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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CILOSTAZOL IN PHARMACEUTICAL FORMULATION USING RP-HPLC

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ABSTRACT

A reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Cilostazol in Bulk and its pharmaceutical formulation. Separation was achieved with a Agilent TC-C_{18}(2) 5µm 4.6×250mm Column using water, acetonitrile and methanol as mobile phase in a ratio of (40:50:10) v/v at flow rate 1.0 mL/min and the Column temperature was 25°C. UV detection was performed at 257 nm. The method is simple, rapid, and selective. The described method of Cilostazol is linear over a range of 15 µg/mL to 35 µg/mL. The method precision for the determination of assay was below 2.0% RSD. The % recoveries were found to be 99.7 to 101.6% for Cilostazol.

Key words: Cilostazol, RP-HPLC.

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INTRODUCTION

Cilostazol is 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone(Fig-1).

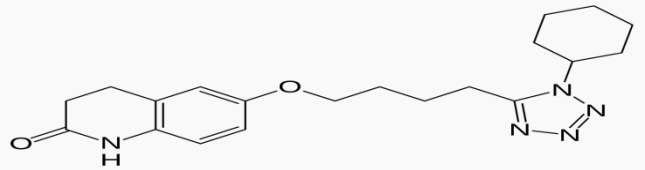

Fig-1 Structure of Cilostazol

Cilostazol is a selective inhibitor of 3-type phosphodiesterase (PDE3) with therapeutic focus on increasing cAMP. An increase in cAMP results in an increase in the active form of 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.On the literature survey most of publications related to HPLC methods have been reported for determination of Cilostazol in pharmaceutical dosage forms (1-8). Hence an attempt has made to develop a HPLC method for the determination of Cilostazol in pharmaceutical dosage forms. The objective of this experiment was to optimize the assay method for estimation of Cilostazol in tablets based on the literature survey made. So here planned to develop accurate, precise, specific method for estimation of Cilostazol in tablets.

MATERIALS AND METHODS

Optimized method

Mobile phase

Prepare a filtered and degassed mixture of water, acetonitrile and methanol in the ratio of 40:50:10 v/v respectively.

Diluent: Methanol

Chromatographic conditions

Column-Agilent TC-C_{18}(2) 5µm 4.6×250mm; Flow rate-1.0ml/min, Detector wave length-257nm, Column temperature-25^{o}C, Injection volume-10µl, Run time-10 mins.

Standard Preparation

10 mg of Cilostazol was accurately weighed and transferred into 10 ml volumetric flask.10 ml of diluent was added and make it to dissolve. From the above solution 1 ml was pipetted out and transferred to 10 ml volumetric flask and make up the volume with diluents. From the above prepared solution 1.5 ml was pipette out and was made upto 10 ml with diluent.

Sample preparation

Portion of powder equivalent to 10 mg of Cilostazol was accurately weighed and transferred into 10 ml volumetric flask.About 10 ml of diluent was added, shaken for 10 minutes on shaker and sonicated for 20 minutes with occasional shakings. The solution was cooled to room temperature and diluted to volume with diluent. The solution was filtered through 0.45 µm PVDF filter.

VALIDATION PARAMETERS

Linearity

Linearity was studied over a range of 15 µg/mL to 35 µg/mL. Each level was injected into chromatographic system .The area of each level was used for calculation of correlation coefficient.

Accuracy

The accuracy study was performed for 50%, 100% and 150% recoveries for Cilostazol. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Precision

The method and system precision study was performed for six injections of Cilostazol. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD.

System Suitability:

The system suitability studies were done with the 25 mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.

Specificity

10 µl of each solution was injected individually the prepared solutions of standard and sample and develop chromatograms check the retention time of individual injection sample in the chromatogram and observe whether the retention time is matching with that of standard.

LOD and LOQ:

The detection limit is characteristic of limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified under stated experimental conditions. The limit of quantification is the lowest amount of analyte in the sample that can be quantitatively determined with definite precision with stated experimental conditions.

Results and discussion

Good Separation was achieved with a Agilent TC-C_{18}(2) 5µm 4.6×250mm Column using water, acetonitrile and methanol as mobile phase in a ratio of (40:50:10) v/v at flow rate 1.0 mL/min and wavelength 257nm.The linearity study was performed the correlation coefficient of Cilostazol was found to be 0.999 respectively (Fig-2)

Table-1 Results from linearity study

S.No	Linearity Level	Concentration (µg/ml)	Peak area
1	I	15	6773478
2	II	20	8946234
3	III	25	11268098
4	IV	30	13210394
5	V	35	15689741
Correlation coefficient			**0.999**

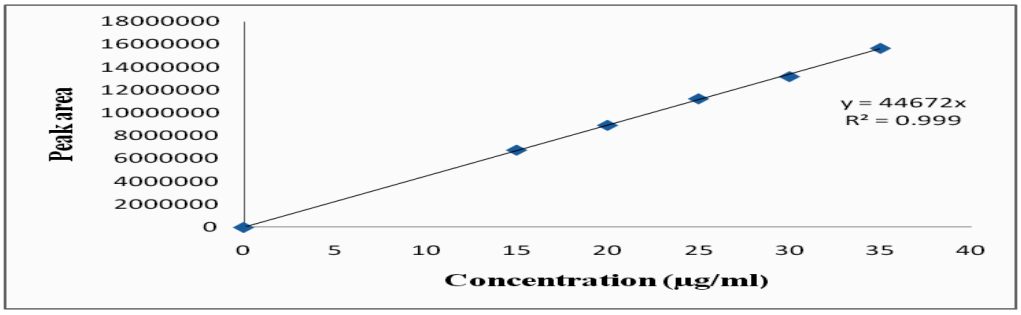

Fig-2 Calibration curve of Cilostazol

The system suitability studies were done with accurately weighing equivalent to 25mg of Cilostazol dosage form. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than indicating that the method is suitable. The chromatogram is recorded and are shown in fig-3

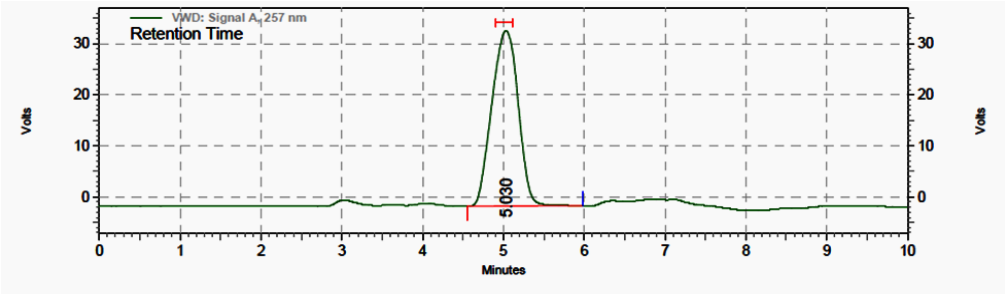

Fig-3 Chromatogram showing system suitability

The specificity test was performed for Cilostazol. It was found that there was no interference of impurities in retention time of analytical peak. The method show excellent specificity with Cilostazol eluting at retention of 5.03 minutes. No interference was observed with mobile phase.The accuracy study was performed for Cilostazol.

The accuracy study was performed for % recovery. The % recovery was found to be 101.6 to 99.70% respectively (Table-2)

Table-2 Results from accuracy study

Level of recovery	Amount of drug spiked(µg/ml)	Drug recovered	%Recovery	Mean	SD	%RSD
50	49.6	49.63	100.3	100.4	0.352	0.34
		49.63	100.3			
		49.66	100.6			
100	99	99.23	101.9	101.6	0.981	0.96
		99.10	100.6			
		99.33	102.5			
150	149.4	149.26	99.6	99.70	0.645	0.64
		149.21	99.8			
		149.45	99.7			

The precision of method and system was determined by replicate injection of sample solution (Table no.3 and 4).

Table-3 Results of method precision

S.No	Peak Name	Peak area
1	Cilostazol	11256987
2	Cilostazol	11269458
3	Cilostazol	11248262
4	Cilostazol	11257386
5	Cilostazol	11269082
6	Cilostazol	11257966
Mean		11259856
SD		22814
%RSD		0.15

Table-4 Results of system precision

S.No	Peak Name	Peak area
1	Cilostazol	11261362
2	Cilostazol	11252438
3	Cilostazol	11262305
4	Cilostazol	11247903
5	Cilostazol	11242736
6	Cilostazol	11252807
Mean		11253258
SD		23218
%RSD		0.19

Table-5 Summary of the results

Validation Parameters	Acceptance Criteria	HPLC Results
Specificity	The peaks of diluent and impurities should not interfere with the main Peak	The peaks of diluent and impurities are not interfering with the main peaks of Cilostazol
Linearity	The Correlation coefficient shall be NLT 0.998	0.999
Accuracy	The % recovery at each spike level should be between 98%-102%	99.7%-101.6%
System Precision	The %RSD of peaks obtained from the 6 replicate injections should be NMT 1.0%	0.19%
Method Precision	The % RSD for the six determinations shall be NMT 2.0.	0.15%
LOD	0.69 µg/ml	-
LOQ	0.21µg/ml	-

CONCLUSION

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Cilostazol. The method enables accurate, precise, and rapid analysis of Cilostazol (Table-5). It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

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