Development and Validation of UV-Visible Spectrophotometric Method for Cilostazol Quantification in Bulk and Formulations

Soheb Tamboli, Krishnamurthy Kamalapurkar, Irfan Dalwale, Kunal Gaikwad, Mohammed Saud

Department of Pharmaceutics, D.S.T.S. Mandal's College of Pharmacy, Solapur

Abstract

A swift, accurate, and economical UV spectrophotometric method has been developed using methanol as a solvent for the quantification of Cilostazol in both bulk and pharmaceutical formulations. The technique operates at a specific wavelength of 265 nm, exhibiting linearity across a concentration range of 10 to 50 µg/ml, with a high correlation coefficient ($R^2 = 0.996$). This method was successfully applied to evaluate the Cilostazol content in various commercially available products, with results consistent with the declared label specifications. The method underwent rigorous statistical validation and recovery assessments to determine its linearity, precision, repeatability, and reproducibility. The findings indicate that this method is appropriate for the routine analysis of Cilostazol in both bulk and commercial products.

Keywords: Cilostazol, Analytical method development, UV- Visible Spectrophotometry

Introduction

Cilostazol(CIL), 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3,4-dihydro-2(1H)-quinolinone is a white crystalline powder slightly soluble in methanol and ethanol and practically insoluble in water, 0.1 mol/L HCl and 0.1 mol/L NaOH [1]. It has a molecular weight of 369.46 g.mol–1, melting range between 150.4 – 160.3 °C and log P of 3.048.[2]

This quinolone derivative, known as 2-oxo-quinolone, acts as an inhibitor of phosphodiesterase (PDE)III, thereby preventing the breakdown of cyclic adenosine monophosphate(cAMP) in both platelets and blood vessels. Through these cellular mechanisms, it functions as a platelet aggregation inhibitor and facilitates the dilation of blood vessels.[3] Consequently, CIL is considered the preferred medication for managing intermittent claudication, a prevalent condition affecting peripheral arterial blood vessels associated with atherosclerosis, particularly in the lower limbs.[4] The constriction or obstruction of the primary arteries supplying blood to the legs results in intermittent claudication, which is a notably painful condition. It is characterized by various symptoms, including discomfort, numbness, paresthesia, cramping, or fatigue in the leg muscles during ambulation, all of which are alleviated after a brief period of rest.[5]

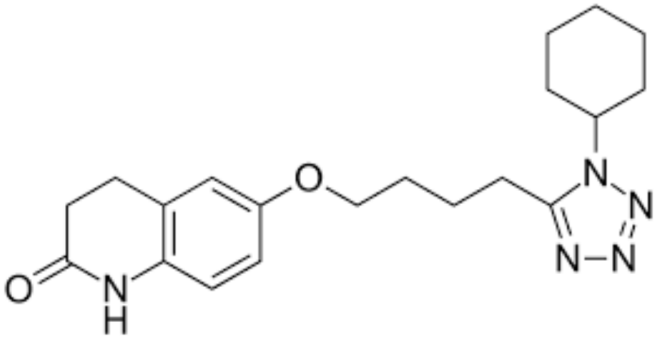

Fig.1 Structure of Cilostazol

A variety of HPLC assay techniques have been reported for the quantification of Cilostazol. A review of existing literature reveals that multiple analytical methods, such as high-performance thin-layer chromatography (HPTLC)[6] and high-performance liquid chromatography (HPLC)[7], UPLC-DAD[8] Method have been employed for the estimation of Cilostazol. Furthermore, recent research has presented several UV spectrophotometric methods for determining Cilostazol using methanol as the solvent.

In this study, a simple, efficient, and economical UV spectrophotometric method was developed using Methanol for the quantification of Cilostazol in both raw materials and commercially available dosage forms. The method was optimized and validated in compliance with the International Conference on Harmonization (ICH) guidelines, demonstrating exceptional specificity, linearity, precision, and accuracy in the measurement of Cilostazol.

Materials and methods

Apparatus

Double beam UV–visible spectrophotometer (Systronics 2201) was used for all absorbance measurements with matched quartz cells, volumetric flask, Pipette, Sonicator etc.

Materials

All chemicals and reagents were of analytical or HPLC grade. Cilostazol was purchased from Balaji Drug Dealers: API & Pharmaceutical Polymers Surat, India, Cilostazol marketed formulation which was used as the reference standard

Preparation of a working standard drug solution

Involved the precise weighing of 10 mg of standard Cilostazol, which was then transferred into a 10 ml volumetric flask. The substance was completely dissolved and the solution was diluted to the calibration mark using Methanol, achieving a final concentration of 1000 µg/ml (Stock-1).

Following this, Stock-1 was further diluted with methanol to create a 100 µg/ml (Stock-2) solution.

Determination of the wavelength of maximum absorbance (λmax)

The Stock-2 sample was examined in full output mode at a moderate scanning speed over the complete range of the UV-VIS Spectrophotometer, which extends from 200 to 800 nm, utilizing a co-solvent system as the blank. Following the acquisition of the spectrum, the λmax was determined. This procedure was conducted three times.

Development of Calibration Curve

The calibration curve was established by employing Stock-2 to prepare five distinct calibration standards with concentrations of 10, 20, 30, 40, and 50µg/mL. The absorbance for each standard was measured at λmax 265 nm, utilizing a fixed wavelength measurement mode. Calibration curves, which depict the relationship between concentration and absorbance, were created using Microsoft Excel. This process was conducted multiple times to verify the reliability and reproducibility of the findings.

Method Validation

The ultraviolet (UV) method established for the quantification of Cilostazol underwent validation concerning various parameters, including linearity, range, precision, robustness, ruggedness, accuracy, limit of quantification (LOQ), and limit of detection (LOD). This validation was conducted utilizing predefined calibration standards, as detailed below.

Linearity and range

The linearity of the proposed UV method was evaluated using five distinct calibration standards. By analyzing these standards, calibration curves were created to demonstrate the relationship between absorbance and concentration, which were subsequently assessed through linear least squares regression. The R^2 value was considered a crucial factor in confirming the linearity of the proposed method. The acceptable range of linearity was determined by the span between the upper and lower concentration limits of the proposed UV method.

Accuracy

Accuracy can be expressed as the percentage of recovery obtained from the analysis of a specified quantity of analyte added to the sample. It may also be defined as the deviation between the mean value and the accepted true value, accompanied by the appropriate confidence intervals.

Intra-day precision and Inter-day precision

The accuracy of the assay method was evaluated regarding repeatability by conducting five independent assays of the Cilostazol test setup, and the percentage relative standard deviation (% RSD) of the measurements was calculated for intraday assessments. The intermediate precision of the method was verified by applying the same procedure over three consecutive days.

Stability study

Samples designated for the repeatability study were stored at room temperature for duration of 24 hours and subsequently analyzed the next day to assess their short-term stability.

Robustness

The evaluation of robustness should be considered during the development phase and is dependent on the particular procedure, which entails deliberate modifications to method parameters. If the measurements are susceptible to variations in analytical conditions, it is crucial to either ensure adequate control over these conditions or to include a warning within the procedure. In the present study, the absorption maxima were adjusted by 1 nm in both directions, and the process was conducted using a standard solution of 30µg/ml.

Limit of Detection (LOD)

In UV method development LOQ was determined by utilizing the following equation.

LOQ = 10xSD/S

Where, S= slope

SD= Standard deviation of Y-intercepts

Estimation of Cilostazol content in marketed formulation

A developed and pre-validated UV-Vis method was successfully utilized to quantify the Cilostazol content in a commercially available formulation. For this investigation, CILODOC Tablets were sourced from the local market in Solapur, and the contents of the tablets were extracted. Suitable dilutions were created using a pre-optimized solvent system. The resulting samples were subsequently analyzed with the pre-validated UV method, and the findings were expressed as the average percent assay.

Results and Discussion

Method development and optimization

The determination of the wavelength associated with maximum absorbance is essential for conducting quantitative UV analysis. Solutions exhibiting absorbance values lower than 1 are

typically deemed appropriate for identifying the wavelength of maximum absorbance. Following this guideline, the maximum wavelength for a Cilostazol solution at a concentration of 100 µg/mL was determined using the full scan mode of a UV-Visible spectrophotometer (refer to Figure 2). The full scan was performed with UV software, which facilitated the identification of λmax. The peak absorbance wavelength for Cilostazol was established at 265 nm.

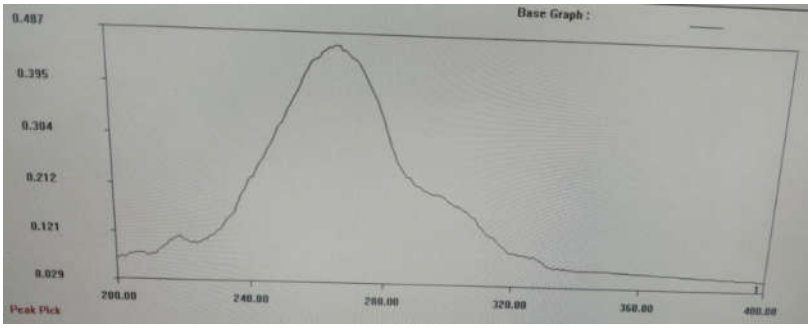

Fig. 2: UV-Visible spectra of Cilostazol

Preparation of calibration curve

To quantify unknown samples using a UV-Visible spectrophotometer or a similar analytical instrument, a reliable calibration curve must be created, along with an equation that defines the relationship between concentration and the measured response. This method is well-regarded for its consistency and reliability when compared to graphical techniques. For accurate quantitative analysis of Cilostazol, a calibration curve was developed using five calibration standards. The absorbance of these standards was measured at 268 nm, utilizing the fixed wavelength mode of the UV-Visible spectrophotometer. The calibration curve was constructed on three separate occasions, and the corresponding results are presented in Table 1.

Table No. 1 Results of Calibration curve

Standard	Conc (µg/ml)	Absorbance
CAL STD-1	10	0.448
CAL STD-2	20	0.504
CAL STD-3	30	0.573
CAL STD-4	40	0.626
CAL STD-5	50	0.674

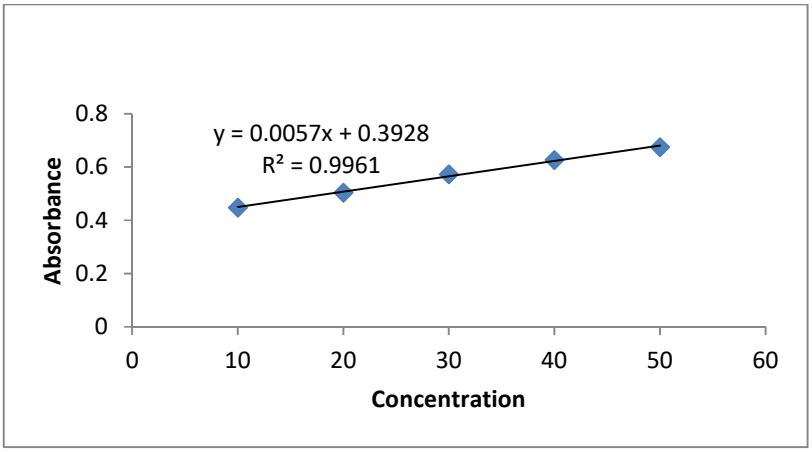

Fig 3: Calibration curve for Cilostazol

Accuracy

Accuracy is a crucial metric that reflects how closely the experimental value matches the true concentration of a substance in a given matrix. It is important to evaluate accuracy across the entire calibration range of the analytical method to confirm the reliability of results at all measurement points. For the UV method used in the analysis of Cilostazol, accuracy was determined through recovery studies. The percentage recovery of Cilostazol was calculated by spiking the analyte at concentrations of 80%, 100%, and 120%.

Table 2: Accuracy data of UV method for Cilostazol

Recovery Level (%)	Concentration of Sample (µg/ml)	Amount added (µg/ml)	% Recovery
80	10	8	98.5
100	30	30	98.8
120	50	60	99.3

Precision

Precision is a key parameter that indicates the degree of variability in measurements, demonstrating the reliability of results produced by a specific analytical technique. A robust analytical method is expected to deliver results that are consistently reproducible. Achieving precision in an analytical procedure is essential for ensuring accurate outcomes. To evaluate the reproducibility and reliability of the UV method, intra- and inter-day precision were assessed at a concentration of 10µg/ml. The results fell within the acceptable limits, showing a relative standard deviation (RSD) of less than 1%.

Table 3: Intra-day precision data of UV method for Cilostazol

Sr. No	Concentration	Absorbance
1	10(µg/ml)	0.446
2		0.448
3		0.447
4		0.446
5		0.445
	Mean	0.4464
	SD	0.001140
	%RSD	0.25

Table 4: Inter-day precision data of UV method for Cilostazol

Sr. no	Concentration	Absorbance (1st day)	Absorbance (2nd day)
1		0.448	0.447
2		0.445	0.446
3	**10**(µg/ml)	0.447	0.449
4		0.449	0.448
5		0.447	0.450
	Mean	0.4472	0.448
	SD	0.001483	0.001581
	%RSD	0.33	0.35

Robustness

Robustness in an analytical method refers to its ability to consistently deliver reliable results despite small, intentional changes to its parameters. This is important because minor, unplanned variations, such as shifts in solvent composition or pH, can happen during regular use and might affect the method's performance. A robust method is designed to ensure that such changes do not significantly impact its effectiveness. For example, a robust method would show minimal variation in the absorption of Cilostazol solution in Methanol when measured across different wavelengths (±2nm).

Table 5: Robustness data of UV method for Cilostazol

Sr. no	Absorbance at 262nm	Absorbance at 268 nm
1	0.447	0.450
2	0.446	0.447
3	0.449	0.451
SD	0.001528	0.002082
%RSD	0.34	0.46

Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The Limit of Quantification (LOQ) refers to the lowest concentration that can be measured with both accuracy and precision. It is usually used as the primary calibration standard. In the proposed UV method, the Limit of Detection (LOD) and LOQ were found to be 0.495 and 1.502 µg/ml, respectively, as shown in Table 7. The relatively low LOQ indicates that the method is well-suited for analyzing samples containing even small amounts of Cilostazol.

Table 7: LOD & LOQ data for UV method for Cilostazol

LOD	0.752 µg/mL
LOQ	2.28 µg/mL

Estimation of Cilostazol

The established UV method was effectively utilized to determine the Cilostazol content in CILODOC Tablet 100 mg. The average percentage assay of Cilostazol in the tablet was measured at 96.4%.

Conclusion

An effective and validated UV-Visible spectrophotometric method for the quantification of Cilostazol has been developed. This technique demonstrated both robustness and reliability, making it suitable for the estimation of Cilostazol.

Conflict of Interest: No conflict of interest

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