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

[1]Naveen Babu Kilaru*, [1]Murali Krishna Javvaji, [2]Rajani Kumar Valluru, [3]Krishna Mohan Chinnala

[1]KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, India.
[2]AXIS Clinicals Limited, Hyderabad, India.
[3]Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, India.

*Corresponding Author Email: naveenbabukilaru@gmail.com

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 µm) stationary phase with mobile phase consisting of Potassium phosphate buffer (pH 3.0 ± 0.05): Acetonitrile (60: 40 v/v) was used at a flow rate of 1.2 mL/min. and column temperature was main 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 (PDE_3) with therapeutic focus on increasing cAMP[1]. 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 effect[2].

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: Reversed 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 Counts asymmetry, capacity, elution time, detection limits, limit of quantization 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

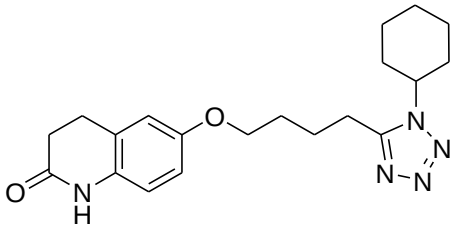

Fig.1.Structure of Cilostazol

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

Chemical formula : $C_{20}H_{27}N_5O_2$

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 tabletsCommercial 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 containingPotassium phosphate buffer (pH 3.0 ± 0.05): Acetonitrile (60:40 v/v) was mixed and degassed in ultrasonic water for 5 minutes finally filtered through 0.45µ membrane filter. This prepared solution was used as mobile phase.

Diluents:
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)
Weigh accurately working standard equivalent to 20 mg of Cilostazol into 200 mL volumetric flask,

add 80 mL of diluent and dissolve, further make up the volume with diluent.

Preparation of sample solution: (0.1mg/ml)

Crush to powder 20 tablets, weigh and transfer the tablet powder equivalent to 20 mg of Cilostazol into 200 mL volumetric flask add 80 mL of diluent, sonicate for 10 min and dilute to volume with diluent. Further filter the solution through 0.45µm pore size 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 0.25mg/mL of Cilostazol solution was accurately prepared by dissolving the active in water. The Cilostazol solution scanned in the 200-400 nm UV regions. The wavelength maximum (λ_{max}) was observed at 250 nm and this wavelength was adopted for absorbance measurement.

Optimized chromatographic conditions:

Column: Phenomenex Synergi polar RP 80A (150 X 4.6 mm, 4 µm

Column temperature: Ambient

Wave length: 259nm

Mobile phase ratio: Potassium phosphate buffer: Acetonitrile (60: 40 v/v)

Flow rate	:	1.2 min/ml
Injection volume	:	20µl
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 was injected into the chromatographic system and measured the area for perindopril and calculated the % assay by using the formulae.

Calculation:

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

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 4.6 mm, 4 µm with a mobile phase composition of Potassium phosphate buffer: Acetonitrile (60: 40 v/v) at a flow rate of 1.2 min/ml. 20µl of sample was injected and the run time was 6 minutes.

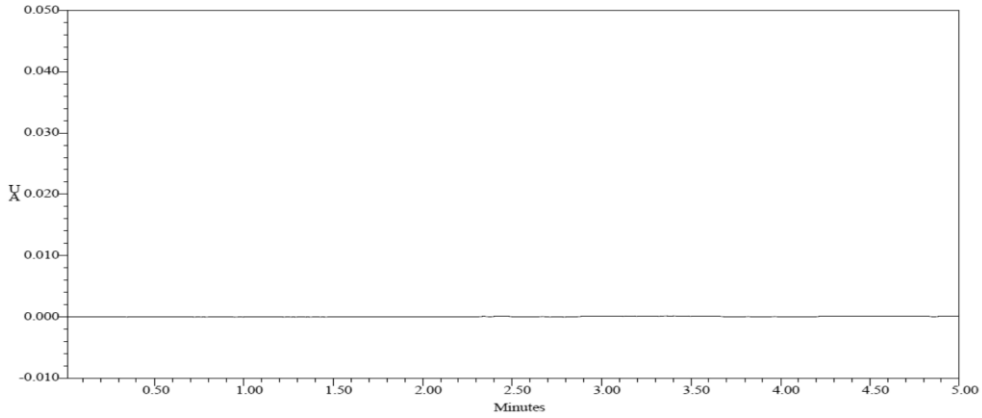

Fig. 2: Chromatogram showing blank preparation (mobile phase)

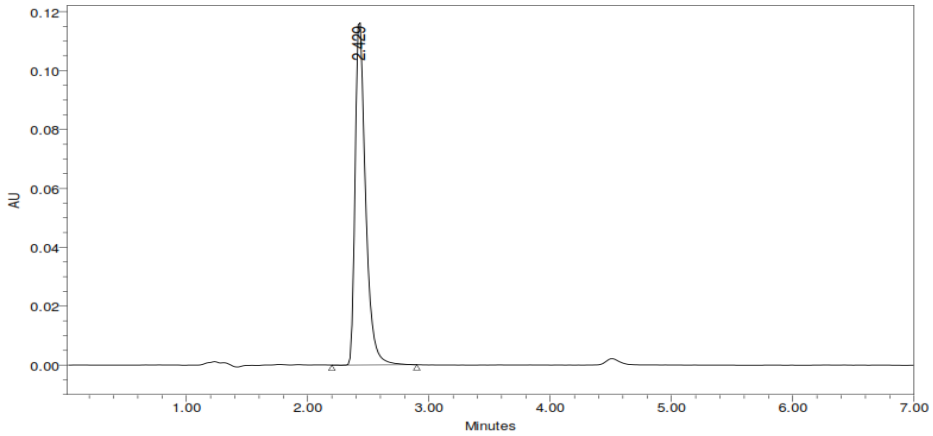

Fig. 3: Chromatogram of Cilostazol standard peak

Linearity:

0.05, 0.07, 0.1, 0.12 and 0.15mg/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
Std. Dev			2.000
%Rsd			0.0

Accuracy:

The standard solution of concentration 50,100 and 150 ppm were injected into chromatographic system. Calculate the amount found and amount added for Cilostazolcalculated the individual % recovery and mean % recovery values.

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(µg/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 Cilostazolin pure form and in tablets. The analytical conditions and solvent system developed provided a good separation forCilostazolwithin 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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***Corresponding Author:**
Dr. Naveen Babu Kilaru
Professor
Department of Pharmaceutical Biotechnology
KVSR. Siddhartha College of Pharmaceutical Sciences
Siddhartha Nagar, Pinnamaneni Polyclinic Road,
Vijayawada, Andhra Pradesh, India-520010.
E-Mail: naveenbabukilaru@gmail.com