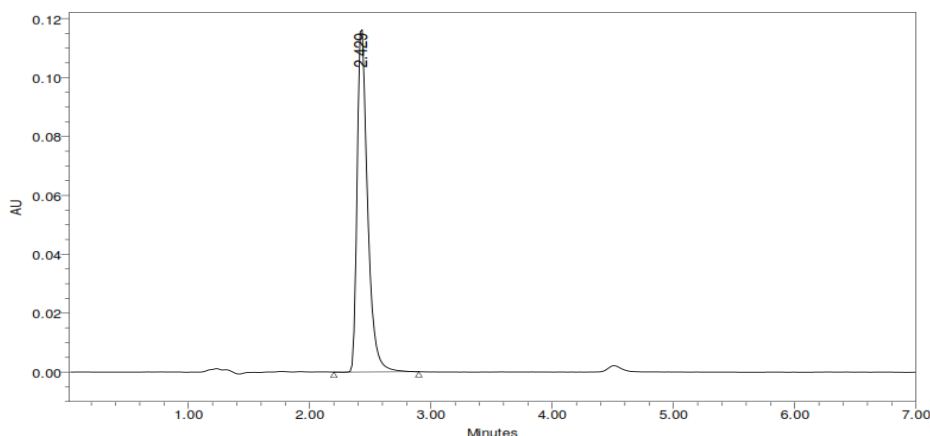


Fig. 3: Chromatogram of Cilostazol standard peak

Linearity:

0.05, 0.07, 0.1, 0.12 and 0.15 mg/ml was injected into the chromatographic system and peak area was measured. Plotted a graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) and the correlation coefficient was calculated.

Acceptance criteria:

Correlation coefficient should be not less than 0.999.

Table 4: Showing the results for the Linearity

Conc.(mg/ml)	RT	Area
0.0495	2.426	1848
0.0742	2.428	2779
0.0990	2.422	3706
0.1188	2.421	4433
0.1485	2.424	5549
Co efficient of correlation (R^2)		0.999

Precision:

The standard solution (0.1 mg/ml) was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria:

The %RSD for the area of five standard injections results should not be more than 2.

Table.5. showing the results for precision

S. No	Conc.(mg/ml)	RT	Area
1	0.1	2.423	3700
2	0.1	2.431	3700
3	0.1	2.448	3699
4	0.1	2.468	3702
5	0.1	2.498	3704
Mean			3701
S. D			2.000
% RSD			0.0

Accuracy:

The standard solution of concentration 50,100 and 150 µg/ml were injected into chromatographic system. Calculated the individual % recovery and mean % recovery of Cilostazol.

Acceptance criteria:

The % recovery for each level should be between 98.0 to 102.0%.

Table 6: Showing Accuracy results for Cilostazol

S. No	Conc(µg/ml)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean% recovery
1	50	1956	5	5.06	101.2%	
2	100	3856	10	10.06	100.6%	100.1%
3	150	5958	15	15.01	100.1%	

System suitability:

The standard I solution was injected one time and standard II solution was injected 5 times.

Table 7: Showing system suitability results for Cilostazol

S. No.	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	1.0	7652	1.1
2	1.2	7829	1.2
3	1.4	7566	1.2

Limit of detection (LOD)

From the above preparation 1ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluents.

Table 8: Showing results for Limit of Detection

Drug Name	y-Intercept	Slope(s)	LOD(µg/ml)
Cilostazol	4.9256	36465	1.675

Limit of quantitation (LOQ)

From the above preparation 0.5ml of solution is transferred to 10ml of volumetric flask and the volume made with the diluent.

Table 9: Showing results for Limit of Quantitation

Drug name	y-Intercept	Slope(s)	LOQ($\mu\text{g/ml}$)
Cilostazol	4.9256	36465	5.026

Assay:

The developed and validated method was applied to the determination of Cilostazolin marketed tablets containing 100 mg of drug per tablet. Three injections of sample were injected into chromatographic system. Assay % was calculated by using the formula mentioned above and it was found to be 99.8%.

Table 10: showing the results of assay

S. No	Name	RT	Area
1	Cilostazol	2.462	3789
2	Cilostazol	2.486	3801
3	Cilostazol	2.425	3763

CONCLUSION:

A simple, rapid, accurate and precise RP-HPLC method was developed for the determination of Cilostazol in pure form and in tablets. The analytical conditions and solvent system developed provided a good separation for Cilostazol within a short analysis time. The method was validated and demonstrated a wide linear dynamic range, a good precision and accuracy. Thus, the method can be proposed for routine analysis laboratories and for quality control.

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