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New UV-Spectrophotometric Method Development and Validation for the Assay of Cilostazol in Pure and Formulations

Suresh T[1], Rambabu K[1]*
1. Department of Chemistry, RVR &JC College of Engineering, Chowdavaram, Guntur, AP, India.

ABSTRACT

A simple, new UV-spectrophotometric method has been developed and validated for the assay of cilostazol in pure and formulations based on measurement of absorption at maximum wavelength of 257.40nm. The developed method exhibited linearity in the range 2.5-15µg/ml with precision is exemplified by relative standard deviation of 0.86% for cilostazol. The percentage mean recovery of cilostazol was found to be in the range of 98.39-99.34% during accuracy studies respectively. The proposed method is further validated statistically in accordance with ICH norms and the validation results allowed the feasibility of the proposed method in the analysis of cilostazol in pure and its formulations.

Keywords: Cilostazol, Formulation's, ICH norm's.

*Corresponding Author Email: thuta.suresh@gmail.com
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INTRODUCTION

Cilostazol[1,2][Figure 1] is a quinolinone derivative used for the treatment of intermittent claudication resulting from peripheral arterial disease. It inhibits cellular phosphodiesterase (more specific for phosphodiesterase III) activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation. The empirical formula of cilostazol is $C_{20}H_{27}N_5O_2$, and its molecular weight is 369.46. Cilostazol is 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3,4-dihydro-2(1H)-quinolinone. Cilostazol occurs as white to off-white crystals or as a crystalline powder that is slightly soluble in methanol and ethanol, and is practically insoluble in water, 0.1 N HCl, and 0.1 N NaOH. As per discussion in the literature reviews narrated that few analytical methods[3-14] for the determination of cilostazol in pharmaceutical dosage forms or in biological fluids were reported. So, far to our present knowledge, only two spectrophotometric methods for the determination of cilostazol in pharmaceutical formulation were available in literature and this fact prompted the author to develop a simple, inexpensive UV-spectrophotometric method for the determination of cilostazol in pure and in dosage forms This present paper deals with the development and validation of the UV-spectrophotometric method for the assay of cilostazol in pure and its formulations (tablets).

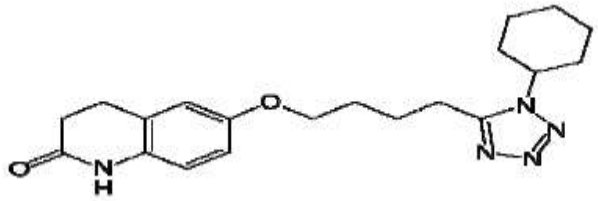

Figure 1: Structure of Cilostazol

MATERIALS AND METHOD

Instrumentation:

Shimadzu UV/Vis Spectrophotometer (Model-2450) equipped with UV probe software was used in the present assay. For dilutions various micropipettes of volumes 10-100µL were used. All weighing experiments were done on Schimadzu Digital Analytical Balance (Japan) and standard glass ware (Borosil Make) was used for preparing of solution.

Chemicals and Reagents:

Cilostazol (99.9% Pure) used was supplied by Dr Reddys Labs, Hyderabad and its formulation in the brand name of Cilokem (strength: 50mg of Cilostazol) from Alkem labs, India Ltd were

purchased from local pharmacy. Methanol of analytical grade was purchased from local vendor and was used for the preparation of standard and sample solutions without further purification.

Diluent:

Methanol of analytical grade was used as diluent in the present assay. It is used as received.

Preparation of Standard Solutions:

Accurately weighed 100mg of cilostazol test standard was transferred to a 100mL volumetric flask containing 25mL of methanol solvent. This was sonicated for about 5 min to dissolve it and the resultant solution was further diluted to 100mL with methanol solvent (Diluent). Working standard solutions in concentration range of 2.5 -15µg/mL were prepared by transferring aliquot of the above stock solution with micropipette to a series of different 100mL and diluted to the mark with the same diluent.

Preparation of Sample Solution:

10 Tablets of dosage form (**Cilokem; 50mg by Alkem Labs**) of cilostazol was weighed and powdered in a mortar. The powder equivalent to 100mg was transferred into a 100mL clean dry volumetric flask, 70mL of diluent was added to it and was shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was latter diluted up to the mark with diluent. Aliquots of this solution was further diluted into a series of 10mL volumetric flask with the same diluent up to the mark, filtered through 0.45µm filter to obtain concentrations that obey within the beers law limit.

RESULTS AND DISCUSSION

Method Development:

Working standard solution (10µg/mL) of cilostazol prepared was subjected to scanning between 200 – 400 nm and the absorption maximum was determined and an optimal response was obtained at 257.40nm. This wavelength of 257.40nm was used for the quantification of standard and in dosage forms of cilostazol respectively. The absorption spectrum so obtained was shown in Figure.2.

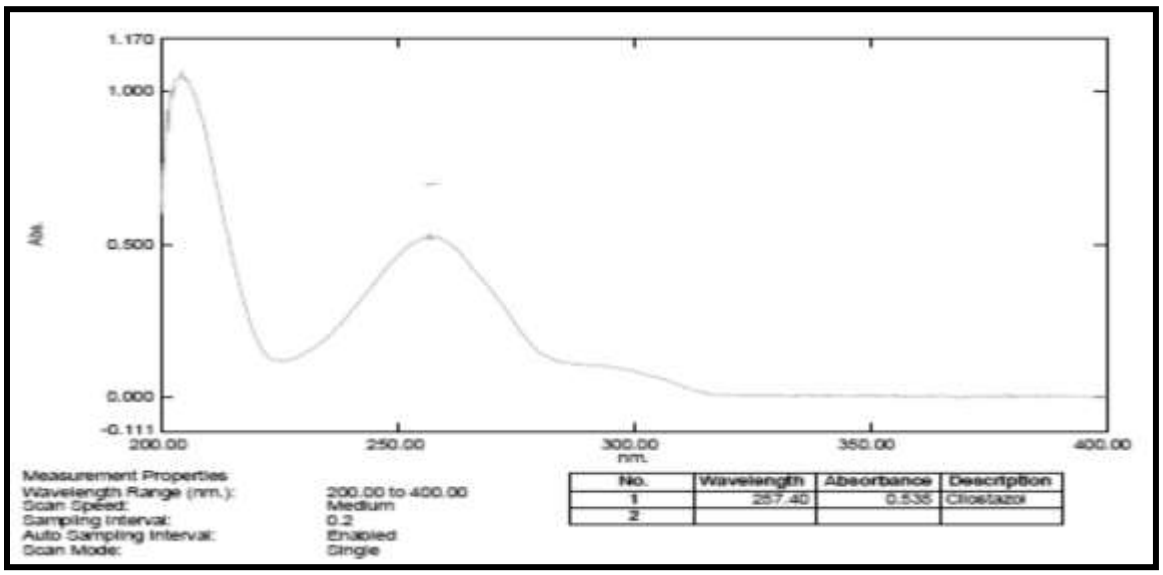

Figure 2: A Typical UV Spectra of Cilostazol

Procedure:

Working standard solutions of cilostazol in concentration range of 2.5 -15µg/mL were placed in an cuvette of UV-spectrophotometer and the absorbance's of the each standard preparation were measured at this fixed wavelength (λmax 257.40nm) and the quantity of cilostazol in standard preparation was calculated. The same procedure was carried for cilostazol in dosage forms.

METHOD VALIDATION:

The developed UV-Spectrophotometric method of cilostazol was validated using the following parameters.

Specificity

Blank Interference:

The interference of blank at the working wavelength was scanned from 200-800nm and was observed the non-interference of blank at the working wavelength of 257.40nm for cilostazol, revealing the specificity of the proposed UV spectrophotometric method for cilostazol.

Linearity:

The linearity of the proposed method was made by determining the absorbance of different working concentrations of cilostazol in triplicate over a range of 25% (2.5µg/mL) to 150% (15.0µg/mL). Correspondingly a calibration graph was plotted by plotting the absorbance recorded verses the concentration and was treated by least-squares linear regression analysis. The results of regression analysis i.e, slope, intercept with correlation coefficient more than 0.9999 [Table.1 and Figure 3] indicated the linearity of the proposed method with optimum value of standard error.

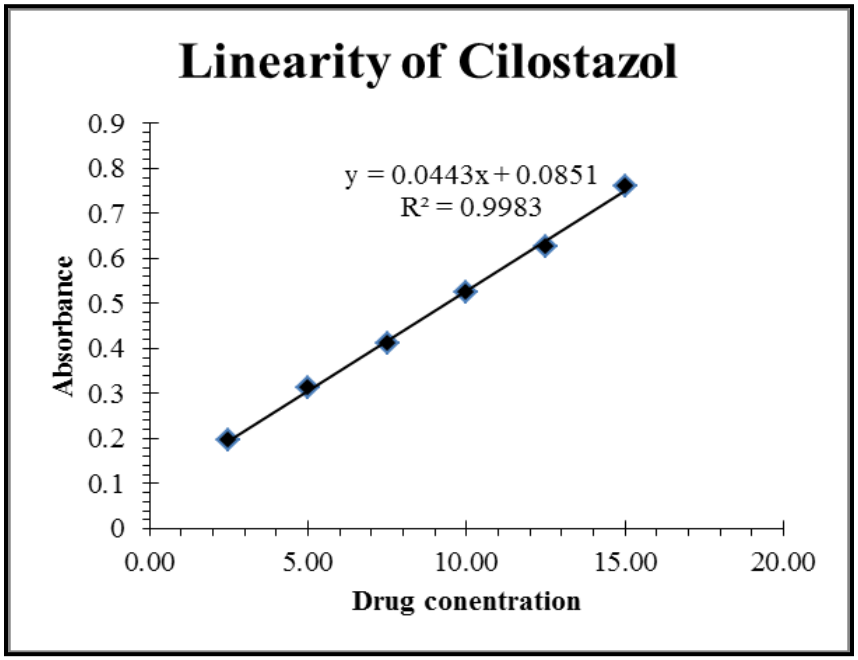

Figure 3: Linearity Curve for Cilostazol

Table 1: Results of Linearity Studies for UV- Spectrophotometric Determination of Cilostazol

% Level	conc	Absorbance
0	0	**0**
25	2.5	0.198
50	5.0	0.312
75	7.5	0.413
100	10.0	0.524
125	12.5	0.627
150	15.0	0.762
Slope,b		0.0.433
Intercept,a		0.0851
Correlation,r2		0.9983
LOD		0.601
LOQ		2.05

LOD and LOQ:

The detection and quantization limits were found to be 0.601 and 2.05µg/ml for cilostazol and were found to be in sub-microgram level indicating the good sensitivity of the proposed method.

Precision:

The precision of the present proposed method was performed in six replicates of fixed concentration of cilostazol and the percentage relative standard deviation (%RSD) was measured. The %RSD of 0.86 tabulated (Table.2) was less than 2.0% (Accordance to ICH norms) revealing good precision of the proposed method.

Table 2: Results of Precision Studies for Cilostazol

S No	Name	Absorbance
1	SOLUTION-1	0.529
2	SOLUTION-2	0.531
3	SOLUTION-3	0.530
4	SOLUTION-4	0.523
5	SOLUTION-5	0.521
6	SOLUTION-6	0.532
AVG*		0.528
STD DEV*		0.00455
% RSD*		0.86

*Average of six determinations considered

Accuracy:

The recovery studies of the proposed method was analyzed in triplicate preparations on composite blend collected from 20 tablets of cilostazol as per the proposed method at three different levels and the results of percentage recoveries were reported Table.3. The percent recovery at each level was ranged from 98.39-99.34% respectively, indicating insignificant interference from the excipients.

Table 3: Results of Recovery Studies for Cilostazol

Accuracy Level	50%	100%	150%
S No	Absorbance	Absorbance	Absorbance
Injection-1	0.262	0.521	0.789
Injection-2	0.260	0.520	1.65
Injection-3	0.264	0.519	1.648
Avg*	0.262	0.520	0.782
AMT. Recovered*	49.67	98.39	148.25
%Recovery*	99.34	98.39	98.83

*Average of three determinations considered

Ruggedness:

The ruggedness of the present proposed method for cilostazol was made by the precision study performed on another instrument by another analyst and the results are reported. The %RSD obtained in this study were found to be not more than 2.0% (Table 4) making the current method rugged.

Table 4: Results of Ruggedness Studies for Cilostazol by the Proposed Method

S NO	Name	Analyst -1	Analyst -2
		Absorbance	Absorbance
1	SCAN-1	0.529	0.529
2	SCAN-2	0.531	0.533
3	SCAN-3	0.530	0.535

4	SCAN-4	0.523	0.525
5	SCAN-5	0.521	0.528
6	SCAN-6	0.532	0.521
AVG*		0.528	0.529
STD DEV*		0.00455	0.005
% RSD*		0.86	0.970

*Average of six determinations considered

Solution Stability:

In this study the absorbance of the same standard and sample solutions of cilostazol in triplicate at intervals of 0 hours, 12hours, and 24 hours were recorded and the cumulative %recovery at each interval was determined. The % recoveries tabulated in Table 5 revealed the stability of the proposed method.

Table 5: Results of Stability Studies of Standard and Sample Solutions of Cilostazol with the Proposed Method

Time Interval (Hrs)	%Recovery	
	Standard(n=3)	Sample(n=3)
0	99.97	99.99
12	99.95	99.96
24	99.96	99.98

Assay of Dosage Forms:

Pharmaceutical assay was carried out on available brand of cilostazol (**Cilokem; Label claim 50mg**) procured from local pharmacy using the developed UV spectrophotometric method and the % assay was calculated and the results were tabulated (Table 6). These results revealed that the proposed UV spectrophotometric method can be used for routine quality control analysis for cilostazol in its pure and in dosage forms.

Table 6: Assay Results of Cilostazol in Market Brands

Market Brand of the Drug	Taken mg	Found* mg	% Assay*
Cilokem	50	49.97	99.94

*Average of three determinations considered

CONCLUSIONS

A simple and validated UV-spectrophotometric method was developed for the assay of cilostazol in pure forms and in pharmaceutical formulations. The developed method resulted in cilostazol exhibiting linearity in the range 2.5-15µg/ml. The precision is exemplified by relative standard deviation of 0.86%. Percentage mean recovery was found to be in the range of 98.39-99.34, during accuracy studies. Accordingly it is concluded that the developed UV spectrophotometric

method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of cilostazol in tablets in various pharmaceutical industries.

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