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METHOD DEVELOPMENT AND VALIDATION BY RP- HPLC METHOD FOR CIOSTAZOL IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
The aim of the present work is to develop an accurate, precise, and cost effective RP-HPLC method for the determination of Cilostazol in bulk and pharmaceutical dosage form. Separation was done with a column Chemsil ODS C_{18} , (250mm × 4.6 I.D; particle size 5µm) at R.T. at a flow rate 1.2ml/min using the mobile phase methanol: water pH 3 (85:15 v/v) at an wavelength 257nm with an chromatographic run time 10 min. The method was linear over the range of 2-10 µg/ml with correlation coefficient of 0.9993 for Cilostazol.

KEYWORDS : *Cilostazol, RP-HPLC and validation*

INTRODUCTION

Cilostazol is a quinolinone derivative and antiplatelet agent with vasidilating properties that has been used in the symptomatic treatment of intermittent claucidation in patient with peripheral ischaemia. Is used to improve the symptoms of certain blood flow problem in the legs. Cilostazol is chemically known as 6 – [4 – (1- cyclohexyl -1H – tetrazol – 5 – yl) -butoxyl] – 3,4 dihydro – 2(1H) – quinolinone.

The present RP-HPLC work was more effective than other methods for estimation of Cilostazol drug in tablet dosage form because require less time ,less sample volume, done at an ambient temperature etc.

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Structure

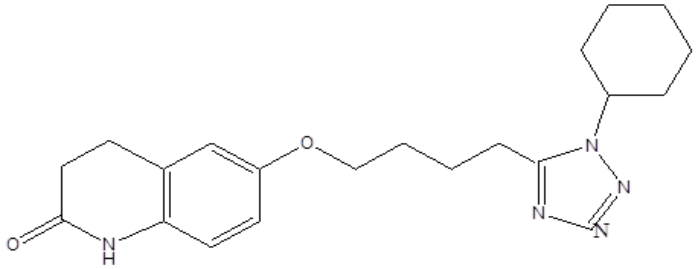

Table no 1 : Properties of Cilostazol drug

Sr. no.	Physical properties	Reported result
1	Colour	White or off white
2	Odour	Odourless
3	Appearance	Crystalline
4	Melting point	159.4- 160.3ºC
5	Category	antithrombotic

MATERIALS AND METHODS

Materials

Cilostazol was a gift sample from pure chem India. Cilostazol tablet used were Slitoz 50mg, Glenmark pharmaceuticals. HPLC grade methanol, water, orthophosporic acid, from Modern lab, Nashik.

Instruments

Waters corp HPLC, column chemsil ODS C_{18} , UV – visible detector, manual inject port, breeze software, precision balance, digital pH meter, Digital ultra sonicator.

Preparation of Mobile Phase

The 10mM orthophosporic acid was prepared and pH adjusted to 3 and filtered through nylon filter paper and mixed with methanol : water in the volume ratio of 85:15 v/v and sonicated for 15 mins to degas the mobile phase.

Preparation of Standard Stock Solution

25mg Cilostazol was accurately weighed and transferred into 25 ml volumetric flask make up the volume up to the mark with the diluent to obtained concentration 1000µg/ml. through this solution prepare further dilution.

Test solution preparation:

Take twenty tablets, each containing 50 mg of Cilostazol . The tablets were extinguished to small powder and quantity of powder parallel to 50 mg of Cilostazol were measured and added in 25 ml volumetric flask make up with methanol and shaked to make transparent solution. The solution was flow by using membrane filter and degassed. From this solution pipette out 2ml transfer in 10ml volumetric flask and fill up to the signal by utilizing methanol as solvent to get 200µg/ml solution.

VALIDATION PARAMETERS

A) Linearity:

Unknown conc. reports that are parallel to the concentration of analyte in samples within a given limit known as linearity.

Determination

Take 6 different concentration and each take 3 replicate. Prepare graph conc. Vs. Area and calculate correlation coefficient and %RSD

B) Accuracy (%Recovery):

The belonging of unknown conc. solutions reports obtained by that method to the observed value is known as accuracy. The % recovery checked by add known conc. of STD solution against test solution .

C) Precision:

The number of test solutions of a same sample giving same results known as precision. Through this calculated SD and RSD

Method for precision:

Determination:

Take either 3 different conc. and each take 3 replicate or take 6 replication of same concentration and calculate precision

D) Robustness:

It is the quantitate of capacity of the method to unchanged by little but intentional difference in method framework and provides an signal of its constant under normal usage.

Determination:

Quantitated by changing different variable which effect on method performance in within limit.

The unknown conc. solution and known conc. solution was injected under variable chromatographic state as shown below.

E)Limit of Detection:

The lower conc. of the analyte in the sample that the method can found but not necessarily measured

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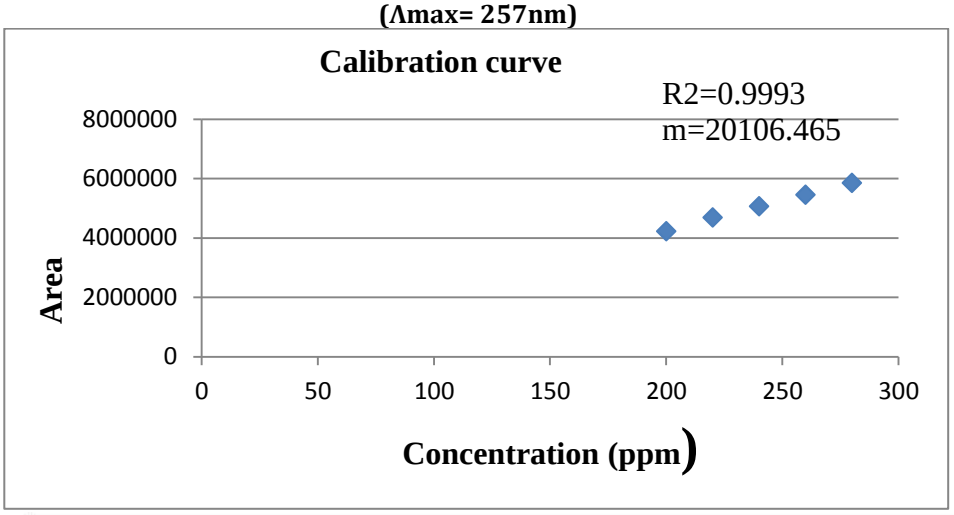
under the given experimental state simply shows that the sample is below or above certain range. Limit test prescribed as percentage or as parts per million. The limit of detection will not only depend on the procedure of analysis but also on type of instrument.

Limit of Quantitation:
The lowest conc. of test sample can be measured under the given experimental conditions .The S/N ratio should not less than 10 and RSD ≤ 2%.

RESULT AND DISCUSSION

Fig no. 1: Calibration curve for Cilostazol in methanol:water (Λmax= 257nm)



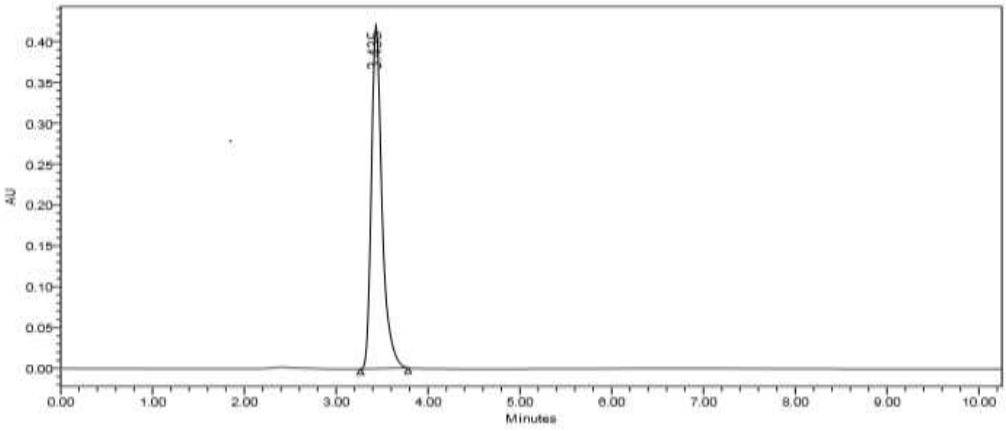


Fig no. 2 chromatogram of Cilostazol

Developed Method

Mobile Phase Composition: Methanol : Water (85:15)	Flow Rate: 1.2 ml/min
Temperature: ambient	pH : 3 (adjusted by10mM orthophosporic acid)
Column name: Chemsil ODSC18 (250 x 4.6 mm, 5.0 µm)	Tailing factor :1.40
Injection Volume : 10 µl	Wavelength: 257nm
Program : Isocratic	Plate count : 4393
Detector: Ultraviolet	Retention time : 3.4 min.

System suitability test for Cilostazol:
The plate count, tailing and %RSD was found to be within limit.
Plate count – more than 2000, tailing less than 2 and %RSD less than 2.

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Table no 2: Results of Method Precision of Cilostazol

Sample Name	Retention Time (min)	Area	Plate count	Tailing
Stand.1	3.442	4395598	4432	1.40
Stand.2	3.396	4359222	4426	1.41
Stand.3	3.432	4418208	4439	1.40
Stand.4	3.372	4415373	4345	1.40
Stand.5	3.379	4403955	4349	1.40
Stand.6	3.579	4398574	4367	1.40
Mean	3.428	4398488	4393	1.40
S.D	0.05723	9495.36	25.807	0.002
%RSD	1.66	0.21%	0.587	0.14

Validation

Linearity : correlation coefficient was found to be 0.9993 (NLT 0.999) and %RSD less than 2.

Table no 3: Results of Method Precision of Cilostazol

Sr. no.	Conc. (µg/ml)	Area (avg.mean)	%RSD
1	200	4412512	0.12
2	220	4685763.6	0.053
3	240	49027856	0.71
4	260	5449454.6	0.64
5	280	5845392.3	0.012
Correlation coefficient (R^2) = 0.9993			

ACCURACY:

The recovery of drug was found to be 98 to 102% within std. limit.

Table no 4: Results of Method Precision of Cilostazol

Cilostazol							
Sr. No.	**Conc. Level**	**Conc. (µg/mL) STD solution**	**Conc. (µg/mL) tablet solution**	**Area**	**SD**	**%RSD**	**% Recovery**
1	80%	1	1.4	4685892	1879.10807	0.04	
		1	1.4	4683527			
		1	1.4	4688832			
				Mean – 4686083.6			100
2	100%	1.4	1.4	4868273	1412.9874	0.02	
		1.4	1.4	4865832			
		1.4	1.4	4864312			
				Mean- 4866139			99.25
3	120%	1.6	1.4	5450304	8774.9913	0.16	
		1.6	1.4	5433024			
		1.6	1.4	5426235			
				Mean- 5436521			99.7

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Precision : The %RSD was found to be less than 2%.

Table no 5: Results of Method Precision of Cilostazol

Sr. No.	Concentration (µg/ml)	Area	RT (min)	Inj.Vol. (µl)	TPN	TF
1	240	4868289	3.435	10	4482	1.40
2	240	4865834	3.345	10	4320	1.40
2	240	4863818	3.391	10	4321	1.40
4	240	4866254	3.345	10	4432	1.41
5	240	4868863	3.435	10	4420	1.40
6	240	4863988	3.444	10	4373	1.40
Mean		4866173.6				
SD		938.355				
%RSD		0.01928				

Robustness

The given method was show same efficiency when changing a small parameter like a change in flow rate, change in mobile phase etc.

Effect of variation in flow rate
Change in flow rate – ± 0.1ml/min

Table no 6: Results of Method robustness of Cilostazol

Sr. No.	System Suitability parameter	Observations			Limits
		1.1ml/min	1.2ml/min	1.3ml/min	
1	peak area response	4868289	4863814	4767482	
2	Theoretical plates	4392	4367	4390	NLT 2000
3	Tailing factor	1.40	1.40	1.39	NMT 2.0
4	Retention Time (Min)	3.391	3.436	3.436	

LOD and LOQ :

Table no 7: Results of Method LOD and LOQ of Cilostazol

Drug name	Correlation Coefficient (R^2)	Slope (s)	LOD (µg/ml)	LOQ (µg/ml)
Cilostazol	0.9993	20106.465	0.15400µg/ml	0.46669µg/ml

Assay

The given method and validated method was applied to the determination of cilostazol in marketed tablet containing 50mg of drug per tablet. The std limit of assay 98 to 102 %. The given method was show result within assay limit.

Table no 8: Results of Method Precision of Cilostazol

Name	Std sample Peak Area	Tablet sample peak Area	Assay(%)
Cilostazol	4854224	4865834	100.2%

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CONCLUSION

The developed method was validated according to ICH Q2R1 guidelines. The developed method was simple, robust and its an cheap method than others method. The given method efficiency was good i.e plate count no. more with short retention time, less tailing i.e. with sharp peak. And also the method give results(%RSD Less than 2, taliling less than 2 and Regression NLT 0.999) within in limit, Hence this method are useful for separation of Cilostazol drug in pure and tablet dosage form.

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