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An analytical review on the quantitative techniques for estimation of cilostazol in pharmaceutical preparations and biological samples

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Abstract: Cilostazol (CLZ) is a quinolinone derivative possessing anti platelet and vasodilating properties and it is used in the treatment of intermittent claudication. It operates by inhibiting the cyclic guanosine monophosphate (cGMP) dependent phosphodiesterase III which leads to availability of cyclic adenosine monophosphate (cAMP) in blood vessels and platelets owing to vasodilation and reduced calcium-induced contractions. This article illustrates the various reported quantitative methods which can be used to determine cilostazol and its metabolites either alone or in combination forms in pharmaceutical preparation or biological matrices like plasma, serum, and urine. The review covers analytical methods like ultraviolet spectroscopy, fluorescence spectroscopy, electrometric methods, nuclear magnetic resonance spectroscopy, high performance liquid chromatography, high performance thin layer chromatography, ultra-high performance liquid chromatography, and tandem mass spectroscopy from the year 1985 to 2019 with a brief explanation on every analytical method. Among the methods, it was found that most researchers opted for UV and HPLC analytical methods for the estimation of cilostazol.

Keywords: cilostazol, phosphodiesterase III inhibitors (PDE III), intermittent claudication, pharmaceutical formulations, biological fluids, analytical methods

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1 Introduction

Intermittent claudication, otherwise known as occlusive peripheral arterial disease, causes pain while walking due to lack of oxygenated blood in the leg muscles.

In the middle of 19th century, veterinary surgeons in France and Germany, discovered lameness in horses and it was assumed that in some cases this was due to lack of oxygen in the arteries of the leg. Bouley, a veterinarian coined the term intermittent claudication in 1831. Benjamin Brodie described a similar syndrome in humans in 1846 [1].

A study for intermittent claudication was referred for the first time at Bispebjerg hospital in Copenhagen, Denmark in 1977 comprising of 100 women and 157 men (257 patients). At follow up after 6.5 years, 44% (113) of the patients were dead of which 5.4% had a history of diabetes. Without the discovery of any suitable drugs at that time, the only treatment was treadmill exercise at elevation [2,3].

The Framingham study was carried out in 1813 men and 2504 women over a span of 34 years to examine the effect of diabetes on intermittent claudication. When compared, patients (both male and female) with diabetes were found to be likely affected with intermittent claudication two to three times more than those without diabetes. This study also revealed an important fact that those with diabetes and intermittent claudication were at high risk of cardiovascular disorders [4].

The study showed that mortality rate in male was twofold more when compared to females. Smoking, high cholesterol, diabetes and hypertension were said to be powerful factors affecting intermittent claudication [5].

The prevalence of intermittent claudication in relation with the 5-year mortality rate was investigated in a population study in Finland with 5738 men and 5224 women. During the 5-year observation period, due typical claudication, 18 men and only 2 women died. However, intermittent claudication increased

cardiovascular disorders owing to more than 26% of the patients to die [6].

Exercise programs were carried out to determine whether it alleviates symptoms of intermittent claudication or underlying cardiovascular disorders. It showed significant improvement in quality of life and with increase in walking time and distance, there was no evidence for complete cure and decrease in mortality [7].

Cilostazol is chemically 6-[4-(1-cyclohexyl-1H-tetrazol-5-yl) butoxy]-3, 4-dihydro-2(1H)-quinolinone. The structure of cilostazol is over viewed in Figure 1.

Cilostazol is an oxo quinolone derivative with anti-platelet, antithrombotic, cardio tonic, and vasodilating properties that has been used in the treatment of patients with peripheral ischemia such as intermittent claudication. Patients suffering from this are said to have pain after walking which disappears at rest but reappears every time while walking [8].

Cilostazol is a phosphodiesterase III inhibitor. PDE IIIs are enzymes located within the sarcoplasmic reticulum that utilize cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) for the cardiac and vascular smooth muscle contractility. Cilostazol inhibits phosphodiesterase III which leads to increase in availability of cAMP in platelets and blood vessels which in turn leads to activation of protein kinase A (PKA). PKA then causes Ca^{2+} entry through L type channels causing inhibition of Ca^{2+} sequestration by the sarcoplasmic reticulum. This mechanism leads to increase in contractility and cardiac output while decreasing peripheral vascular resistance leading to vasodilation and inhibition of platelet aggregation to occur. Recent findings have shown that cilostazol inhibited collagen induced platelet aggregation by inhibiting adenosine uptake in platelets and erythrocytes [9-11].

Experiments were carried out *in-vitro* and *in-vivo* to determine the anti-thrombotic properties of cilostazol in mice, rabbits, dogs, and humans. It was used to prevent death in mice by inhibiting platelet aggregation [12].

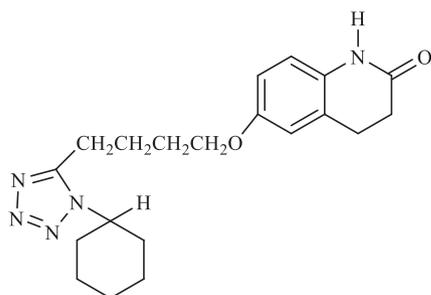


Figure 1: Structure of cilostazol.

Shuichi et al. [13] carried out experiments which determined that cilostazol can be used for reocclusion therapy after major thrombolytic surgeries. Tanaka et al. [14] carried out an experiment to determine the effect of cilostazol as a vasodilator on rabbit arterial smooth muscle strips. At present, clinical trials and investigations are being conducted to determine whether the phosphodiesterase III inhibitors like cilostazol can be used to treat Alzheimer's disease [15].

The main goal of the current review is to collate the reported quantitative analytical techniques apart from the official methods on cilostazol either alone or in combination in raw, bulk, and pharmaceutical dosage forms like tablet, capsules, nanoparticle suspensions, and biological samples like rat plasma, rabbit plasma, human plasma, human serum, and human urine.

The current review acts as quick reference to various analytical techniques reported for estimation of cilostazol like ultraviolet spectroscopy, fluorescence spectroscopy, electrometric methods, nuclear magnetic resonance spectroscopy, high performance liquid chromatography, high performance thin layer chromatography, ultra-high performance liquid chromatography, and tandem mass spectroscopy from the years 1985 to 2019 with a brief explanation on every analytical method.

2 Official method of analysis

Cilostazol is official in US Pharmacopeia [16] and is marketed under the name Pletal whose dose is 100 mg taken twice a day, at least half an hour before breakfast and dinner. It is contraindicated in patients with congestive heart failure, hypersensitivity, and haemostatic disorders [17].

According to United States Pharmacopoeia, the official methods for analysis of cilostazol are as follows:

1. UV spectroscopic method

The standard solution should be prepared by dissolving 28 mg of cilostazol in methanol and making serial dilutions. The test solution should be prepared with final concentration being 5.6 mg of cilostazol per mL. UV absorption is carried out at the wavelength of about 257 nm on the test solution in comparison with the standard solution, using a 1 cm cell and medium as the blank.

2. Assay

An internal standard is a solution of benzophenone in methanol having a known concentration of 4 mg/mL. A standard solution should have final concentration

of 0.1 mg of USP Cilostazol RS and 0.04 mg of the internal standard per mL in a filtered and degassed mixture of water, acetonitrile, and methanol (10:7:3, v/v/v). The test solution should be prepared by dissolving 50 mg powder equivalent to obtain similar final concentration as the standard solution.

3. Chromatographic conditions

HPLC is equipped with a 254 nm detector and a 4.6 mm × 150 cm column. The flow rate is about 1 mL/min. The column temperature is maintained at an ambient temperature. The injection volume is about 10 mL. The resolution R , between the cilostazol and benzophenone peaks eluted should not be less than 9.0 and the relative standard deviation should not be more than 1.5%.

3 Review of quantitative techniques for estimation of cilostazol in pharmaceutical and biological preparations

3.1 UV spectroscopy

Spectroscopy is the branch of analytical chemistry that deals with the interaction between matter and electromagnetic radiation based on the amount of radiation produced or absorbed by the matter of interest [18]. Absorption measurements employing ultraviolet or visible radiation find widespread application to quantitative and qualitative analysis [19].

Cilostazol has been quantitatively analyzed by many researchers either alone or in combination with other drugs. Cilostazol has also been reported by various techniques like colorimetric spectroscopy methods [20,21], forced degradation studies [23], derivative spectroscopic analysis [29,30], multivariate calibration techniques [30], and spectrokinetics [31]. The important findings of these techniques are discussed in the following section.

Alhamidehoballah [20] reported two colorimetric methods in which the former method was based on oxidative coupling using resorcinol and potassium periodate in acid-medium. The reddish pink colored complex formed absorbs at 541 nm. The effects of various factors such as temperature, acid, pH, oxidizing agents, surfactants, and stability of the complex was observed. The latter method was based on the formation of violet and green colored metal complexes with cobalt (II), copper (II), and nickel

(II) in phosphate buffer and pink coloured complex with ferric chloride in acetate buffer which absorbs at 572, 504, 328, and 536 nm, respectively. Both the methods were validated as per the International Council for Harmonisation (ICH) guidelines. Kuruba et al. [21] outlined a spectroscopic method which relies on the formation of a bright yellow coloured hydrazone with 2, 4-dinitrophenylhydrazine (DNPH reagent). The λ_{\max} was found to be 355 nm. The method complies with Beer's law at the concentration range of 2-20 $\mu\text{g/mL}$. The validation parameters specific to colorimetric method has been reported in Table 1.

Basniwal et al. [22] developed a validated method for determining cilostazol by linear regression equation method and standard absorptivity method at a wavelength of 258.2 nm using 50%, v/v methanol as the solvent. At the linearity range of 0-25 $\mu\text{g/mL}$, it obeys Beer Lambert's law and shows purity of 100-102.4% and 98.7-101.1% for both methods, respectively. A validated spectrophotometric method for the determination of cilostazol in bulk and pharmaceutical preparations including forced degradation studies was exemplified by Chaitanya et al. [23]. The absorption maximum was found to be 256 nm. The linearity was found to be in the range of 3-24 $\mu\text{g/mL}$ and the correlation coefficient was 0.9929. The method was found to be stable on undergoing forced degradation with acid, alkali, and oxidizing agent.

Fursule et al. [24] proposed a validated method for the quantification of quetiapine fumarate (QTF) and cilostazol in pharmaceutical formulations. QTF in water showed λ_{\max} at 290 nm and linearity was observed in the range 6-54 $\mu\text{g/mL}$ with r^2 being 0.9999. Cilostazol in 30% v/v acetonitrile showed λ_{\max} at 258 nm and linearity was observed in the range 3-21 $\mu\text{g/mL}$ with r^2 being 0.9993. Three UV spectroscopic validated methods for the estimation of telmisartan (TELM) and cilostazol were published by Mashru et al. [25] The first method is by simultaneous equation method where the samples were analyzed at 258 and 296 nm, respectively. The second method is absorbance ratio method in which the Q value is determined by selecting one isoabsorptive point, 237.5 nm for telmisartan and λ_{\max} of cilostazol at 258 nm. The third method is dual wavelength method in which cilostazol was determined at the range of 1-40 $\mu\text{g/mL}$ and telmisartan at the range of 1-25 $\mu\text{g/mL}$.

Similarly, Patel et al. [26] carried out two simple validated simultaneous UV spectroscopic estimation methods for aspirin and cilostazol. Method I was based on simultaneous equation method where the samples were analyzed in methanol at 226 and 257 nm, respectively. The second method was based on absorbance ratio method in which

Table 1: Colorimetric method for analysis of cilostazol

Matrix	Complex formation of cilostazol with	Linearity range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Precision (%RSD)	Accuracy (%Recovery)	Robustness	Ref.
Aqueous solution	Resorcinol	10-25	0.99	0.173	0.83	100.0 \pm 0.49	No significant change	[20]
	Cobalt	5-20	0.056	0.105	0.67	99.98 \pm 0.51		
	Nickel	1-20	0.068	0.104	0.56	100.02 \pm 0.81		
	Copper chloride	3-30	0.043	0.133	0.71	99.96 \pm 0.48		
	Ferric chloride	5-25	0.037	0.151	0.68	99.98 \pm 0.42		
Aqueous solution	DNPH reagent	2-20	0.1079	0.3594	0.033	99.99 \pm 0.41	No significant change	[21]

the Q (absorbance ratio) value was determined by selecting one isoabsorptive point, 239.5 nm for cilostazol and λ_{max} of aspirin at 226 nm. For both aspirin and cilostazol, the linearity was determined in the concentration range of 2-24 $\mu\text{g/mL}$. The method published by Patel et al. [27] is quite similar to the above two literatures. The quantification of telmisartan and cilostazol is carried out by two methods - simultaneous equations method, in which the absorbance of both the drugs were determined at 296 and 257 nm, respectively, and Q-absorbance ratio method in which absorbance of both the drugs were determined at an isoabsorptive point, 274.45 nm and the λ_{max} of cilostazol at 257 nm. The linearity was found to be in the range of 2-12 $\mu\text{g/mL}$ for telmisartan and 5-30 $\mu\text{g/mL}$ for cilostazol.

Paras et al. [28] proposed a validated spectrophotometric method for the simultaneous estimation of imipramine (IMI) and cilostazol by the absorbance ratio technique where the Q ratio is found by calculating the ratio of the determined absorbencies, one of which is an isoabsorptive point while the other is the λ_{max} of the drug. The selected wavelengths are 233.4 nm which is the isoabsorptive point and 258 nm which is the absorption maxima of cilostazol. The linearity range was 10-35 $\mu\text{g/mL}$ for both the drugs.

Shah et al. [29] illustrated the simultaneous determination of cilostazol and zonisamide by zero order derivative spectrophotometric method. The absorption maximum for cilostazol and zonisamide was found to be 257.60 and 283.80 nm, respectively. The linearity is found to be in the range of 4-20 $\mu\text{g/mL}$ and correlation coefficient was found to be 0.9999 for both the drugs. The proposed method was validated according to ICH Q2R1 guidelines.

A multivariate calibration method, which finds its application in spectral investigation due to its simultaneous incorporation of many wavelengths was reported by Mashru et al. [30] for the determination of cilostazol and telmisartan consisting of PCR (principal component regression), PLS (partial least square), CLS (Classical least square), and ILS (Inverse least square). The absorbance for a sample set containing 5-25 $\mu\text{g/mL}$ of cilostazol and telmisartan in methanol respectively, in the wavelength range of 210-314 nm was measured with a 2 nm interval for creating zero-order spectra on which the chemometric calibrations were carried out. Maximum absorbance was achieved at 258 nm for cilostazol and 296 nm for telmisartan. Unscrambler X 10.3 and MATLAB 6 were used for carrying out the chemometric calculations. Root Mean Square Error of Prediction (RMSEP) of cilostazol for PCR, PLS, CLS, and ILS was 0.067408, 0.067408, 0.111665, and 0.106792 while for telmisartan, it was observed to be 0.012087, 0.012087, 0.071533, and 0.029835.

Elango et al. [31] proposed a unique study outlining the spectrokinetics techniques for the determination of cilostazol. Spectrokinetics, also known as Raman spectrokinetics is used to study the chemical properties of the drug combining Raman spectroscopy along with temporary kinetic studies to obtain the overall reaction rates [32]. The techniques used in this method are UV, FT-IR, GC-MS, and Far-IR to study the effect of DDQ (2, 3-dichloro-5, 6-dicyanobenzoquinone) and iodine with cilostazol. Cilostazol forms intermediate charge transfer complexes with both iodine in methanol and DDQ in acetonitrile at 360 and 589 nm, respectively, which were discovered by Job's method of continuous variation to establish the stoichiometry of the complexes. The validation parameters have been reported in Table 2.

UV spectroscopic method is one of the most commonly opted methods of analysis due to its simplicity and inexpensive property. However, researchers have made efforts to use different techniques like derivative spectroscopy, multivariate calibration, spectrokinetics, degradation and stability studies to further enhance the research. The most commonly employed solvent in this method is methanol.

3.2 Fluorescence spectroscopy

Fluorescence spectroscopy also known as fluorimetry is based on the principle of measurement of light/fluorescence emitted from a sample caused due to excitation of electrons.

Attia et al. [33] proposed a spectro fluorescence method in which terbium acts as an optical sensor to estimate cilostazol in pharmaceutical and biological preparations like human serum and urine by complex formation due to the excited-state interactions between cilostazol and Tb^{3+} . At 545 nm, high luminescence intensity peak of the terbium complex in acetonitrile was obtained. Linearity was observed at a concentration range from 1.0×10^{-9} to 1.0×10^{-6} mol/L with correlation coefficient being 0.998.

A sensitive spectro fluorimetric method was developed for the estimation of cilostazol and clopidogrel (CLG) in pharmaceutical preparations and biological fluids like human plasma based on the quenching of the complex formed between these tertiary amine containing drugs and fluorescein dye by Ibrahim et al. [34]. They contribute to decreasing the fluorescence intensity of the dye which is directly proportional to the concentration. The determination was carried out in range of 0.001-0.08 $\mu\text{g}/\text{mL}$ for cilostazol and 0.01-0.50 $\mu\text{g}/\text{mL}$ for clopidogrel.

Fluorimetry has been a choice for conducting analysis studies due to its high sensitivity, specificity, and low detection limits. Due to high emission of the fluorophore, it is applicable in clinical and pharmacological analysis using biological samples like plasma, urine, and serum. Molecular arrangements and complex formation can also be studied due to fluorescence quenching.

3.3 NMR spectroscopy

The study of absorption of radio-frequency radiation by nuclei is called nuclear magnetic resonance (NMR), which observes the spin of the nuclei when placed in a magnetic flux. It has proved to be one of the most powerful tools available for determining the structure of both organic and inorganic species [35].

Yang et al. [36] established a method for determining the enantiomeric purity of cilostazol in aqueous pharmaceutical preparation by proton nuclear magnetic resonance (^1H NMR). The complexes were formed with CDCl_3 in the presence of the internal standard 1, 2, 4, 5-tetrachloro-3-nitrobenzene. The test parameters were optimized. At the concentration range of 5–30 mg for cilostazol, the ratio of sample area and internal standard area was found to be linear with the ratio of sample mass and internal standard mass.

There is a wide scope for research available with NMR technique where impurity profiling, optical purity, structural elucidation of metabolites, and determination of quality of drug and excipients and can be carried out.

3.4 Electrochemical analysis

Voltammetry comprises a group of electro analytical methods in which information about the analyte is derived from current-voltage curves i.e., plots of current as a function of applied potential, obtained under conditions that encourage polarization of the indicator or working electrode [37].

Only one method has been reported for estimation of cilostazol by electrochemical analysis. In this method differential pulse voltammetry was used to estimate the amount of cilostazol in pharmaceutical and biological samples like human urine by Wassel et al. [38] using carbon paste electrode (CPE) and glassy carbon electrode (GCE) in Britton-Robinson buffer (pH 2.0 to 12.0). The adsorptive and electrochemical oxidation behavior of cilostazol has been studied. The linear calibration for CPE and GCE was obtained from 4.0×10^{-7} to 6.40×10^{-6} M and

Table 2: UV Spectrophotometric methods for analysis of cilostazol either alone or in combination

Matrix	Compound	Linearity range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Precision (%RSD)	Accuracy (%Recovery)	Robustness	Ref.
Aqueous solution	Cilostazol	0-25	NA	NA	0.611	100.0 ± 0.66	No significant change	[22]
Aqueous solution	Cilostazol	3-24	2.222	6.732	NA	NA	NA	[23]
Aqueous solution	Cilostazol	3-21	NA	NA	0.58	98.84 ± 0.95	No significant change	[24]
Aqueous solution	Quetiapine fumarate	6-54	NA	NA	0.46	99.23 ± 0.82	No significant change	[24]
Aqueous solution	Cilostazol	1-40	0.3355	1.0166	0.5770	101.81 ± 0.06	No significant change	[25]
	Telmisartan	1-25	0.0085	0.2587	0.7287	101.33 ± 0.35		
	Cilostazol	1-40	0.9181	2.7823	1.1689	100.50 ± 0.04		
	Telmisartan	1-25	0.1297	0.6555	1.2821	101.25 ± 0.85		
	Cilostazol	1-40	0.3668	1.1115	0.8111	100.80 ± 0.09		
	Telmisartan	1-25	0.1297	0.3931	1.8552	100.83 ± 0.88		
Synthetic mixture	Cilostazol		0.486	2.181	NA	101.58 ± 0.89	NA	[26]
	Aspirin	2-24	0.378	2.086	NA	99.30 ± 0.97		
	Cilostazol		1.359	2.915	99.12 ± 1.00			
	Aspirin		1.206	3.928	98.05 ± 1.20			
Synthetic mixture	Cilostazol	5-30	0.086	0.263	0.58	99.62 ± 0.6742	No significant change	[27]
			0.086	0.263		100.45 ± 0.639		
	Telmisartan	2-12	0.155	0.470	0.69	98.40 ± 0.7438		
			0.126	0.382	0.65	99.46 ± 0.5376		
Aqueous solution	Cilostazol	10-35	0.026	0.077	0.20	98.45 ± 1.52	No significant change	[28]
	Imipramine		0.069	0.209	0.48	98.03 ± 0.865		
Synthetic mixture	Cilostazol	4-20	NA	NA	0.105	100.05 ± 0.272	NA	[29]
	Zonisamide				0.208	100.69 ± 0.177		
Synthetic mixture	Cilostazol	5-25	NA	NA	1.76	98.77 ± 2.54	NA	[30]
	Telmisartan				1.22	99.79 ± 1.84		

NA: not available.

from 5×10^{-7} to 8.7×10^{-6} M with the % recoveries being in the range of 99.50-100.80% and 99.20-100.10%, respectively.

There are no capillary electrophoresis methods reported for cilostazol. CE coupled with MS would provide good sensitivity.

3.5 High performance thin liquid chromatography (HPTLC)

Chromatography is a separation technique, based on the affinities between the stationary phase and the mobile phase which is widely employed by researchers for the determination and estimation of compounds in a mixture. HPTLC is a method of separation in which aluminium plates previously coated with silica gel is used as the stationary phase with a range of solvents in specific ratios as the mobile phase. The chromatographic and validation parameters been reported in Table 3.

Khan et al. [39] optimized a stability-indicating HPTLC method for the quantification of cilostazol in which, the stationary phase was silica gel pre-coated HPTLC aluminium plates and the mobile phase was toluene:ethyl acetate:methanol:ammonia in the ratio 3.5:2:0.8:0.3, v/v/v/v. The Rf value was found to be 0.52 ± 0.02 , which was compact and precise. At 258 nm densitometric analysis was carried out. This method was validated as per ICH guidelines. Forced degradation studies like acid, alkali, oxidative and thermal degradation were carried out and the results indicate degradation in acid and alkaline media.

A precise HPTLC method for estimation of cilostazol in pharmaceutical formulation was developed and validated by Kurian et al. [40]. The stationary phase used was silica gel 60F₂₅₄ pre-coated aluminium plates and the solvent system used was hexane:acetone:chloroform in the ratio of 5:2:3, v/v/v. At Rf value 0.15 ± 0.01 , this method was found to give compact spots. Cilostazol was analyzed densitometrically at 254 nm. The method showed satisfactory linearity over a concentration range of 1-10 µg/spot.

Patel et al. [41] reported a validated HPTLC method for the estimation of cilostazol and aspirin. The stationary phase used was silica gel G60 F₂₅₄ pre-coated aluminium HPTLC plates (10 cm × 10 cm) and mobile phase used was methanol:ethyl acetate:toluene:solvent ether in the ratio of 1:2:2:0.5, v/v/v. The method showed satisfactory linearity in the concentration range of 75-600 ng/mL for aspirin and 100-800 ng/mL for cilostazol.

An extensive research for the determination of cilostazol and its differentiation from its degraded products by stability indicating assay methods (SIAMs) through HPLC

Table 3: HPTLC methods for analysis of cilostazol either alone or in combination

Analyte	Stationary Phase	Linearity range	LOD (ng/mL)	LOQ (ng/mL)	Precision (%RSD)	Accuracy (%Recovery)	Robustness	Ref.
CLZ	Silica gel pre-coated HPTLC aluminium plates	300-1800 ng/spot	15.69	45.07	0.52	99.83 ± 0.41	No significant change	[39]
CLZ	Silica gel 60F ₂₅₄ pre-coated aluminium plates	1-10 µg/spot	0.089	0.269	0.47	99.18 ± 0.84	No significant change	[40]
CLZ	Silica gel G60 F ₂₅₄ pre-coated HPTLC plates (10 cm × 10 cm)	100-800 ng/mL	NA	NA	0.60	99.79 ± 0.89	No significant change	[41]
ASP		75-600 ng/mL			1.24	99.04 ± 0.51		
CLZ	0.2 mm silica gel F ₂₅₄ coated aluminium plates	0.6-14 µg/spot	0.14 µg/spot	0.43 µg/spot	1.08	99.88 ± 1.10	No significant change	[42]

NA: not available.

and HPTLC methods was carried out by Fayed et al. [42]. For HPTLC, separation was carried out on 0.2 mm silica gel F₂₅₄ coated aluminum plates with methanol:ethyl acetate:water (95:5:1.5, v/v/v). The R_f value was found to be 0.82 at the concentration range of 0.6-14 µg/spot. The above method was validated according to the ICH guidelines.

HPTLC can be used to carry out separation with satisfactory resolution especially when the limits of detection and quantification are very low. Densitometric analysis has also been carried out through HPTLC. However, it is a technique which involves lots of preparative stages and knowledge in handling the process. Hence, it is usually not a first option for researchers.

3.6 High performance liquid chromatography

HPLC is the most common, reliable, and efficient separation technique used to identify and estimate the compounds based on the principle of adsorption and affinities between the stationary and mobile phase.

Cilostazol has been analyzed by high performance liquid chromatography in pharmaceutical dosage forms in single [43-49] and in combination with either other drugs or its own metabolites and degraded products [42,50-54]. Chromatographic and validation parameters specific to every method are reported in Table 4.

Cilostazol has also been analyzed by high performance liquid chromatography in biological matrices of humans, rats and rabbits like plasma, serum, and urine [55-63]. Chromatographic and validation parameters of all methods are reported in Table 5.

3.6.1 High performance liquid chromatography in pharmaceutical preparations

Cilostazol in tablet dosage forms was determined by a simple and validated method devised by Lestari et al. [43] using LiChrospher 100 RP-18 column and methanol:acetonitrile:water in the ratio 20:50:30, v/v/v as the mobile phase at a flow rate of 1 mL/min. The λ_{\max} used was 260 nm and detected by UV DAD system. The correlation coefficient was found to be 0.9996.

Jadhav et al. [44] reported a validated RP HPLC method for the quantification of cilostazol and its related substances in bulk and formulations. The separation was carried out on Interstil C18 column at a flow rate of 1 mL/min using water:acetonitrile in the ratio 50:50, v/v as the mobile

phase and detected on DAD system at 210 nm. The correlation coefficient was found to be 0.9996. In this method, three impurities, Imp 1, Imp 2, and Imp 3 were observed. Cilostazol was also subjected to forced degradation techniques like hydrolysis, thermal, photolysis, and oxidation which showed that it was affected by alkaline medium. Rambabu et al. [45] proposed a stability indicating validated method for the determination of cilostazol using Inertsil C18 column and 50 mM sodium hydrogen phosphate dibasic dihydrate buffer (pH 3.0):acetonitrile (50:50, v/v) at a flow rate of 1 mL/min and detected at 257.40 nm. The correlation coefficient was observed to be 0.9999. This method was found to be stable on undergoing forced stress conditions like hydrolysis, thermal, and oxidation. Kurien et al. [46] published a validated stability indicating method with the purpose of differentiating cilostazol from its impurities. The chromatographic separation was carried on a RP C₁₈ column (250 mm × 4.6 mm, 5 µm) at a flow rate of 1 mL/min using acetonitrile and acetic acid (50:50, v/v) as the mobile phase. At 260 nm, it was detected by UV diode array detector. Linearity was observed in the range of 10-200 µg/mL and correlation coefficient was found to be 0.99961. On undergoing stress conditions, cilostazol was found to be stable against hydrolysis, photochemical and thermal degradation but shows significant degradation in the presence of peroxides.

For the determination of cilostazol dispersible tablets, Wen [47] carried out a gradient elution method on a C8 column (250 mm × 4.6 mm, 5 µm) at 254 nm with the solvent system being acetonitrile:water.

Cilostazol in polymeric nanoparticle suspensions has been estimated by an effective and validated method developed by Farago et al. [48] using RP-C18 column and acetonitrile:water in the ratio 47:53, v/v as the mobile phase at a flow rate of 1 mL/min. The λ_{\max} used was 254 nm and detected by UV DAD system. The correlation coefficient was found to be 0.9989.

Basniwal et al. [49] optimized a validated stability-indicating HPLC method for determination of cilostazol. The chromatographic separation was carried out on C18 column employing 10 mM phosphate buffer (pH 6.0):acetonitrile:methanol (20:40:40, v/v/v) at the flow rate of 1.3 mL/min, detected at 248 nm with RT at 5.7 ± 0.1 min. The proposed method showed good linearity in the concentration range 100-3200 ng/mL with correlation coefficient being 0.9998. The stability studies showed that cilostazol was stable in all stress conditions.

For the simultaneous estimation of aspirin and cilostazol, Ambekar et al. [50] outlined a gradient elution RP-HPLC method in which Nova-pack C18 (4.6 mm × 250 mm, particle size 4 µm) column and

Table 4: HPLC methods for analysis of cilostazol either alone or in combination

Matrix	Analyte	Linearity range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)	Precision (%RSD)	Accuracy (%Recovery)	Robustness	Ref.
Aqueous solution	CLZ	70-700	1.83	5.48	0.72	99.9 \pm 1.3	No significant change	[43]
Aqueous solution	CLZ	25-150	NA	NA	0.70	99.02 \pm 1.50	No significant change	[44]
Aqueous solution	CLZ	5-17.5	0.0481	0.160	0.36	99.84 \pm 0.97	No significant change	[45]
Aqueous solution	CLZ	10-200	3	10	NA	NA	NA	[46]
Aqueous solution	CLZ	NA	0.1 ng/mL	0.2 ng/mL	NA	NA	NA	[47]
PolymERIC nanoparticle suspension	CLZ	10-70	0.41	1.23	0.7	100.81 \pm 0.97	No significant change	[48]
Aqueous solution	CLZ	100-3200 ng/mL	NA	NA	0.46	99.58 \pm 1.09	No significant change	[49]
Aqueous solution	CLZ	5-100	0.37	1.14	0.42	98.95 \pm 0.95	No significant change	[50]
	ASP	2-40	0.25	0.78	0.21	99.25 \pm 1.20		
Synthetic mixture	CLZ	2-10	0.038	0.117	0.68	99.65 \pm 0.40	No significant change	[51]
	TELMI	4-20	0.041	0.125	0.60	100.80 \pm 0.62		
Aqueous solution	CLZ	6-18	NA	NA	0.58	99.61 \pm 0.50	No significant change	[52]
	IMI	6-18			0.32	99.78 \pm 0.65		
Aqueous solution	CLZ	10-100	NA	NA	0.64	98.97 \pm 0.50	No significant change	[53]
	CLG	7.5-75			0.56	98.94 \pm 0.56		
Synthetic mixture	CLZ	0.5-15	0.04	0.11	0.55	99.41 \pm 0.50	No significant change	[54]
	TELMI	0.25-20	0.06	0.20	0.63	99.15 \pm 0.50		
Aqueous solution	CLZ	1.0-31.0	0.24	0.72	0.69	99.96 \pm 0.46	No significant change	[42]

NA: not available.

Table 5: HPLC methods for analysis of cilostazol in biological matrices

Analyte	Matrix	Linearity (ng/mL)	LOD	LOQ (ng/mL)	Precision (%RSD)	Accuracy (%Recovery)	Robustness	Ref.
CLZ	Human plasma	5-1200	NA	5	0.051	91.23 ± 6.3	No significant change	[55]
CLZ	Human plasma	25-2000	NA	NA	0.059	71.25 ± 2.3	No significant change	[56]
CLZ	Human plasma	25-2000	NA	25	6.53	93.94 ± 9.25	No significant change	[57]
CLZ	Human plasma	0.2-2 µg/mL	0.005 µg/mL	0.05 µg/mL	2.61	96.02 ± 4.25	No significant change	[58]
CLZ	Human serum	20-2000	NA	20	1.82	99.22 ± 2.50	No significant change	[59]
CLZ	Human plasma	20-600	NA	20	1.5	98.5 ± 6.20	No significant change	[60]
CLZ	Human urine	100-300	NA	100	2.1	96.30 ± 2.30	No significant change	[61]
CLZ	Human liver microsomal solutions	100-2000	NA	1000	2.8	81.8 ± 5.29	No significant change	[62]
CLZ	Rat plasma	25-2500	NA	25	2.6	91.9 ± 0.23	No significant change	[63]
ROS			NA	25	1.0	94.7 ± 2.23		
DCLI		20-2000	NA	20	1.1	97.0 ± 6.25		

NA: not available.

acetonitrile:ammonium formate (10 mM) pH 3.5 mobile phase at a flow rate of 0.7 mL/min was used. The detection wavelength was 254 nm using DAD system. The described method was found linear over a range of 5-100 µg/mL for cilostazol and 2-40 µg/mL for aspirin. When degradation studies were carried out, it was observed that aspirin was susceptible to hydrolysis (acid, alkaline, neutral) and oxidation while cilostazol showed extensive degradation in hydrolysis (acid, alkaline) and oxidation methods.

Patel et al. [51] established a sensitive gradient RP-HPLC method for the simultaneous quantification of cilostazol and telmisartan using Enable C18G (250 mm × 4.6 mm, 5 µm) column and potassium dihydrogen phosphate buffer (10 mM):methanol:acetonitrile in the ratio of 30:10:60, v/v/v as the mobile phase. The flow rate was 1.0 mL/min and the wavelength used was 257 nm. The method was found to be linear in the range of 2-10 µg/mL for the telmisartan and 4-20 µg/mL for the cilostazol. The methods were validated as per ICH guidelines.

Nayak et al. [52] published a validated RP-HPLC method for the determination of cilostazol in combined dosage forms with imipramine. The chromatographic separation was carried out by LC- 20 AT C18 (25 cm × 0.46 cm) Hypersil BDS column with buffer (pH 4.5) and methanol in the ratio of 20:80, v/v as the mobile phase, at a flow rate of 1 mL/min. Detection was monitored at 222 nm using UV detector. Linearity was observed at 6-18 µg/mL for both cilostazol and imipramine.

Thomas et al. [53] presented a validated method for the estimation of clopidogrel and cilostazol using C8 column as stationary phase and 0.025 M phosphate buffer:methanol:acetonitrile (20:40:40, v/v/v) as mobile phase at a flow rate of 1 mL/min with UV detection at 237 nm. The correlation coefficient was observed to be 0.0999 and linearity was found in the concentration range of 10-100 mg/mL for cilostazol and 7.5-75 mg/mL for clopidogrel. When subjected to forced degradation, it was observed that while cilostazol was only affected by oxidation, clopidogrel was susceptible to acid and alkaline hydrolysis.

Ibrahim et al. [54] illustrated a unique stability-indicating assay method using RP-HPLC for the estimation of cilostazol and telmisartan in raw and pharmaceutical preparations and differentiated from its degraded products. The chromatographic separation was carried out on monolithic C8 column (3 mm × 4.6 mm i.d., 2 µm pore size highly porous) with acetonitrile:0.03 M dihydrogen phosphate buffer (40:60, v/v) at flow rate of 1 mL/min and detected at 257 nm. Good linearity was obtained in the concentration range of 0.5-15 µg/mL for cilostazol and 0.25-20 µg/mL for telmisartan. Cilostazol when subjected

to stress conditions was found to be stable in all except oxidation. Hence, the drug and its degraded product due to oxidation can be separated precisely by this method using derivative spectroscopy. The linearity range of cilostazol and oxidative degradate at 227 and 257 nm were found to be 1-35 $\mu\text{g/mL}$ and 2-50 $\mu\text{g/mL}$. All parameters of validation according to the ICH were carried out.

An extensive research for the determination of cilostazol and its differentiation from its degraded products by SIAMs through HPLC and HPTLC methods was carried out by Fayed et al. [42]. For HPLC, separation was carried out on Xterra RP 18 column with acetonitrile:ammonium acetate (40:55, v/v) at 1 mL/min and detected through photodiode array detection at 254 nm. Stress studies carried out on cilostazol showed that it was susceptible to acid, alkaline, and oxidative degradation which was differentiated from cilostazol by derivative and chemometric methods namely, partial least squares and concentrations residual augmented classical least squares method applied on zero order spectra were developed. For third order spectroscopy, the concentration range of the sample was 2-34 $\mu\text{g/mL}$ scanned at the range of 200-350 nm using 400 as the scaling factor. The optimum wavelength for third derivative and first derivative was found to be 275 and 255 nm, respectively.

3.6.2 High performance liquid chromatography in biological matrices

Bramer et al. [55] proposed a tandem spectroscopy method for the quantitation of cilostazol and its three active metabolites which was cross validated by a comparative HPLC method. The separation in HPLC was carried out on TSKGEL ODS-80TM column (150 mm \times 4.6 mm, 5 μm with the solvent system as phase A acetonitrile:2 mM ammonium acetate (10:90, v/v) and phase B acetonitrile:2 mM ammonium acetate (90:10, v/v) pumped in gradient elution at 1 mL/min detected at 254 nm. Correlation coefficient was found to be 0.988.

Akiyama et al. [56] developed a RP-HPLC method for the determination of cilostazol in biological fluids like human plasma obtained from healthy male volunteers. The chromatographic separation was carried out on $\mu\text{Bondapak C18}$ column (30 cm \times 3.9 mm I.D., particle size 10 μm) with acetonitrile-water (42:58, v/v) as the mobile phase at a flow rate of 1.7 mL/min through UV detector at 254 nm.

Yeon et al. [57] exemplified a unique HPLC method to estimate cilostazol in human plasma with an on-line triple column switching system. The plasma after extraction and

suitable preparation is primarily injected into a MF Ph-1 column (20 mm \times 4 mm I.D.) which is then transferred to an intermediate C18 column (35 mm \times 2 mm I.D.) at a flow rate of 1 mL/min using acetonitrile in water (10:90, v/v). The main separation was carried out on a semi micro-bore C18 column (250 mm \times 1.5 mm I.D.) with (40:60, v/v) acetonitrile:water at a flow rate of 150 $\mu\text{L/min}$. The RT of cilostazol was 19 min. It was observed to be linear over the range of 25-2000 ng/mL with the correlation coefficient being 0.999.

Pareek et al. [58] proposed *in-vitro* and *in-vivo* methods for the determination of cilostazol, where the *in-vitro* study was carried out using human plasma from healthy volunteers, the *in-vivo* method was used to study pharmacokinetics and carried out on rabbit plasma. Cilostazol was extracted from human plasma by protein precipitation method and separation is carried out on a Phenomenex C18 column (250 mm \times 4.60 mm i.d., 0.5 μm particle size) at flow rate of 1.5 mL/min with acetonitrile:50 mM acetate buffer:water in the ratio 50:20:30, v/v/v as the solvent system. Detection was observed at 248 nm. The linearity analysis was excellent over the range 0.2-2 $\mu\text{g/mL}$. Stability studies were carried out with the QC samples at temperatures they are required to occur naturally during analysis. In the pharmacokinetic studies of cilostazol, *in-vivo* studies were carried out on male albino rabbit plasma and calculated C_{max} , AUC, T_{max} , $T_{1/2}$, and K_{el} . The method was carried out as per ICH guidelines and can be used for bioequivalence study.

A validated HPLC method for the estimation of cilostazol in human serum was devised by Joti et al. [59] where the stationary phase was C-18 column and the solvent system was water and acetonitrile (60:40, v/v) at 254 nm with a flow rate of 1.0 mL/min employing diazepam as internal standard. Linearity was observed in the range of 20-2000 ng.

Fu et al. [60] reported a validated HPLC method for the simultaneous estimation of cilostazol and its seven metabolites in human plasma. The extraction was made by liquid-liquid and liquid-solid extraction techniques and the chromatographic separation was carried out by ODS-80TM column with gradient elution using ammonium acetate buffer and acetonitrile at different ratios at 1 mL/min through UV detection at 254 nm. The method was observed to be linear over a range of 20-600 ng/mL and correlation coefficient being 0.998. The long-time stability of the plasma was observed for one year.

Tata et al. [61] published the only validated method for the simultaneous quantification of cilostazol and its eight metabolites in human urine. Following liquid-liquid extraction, the chromatographic separation was carried

out on TSKGEL ODS-80TM column (150 mm × 4.6 mm i.d., 5 μm) preceded by Cosmosil column (150 mm × 4.6 mm i.d., 5 μm) where the mobile phase acetonitrile:100 mM ammonium acetate buffer in different ratios were used in gradient elution at 1 mL/min and detection carried out at 254 nm. The correlation coefficient was 0.995. The method tested long term stability for three months.

Bramer et al. [62] exemplified a validated HPLC method for determining cilostazol and its four primary metabolites in human liver microsomal incubation mixtures. Following extraction by protein precipitation and inclusion of five metabolic inhibitor probes, separation was carried out on TSK Gel ODS80TM column (150 mm × 4.6 mm, 5 μm) preceded by Cosmil C-18 column (150 mm × 4.6 mm, 5 μm) in tandem and detected at 254 nm with gradient elution of acetonitrile and ammonium acetate buffer acetonitrile:100 mM ammonium acetate buffer phase A (8:92, v/v), phase B (60:40, v/v) at 1 mL/min. The linearity was observed in ranges of 1000-20000 ng/mL for cilostazol, 100-4000 ng/mL for three metabolites and 100-2000 ng/mL for the remaining metabolite.

A validated HPLC method for simultaneous quantitation of rosiglitazone (ROS), cilostazol and its active metabolite 3, 4-dehydro-cilostazol (DCLI) in rat plasma extracted by liquid-liquid extraction was optimized by Varanasi et al. [63] The separation was carried out on Hypersil BDS C18 column (150 mm × 4.6 mm, 5 μm) pumped at 1.2 mL/min with acetonitrile:potassium di-hydrogen phosphate (35:65, v/v) and detected at dual wavelength, 226 nm for rosiglitazone and 3, 4-dehydro-cilostazol while 257 nm for cilostazol respectively. It shows good linear relations in the concentration range of 20-2000 ng/mL for 3, 4-dehydro-cilostazol and 25-2500 ng/mL for rosiglitazone and cilostazol.

HPLC is the most reported analytical technique for cilostazol as it is not only reliable, precise, time and cost efficient but can also be used for analysis of both pharmaceutical and biological samples. The most common column used in HPLC analysis of cilostazol was observed to be C18 column with mobile phase being a mixture of acetonitrile with water, methanol, or buffer.

3.7 Liquid chromatography-tandem mass spectrometry

Liquid chromatography-tandem mass spectrometry is a trending hyphenated technique which is used for determination of the analyte in biological samples like plasma, urine, serum, hair, etc. It gives rise to highly sensitive and accurate estimation methods with the help of isotopic

internal standards [64]. Cilostazol has been analyzed by LC-MS/MS in biological matrices of humans and rats like plasma, serum, and urine [55,65-68]. The chromatographic and validation parameters specific to every method is reported in Table 6.

Bramer et al. [55] presented a tandem spectroscopy method for the quantitation of cilostazol and its three metabolites in human plasma prepared by liquid-liquid extraction followed by solid-phase extraction (SPE) which was validated by a comparative HPLC method. The solvent system phase A acetonitrile:2 mM ammonium acetate (10:90, v/v) and phase B acetonitrile:2 mM ammonium acetate (90:10, v/v) pumped in gradient elution in the Supelcosil LC-18-DB column (250 mm × 2.1 mm i.d., 5 μm) at 1 mL/min and was detected at 254 nm. The calibration curves were generated using the analyte-to-internal standard peak area ratios by weighted (1/x) linear regression. The concentrations of calibration standards were back-calculated using the following equation:

$$\text{Analyte concentration} = \frac{\left(\frac{\text{Analyte peak height}}{\text{IS peak height}} - \text{Intercept} \right)}{\text{Slope}} \quad (1)$$

Correlation coefficient was found to be 0.999 in the concentration range of 5-200 ng/mL. The method was validated with respect to specificity, linearity, sensitivity, extraction recovery, inter-batch and intra-batch precision and accuracy, stability (freeze-thaw, bench top, and long term), and dilution integrity.

Yerramilli A et al. [65] optimized a validated LC-MS/MS method for the determination of cilostazol and its primary metabolite 3, 4-dehydro cilostazol in human plasma using mosapride as an internal standard. The chromatographic separation is brought about by pumping 5 mM ammonium acetate buffer in acetonitrile (10:90, v/v) through Interstil C18 column (5 μm, 150 mm × 4.6 mm) at 1.2 mL/min. This method achieved a low run time of 2.5 min which enables the analyst to run up to 400 samples a day. The calibration curves were generated using the analyte-to-internal standard peak area ratios by weighted (1/x²) least squares linear regression. The back-calculated standard concentration was within 15% deviation from the nominal value except at the LLOQ, for which the maximum acceptable deviation was set at 20%. The method was validated with respect to linearity, sensitivity, extraction recovery, inter-batch and intra-batch precision and accuracy, auto sampler carry over test, and stability (freeze-thaw, bench top, and long term).

Table 6: LC-MS/MS methods for analysis of cilostazol in biological matrices

Analyte	Matrix	Internal Std	Ion source	Metabolite	Precursor to production transition (m/z)	Linearity	LLOQ (ng/mL)	Ref.
CLZ	Human plasma	OPC-3930	ESI+ve	OPC-13015 OPC-13213 OPC-13217	370.2→288.2(CLZ) 354.3→272.0 (IS) 368.2→286.2 (M1) 386.2→288.2 (M2) 386.2→288.2 (M3)	5-1200	5	[55]
CLZ	Human plasma	Mosapride	ESI+ve (MRM)	3,4-dehydro cilostazol (DCLI)	370→288(CLZ) 368→286(DCLI) 422→198 (IS)	5-2000	5	[65]
CLZ	Rat plasma	Repaglinide	ESI+ve (MRM)	3,4-dehydro cilostazol (DCLI)	370→288.3 368.3→286.2 (DCLI) 453.3→230.4(IS)	20–2000	20	[66]
NTG					318→166.2 453.3→230.4(IS)			
CLZ	Rat plasma	Midazolam	ESI+ve (SIM)	NA	370 325.9(IS)	10-2000	10	[67]
CLZ	Rat plasma	Glimepride	ESI+ve (SRM)	3,4-dehydro cilostazol (DCLI)	446.4→321.1 368.3→286.2 (DCLI) 491.4→352.2(IS)	25-2080	25	[68]
GPZ					370.2→288.3 491.4→352.2(IS)			

NA: not available.

IS: internal standard.

ESI+: electrospray ionization positive mode.

Varanasi et al. [66] illustrated a validated LC-MS/MS technique in the multiple reaction monitoring mode which can be used for further pharmacokinetic studies for the simultaneous estimation of nateglinide (NTG), cilostazol and its active metabolite 3, 4-dehydro cilostazol in wistar rat plasma extracted through liquid-liquid extraction. The separation was carried out by isocratic elution of acetonitrile:2 mM ammonium acetate buffer (90:10, v/v) through Hypurity C18 column (50 mm × 4.6 mm i.d., 5 μm) at 0.4 mL/min. The calibration curves were generated using the analyte-to-IS peak area ratios by weighted (1/x²) least squares linear regression. The percentage stability was found to be acceptable by comparing the mean of back-calculated concentration of all analytes from the stored stability samples with that of freshly spiked QC samples. The method was validated with respect to linearity, sensitivity, extraction recovery, inter-batch and intra-batch precision and accuracy, auto sampler carry over test, and stability (freeze-thaw, bench top, and long term, and dry residue stability).

Wang et al. [67] established a tandem spectroscopy method in the selected ion monitoring mode

for estimation of cilostazol in wistar rat plasma using protein precipitation technique with midazolam as internal standard. The analyte was chromatographed on an Agilent Zorbax SB-C18 (2.1 mm × 50 mm, 5.0 μm) column with gradient elution of acetonitrile:0.1% formic acid, v/v at 0.4 mL/min. Linearity was observed over the range of 10-2000 ng/mL for cilostazol in rat plasma. It was assessed by weighted (1/x) linear regression of calibration curves generated, using analyte internal standard peak area ratios. The method was validated with respect to linearity, sensitivity, extraction recovery, matrix effect, inter-batch and intra-batch precision and accuracy, auto sampler carry over test, and stability (freeze-thaw, bench top, and long term).

Manikandan et al. [68] outlined a tandem mass spectrometry in the selective reaction monitoring mode for the determination of drug-drug interaction between glipizide (GPZ), cilostazol and its active metabolite 3, 4-dehydro-cilostazol in rat plasma using liquid-liquid extraction technique employing glimepride as the internal standard. The analyte was chromatographed on Hypurity C 18 column (50 mm × 4.6 mm i.d., 5 μm) an with isocratic

elution of acetonitrile:2 mM ammonium acetate buffer (90:10, v/v) at 0.4 mL/min. Linearity was observed over the range of 25-2000 ng/mL for cilostazol in rat plasma. Linearity was assessed by weighted ($1/x^2$) linear regression of calibration curves generated, using analyte internal standard peak area ratios. The method was validated with respect to linearity, sensitivity, extraction recovery, inter-batch and intra-batch precision and accuracy, auto sampler carry over test, dilution integrity, and stability (freeze-thaw, bench top, and long term).

The hyphenated techniques have turned out as a precious discovery for the analysis of biological fluids. LC-MS/MS is the right choice for analyzing cilostazol and its metabolites or impurities in biological samples as it gives high sensitivity. However, as the instrument and the process are expensive, requires a lot of care and experience, and is thus not most commonly opted by researchers.

3.8 Ultra high performance liquid chromatography

UHPLC is a modern form of separation technique which uses stationary phase consisting of smaller particles, narrow columns, and high pressure almost up to 100 MPa for separation of particles less than 2 μm in diameter as opposed to HPLC. The main focus is on speed, sensitivity, and resolution [69]. The validation parameters specific to the method is reported in Table 7.

Bhatt et al. [70] published a unique sensitive ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method, simultaneously quantifying cilostazol and its active metabolite 3, 4-dehydro cilostazol (DCIL) using deuterated internal standards such as CIL-d11 and DCIL-d11. The plasma samples were collected from 30 healthy subjects and extracted by solid phase extraction technique. The chromatographic separation was carried out using acetonitrile and 2.0 mM ammonium formate (80:20, v/v) as the mobile phase and

BEH C18 (50 mm \times 2.1 mm, 1.7 μm) UHPLC column. The method was validated as per ICH guidelines. UHPLC is one of the hyphenated techniques employed for analysis of biological samples, but they have shorter columns, rapid flow rates, greater sensitivity and specificity, less solvent consumption, and better resolution.

4 Discussion

Cilostazol is used to treat peripheral ischemia, otherwise known as intermittent claudication. A wide spectrum of reported analytical techniques can be obtained for the estimation of cilostazol in pharmaceutical preparations and biological matrices.

The logP value of cilostazol is 3.38, which indicates that it is lipophilic in nature. In the Figure 2, the solvent usage profile has been displayed which shows that the most common solvents used in the analysis of cilostazol were acetonitrile and methanol. The chosen solvents are said to be easily available, non-carcinogenic, easy to handle, and economical.

An overview of all the reported analytical methods used for the determination of cilostazol is presented in Figure 3 from which we can establish that HPLC is the most common analytical technique employed for estimation of cilostazol in both pharmaceutical preparations as well as biological matrices. HPLC is cost and time efficient and has good separation potential. For biological samples, we propose LC-MS/ESI/MS method as it has both high separation and sensitivity, which compensates for its high cost. For pharmaceutical preparations, HPLC method is preferable as it is economical and does not require high level of detection.

There are no capillary electrophoresis methods reported for cilostazol. CE coupled with MS would provide good sensitivity. Furthermore, focus could be on developing rapid assay techniques to determine multiple anti-platelet drugs in a matrix. Impurity profiling of cilostazol and its active metabolites has also not been achieved.

Table 7: UHPLC methods for analysis of cilostazol in biological matrices

Matrix	Ion source	IS	m/z	Linearity range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Precision (%RSD)	Accuracy (% Recovery)	Ref.
Human plasma	ESI+ve (MRM)	CIL-d11	370.3 \rightarrow 288.3	0.5-1000	0.17	0.50	0.84	98.8 \pm 1.7	[70]
			381.2 \rightarrow 288.3(IS)						
		DCIL-d11	368.2 \rightarrow 286.3	0.5-500			0.71	98.0 \pm 2.7	
			379.2 \rightarrow 286.2(IS)						

ESI+: electrospray ionization positive mode.

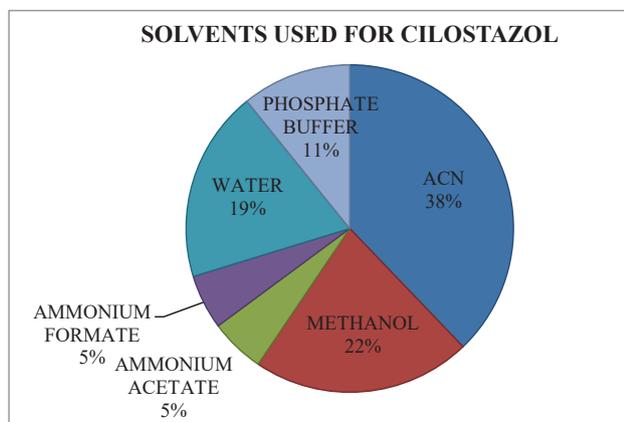


Figure 2: Graph displaying the various solvents used for cilostazol.

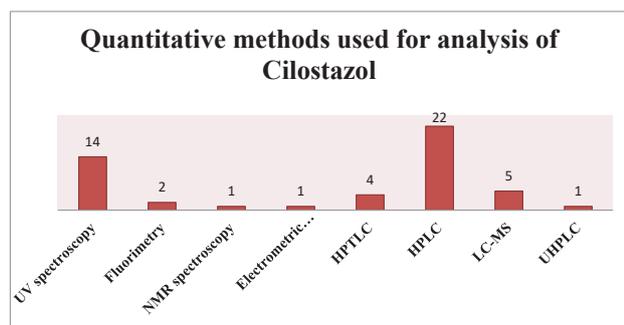


Figure 3: Graph displaying the analytical methods used for determination of cilostazol.

5 Conclusion

In conclusion, various analytical techniques are available for the estimation of cilostazol and its metabolites either alone or in combination forms in pharmaceutical preparation or biological matrices like plasma, serum, and urine. Varying formulations like tablets, capsules, disintegration tablets, polymeric nanoparticles suspensions etc. have been encompassed for evaluation. The suggested UV methods were found to be cost and time efficient for the routine analysis but the reported HPLC techniques were simple, accurate, reliable, and sensitive for analyzing cilostazol in both pharmaceutical and biological matrix. However, for increased sensitivity, the LC-MS/MS techniques were found to be well received. Modern trends and techniques like new software, Raman spectrokinetics, and in line column switching, to name a few have also been implemented. Further research may be carried out to develop a method using environment friendly solvent and focus on the estimation of impurities in both dosage forms and biological matrices. This review summarizes all the reported analytical techniques for the determination

of cilostazol from 1985 to 2019. Cilostazol could further be potentially used in the treatment of Alzheimer's.

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Conflict of interest: Authors state no conflict of interest.

References

- [1] Boyd AM, Ratcliffe AH, Jepson RP, James GWH. Intermittent claudication a clinical study. *J Bone Joint Surg.* 1949;31(3):325-55.
- [2] Tonnesen KH, Noer I, Paaske W, Sager PH. Classification of peripheral occlusive diseases based on symptoms, signs and distal blood pressure measurements. *Acta Chir Scand.* 1980;146(2):101-4.
- [3] Jelnes R, Gaardsting O, Jensen KH, Bnkggaard N, Schroeder T, Tonnesen KH. Fate in intermittent claudication: outcome and risk factors. *Br. Med. J.* 1983;293:1137-40.
- [4] Brand FN, Abbott RD, Kannel WB. Diabetes, Intermittent Claudication, and Risk of Cardiovascular Events: The Framingham Study. *Diabetes.* 1989;38:504-9.
- [5] Kannel WB, McGee DL. Update on Some Epidemiologic Features of Intermittent Claudication: The Framingham Study. *J Am Geriatr Soc.* 1985;33(1):13-9.
- [6] Reunanen A, Takkunen H, Aromaa A. Prevalence of Intermittent Claudication and Its Effect on Mortality. *Acta Med Scand.* 1982;211:245-56.
- [7] Lane R, Harwood A, Watson L, Leng GC. Exercise for intermittent claudication. *Cochrane Db Syst Rev.* 2017;12:1-142.
- [8] Sorokin EM, Markham A. Cilostazol. *Drug Aging.* 1999;14(1):63-71.
- [9] Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. *Br J Clin Pharmacol.* 2011;72(4):634-46.
- [10] Schror K. The pharmacology of cilostazol. *Diabetes Obes Metab.* 2002;4(2):S14-9.
- [11] Sun B, Le SN, Lin S, Fong M, Guertin M, Liu Y, et al. New Mechanism of Action for Cilostazol: Interplay between Adenosine and Cilostazol in Inhibiting Platelet Activation. *J Cardiovasc Pharm.* 2002;40(4):577-85.
- [12] Kimura Y, Tani T, Kanbe T, Watanabe K. Effect of cilostazol on platelet aggregation and experimental thrombosis. *Arznei-Forschung.* 1985;35(7A):1144-9.
- [13] Saitoh S, Saito T, Otake A, Owada T, Mitsugi M, Hashimoto H, et al. Cilostazol, a novel cyclic AMP phosphodiesterase inhibitor, prevents reocclusion after coronary arterial

- thrombolysis with recombinant tissue-type plasminogen activator. *Arterioscler Thromb*. 1993;13(4):563-70.
- [14] Tanaka T, Ishikawa T, Hagiwara M, Onoda K, Itoh H, Hidaka H. Effects of cilostazol a selective cAMP phosphodiesterase inhibitor on the contraction of vascular smooth muscle. *Pharmacology*. 1988;36:313-20.
- [15] Prickaerts J, Heckman PRA, Blokland A. Investigational Phosphodiesterase inhibitors in phase I and phase II clinical trials for Alzheimer's disease. *Expert Opin Inv Drug*. 2017;1-48.
- [16] Cilostazol. Official monographs, USP43-NF38, 2020.
- [17] Pletal cilostazol tablets. NDA 20-863/S-021.
- [18] Herrmann R, Onkelinx C. Quantities and units in clinical chemistry: Nebulizer and flame properties in flame emission and absorption spectrometry. *Pure Appl Chem*. 1986; 58:1737-42.
- [19] Skoog DA, West DM. Ultraviolet and visible absorption measurements. In: Skoog DA, West DM. Principles of instrumental analysis. 2nd ed. New York: Holt. Rinehart and Winston. 1980;2:169.
- [20] Alhamidehoballah SA. Spectrophotometric methods for determination of cilostazol in pure and dosage forms. *Int J Res Pharm Chem*. 2015;5(1):17-26.
- [21] Kuruba SA, Hanamshetty PC. A simple spectrophotometric quantitative determination of cilostazol in bulk and pharmaceutical dosage forms using DNPH reagent. *J Appl Pharm. Sci*. 2015;5(12):117-21.
- [22] Basniwal PK, Kumar V, Shrivastava PK, Jain D. Spectrophotometric determination of cilostazol in tablet dosage form. *Trop J Pharm Res*. 2010;9(5): 499-503.
- [23] Chaitanya T, Ramya B, Archana M, Himavani KV, Ramalingam P, Reddy PY. Forced degradation studies of cilostazol by validated UV-spectrophotometric method. *J Pharm Res*. 2011;4(11):4094.
- [24] Fursule RA, Rupala DK, Khan MG, Shirkhedkar AA, Surana S J. Determination of quetiapine fumarate and Cilostazol in bulk and tablet by UV-Spectrophotometry. *Biosci Biotechnol Res Asia*. 2008;5(1):461-3.
- [25] Damor D, Mittal K, Patel B, Mashru RC. Method development and validation of simultaneous estimation of cilostazol and telmisartan. *Res Rev J Pharm Anal*. 2015;4(3):41-8.
- [26] Patel JV, Patel CN, Anand IS, Patel PU, Prajapati PH. Simultaneous spectrophotometric estimation of cilostazol and aspirin in synthetic mixture. *Int J Chem Sci*. 2008;6(1):73-9.
- [27] Patel R, Maheshwari DG. UV spectrophotometric methods for simultaneous estimation of telmisartan and cilostazol in synthetic mixture. *Pharma Sci Monit*. 2015;6(1):276-91.
- [28] Brahmabhatt PM, Prajapati LM, Joshi AK, Kharodiya ML. Q-absorbance ratio method for simultaneous estimation of cilostazol and imipramine in combined dosage form. *World J Pharm Res*. 2015;4(4):1786-94.
- [29] Shah AB, Zarna D, Jain VC. Development and validation of UV spectroscopic simultaneous method for zonisamide and cilostazol in synthetic mixture. *Pharma Sci Monit*. 2019;10(3):53-65.
- [30] Damor D, Patel BP, Mittal K, Mashru RC. Simultaneous estimation of cilostazol and telmisartan using PCR, PLS, CLS and ILS. *World J Pharm Res*. 2015;4(7):693-709.
- [31] Pandeewaran M, El-Mossalamy EH, Elango KP. Spectroscopic studies on the interaction of cilostazol with iodine and 2, 3-dichloro-5, 6-dicyanobenzoquinone. *Spectrochim Acta A*. 2011; 78:375-82.
- [32] Moncada J, Adams WR, Thakur R, Julin M, Carrero CA. Developing a Raman Spectrokinetic approach to gain insights into the Structure-Reactivity Relationship of supported metal oxide catalysts. *ACS Catal*. 2018;8(10):8976-86.
- [33] Attia MS, Mahmoud WH, Youssef AO, Mostafa MS. Cilostazol Determination by the enhancement of the green emission of Tb³⁺ Optical Sensor. *J Fluoresc*. 2011;21:2229-35.
- [34] Ibrahim F, Sharaf El-Din M, El-Aziz HA. New validated fluorescence quenching based procedure for the determination of cilostazol and clopidogrel in bulk, tablets and biological fluids, with application of Stern-Volmer equation. *Br J Pharm Res*. 2016;12(6):1-15.
- [35] Skoog DA, West DM. Nuclear magnetic resonance spectroscopy. In: Skoog DA, West DM. Principles of instrumental analysis. 2nd ed. New York: Holt. Rinehart and Winston. 1980;2:377.
- [36] Yang J, Yao T, Wang J, Zhang D. Determination of Cilostazol by ¹H NMR. *Huaxue Fenxi Jiliang*. 2012;4.
- [37] Skoog DA, West DM. Voltammetry and Polarography. In: Skoog DA, West DM. Principles of instrumental analysis. 2nd ed. New York: Holt. Rinehart and Winston. 1980;2:605.
- [38] Wassel A, Amin AS, Ahmed IS, Dessouki HA. Electrochemical behavior and determination of Cilostazol in pure, urine and in pharmaceutical formulations. *Anal Bioanal Electrochem*. 2012;4(2):197-211.
- [39] Khan MG, Jain PS, Surana SJ. Development and validation of stability-indicating high performance thin-layer formulation chromatography method for estimation of cilostazol in bulk and in pharmaceutical formulation. *Anal Chem-Indian J*. 2013;12(2):76-82.
- [40] Kurien J, Jayasekhar P, John J. HPTLC determination of cilostazol in pharmaceutical dosage forms. *Int J Adv Res*. 2014;2(2):952-7.
- [41] Patel JV, Patel CN, Patel PU, Prajapati PH, Anand IS, Patel MB. Simultaneous estimation of cilostazol and aspirin in synthetic mixture using HPTLC method. *Int J Chem Sci*. 2008;6(3):1377-84.
- [42] Fayed AS, Shehata MA, Ashour A, Hassan NY, Weshahy SA. Validated stability-indicating methods for determination of cilostazol in presence of its degradation products according to the ICH guidelines. *J Pharm Biomed Anal*. 2007;45,407-16.
- [43] Lestari AD, Palupi T, Oktarina B, Yuwono M, Indrayanto G. HPLC determination of cilostazol in tablets, and its validation. *J Liq Chromatogr R T*. 2004;27(16):2603-12.
- [44] Jadhav AS, Pathare DB, Shingare MS. A validated stability indicating high performance reverse phase liquid chromatographic method for the determination of cilostazol in bulk drug substance. *Drug Dev Ind Pharm*. 2007;33:173-9.
- [45] Rambabu K, Suresh T. Isocratic reversed phase liquid chromatographic method validation for the determination of cilostazol in pure and formulations. *Int J Pharm Pharm Res*. 2015;4(3):180-192.
- [46] Kurien J, Jayasekhar P. Stability indicating HPLC determination of cilostazol in pharmaceutical dosage forms. *Int J Pharm Bio Sci*. 2014;5(1):176-186.
- [47] Wen RC. Determination of Related Substances of Cilostazol Dispersible Tablets by HPLC. *J Harbin Univ Commerce-Nat Sci Ed*. 2016;2(5):157-60.

- [48] Farago PV, Gomes MLS, Klein T, Simionatto M, Nadal JM, Zanin SMW, et al. A simple RP-HPLC/UV method for determination of cilostazol in polymeric nanoparticles suspensions: development and validation. *Lat Am J Pharm.* 2015;34(4):803-9.
- [49] Basniwal PK, Shrivastav PK, Jain D. Hydrolytic Degradation Profile and RP-HPLC Estimation of Cilostazol in Tablet Dosage Form. *Indian J Pharm Sci.* 2008;70(2):222-4.
- [50] Ambekar AM, Kuchekar BS. Validated new gradient stability-indicating LC method for the simultaneous estimation of cilostazol and aspirin in bulk and tablet formulation. *Eur J Biomed Pharm Sci.* 2014;1(2):149-64.
- [51] Patel R, Maheshwari DG. A New RP-HPLC Method for Simultaneous Estimation of Telmisartan and Cilostazol in Synthetic Mixture. *Int J Rec Sci Res.* 2015;6(4):3306-10.
- [52] Nayak JR, Luhar SV, Narkhede SB. Simultaneous Estimation and Determination of imipramine and cilostazol by UV Spectrophotometric and RP-HPLC Method. *J Pharm Sci Biosci Res.* 2016;6(3):270-5.
- [53] Thomas A, Bhosale S, Nanda R. Formulation of solid dosage form containing clopidogrel and cilostazol and its HPLC analysis. *Int J Pharm Pharm Sci.* 2017;9(6):12-8.
- [54] Ibrahim F, Sharaf El-Din M, El-Aziz HA. Selective Methods for Cilostazol Assay in Presence of its Oxidative Degradation Product and Co Formulated Telmisartan Application to Tablet Formulation. *J Chromatogr Sep Tech.* 2016;7(5):1-11.
- [55] Bramer SL, Tata PNV, Vengurlekar SS, Brisson JH. Method for the quantitative analysis of cilostazol and its metabolites in human plasma using LC/MS/MS. *J Pharm Biomed Anal.* 2001;26:637-50.
- [56] Akiyama H, Kudo S, Odomi M, Shimizu T. High-performance liquid chromatographic procedure for the determination of a new antithrombotic and vasodilating agent, cilostazol, in human plasma. *J Chromatogr.* 1985;338:456-9.
- [57] Yeon KJ, Park YJ, Park KM, Park JS, Ban E, Kim MK, et al. High Performance Liquid Chromatographic Analysis of Cilostazol in Human Plasma with On Line Column Switching. *J Liq Chromatogr R T.* 2005;28(1):109-20.
- [58] Pareek D, Jain S, Basniwal PK, Jain D. RP-HPLC Determination of Cilostazol in Human Plasma: Application to Pharmacokinetic Study in Male Albino Rabbit. *Acta Chromatogr.* 2014;26(2):283-96.
- [59] Joti JJ, Haque MA, Islam A SM, Islam MS. Validation and Optimization of a Simple RP-HPLC Method for Determination of Cilostazol in Human Serum. *Indian J Novel Drug Del.* 2011;3(2):143-8.
- [60] Fu CHJ, Tata NV, Okada K, Akiyama H, Bramer SL. Simultaneous quantitative determination of cilostazol and its metabolites in human plasma by high-performance liquid chromatography. *J Chromatogr B.* 1999;728:251-62.
- [61] Tata NV, Fu CHJ, Bramer SL. Determination of cilostazol and its metabolites in human urine by high performance liquid chromatography. *J Pharm Biomed Anal.* 2001;24:381-9.
- [62] Bramer SL, Tata NV, Fu CHJ, Browder NJ, Chow PC. The quantitative determination of cilostazol and its four metabolites in human liver microsomal incubation mixtures by high-performance liquid chromatography. *J Pharm Biomed Anal.* 1998;18:441-51.
- [63] Varanasi VS, Veeraraghavan S, Potharaju S, Thappali RS, Raghavan R, Vakkalanka VS. Validated high performance liquid chromatographic method for simultaneous determination of rosiglitazone, cilostazol, and 3, 4-dehydro-cilostazol in rat plasma and its application to pharmacokinetics. *Arznei-Forschung.* 2008;58(6):288-96.
- [64] Maheswari G, Kumari SK, Rahaman AS, Revathi S, Neeharika M. A review on LC-MS/MS in bioanalytical studies. *World J Pharm Res.* 2013;2(6):274-8.
- [65] Yerramilli A, Shukla M, Mudigond K, Shrivastava W, Datla PV. Simultaneous quantification of cilostazol and its primary metabolite 3, 4-dehydro cilostazol in human plasma by rapid liquid chromatography/tandem mass spectrometry. *Anal Bioanal Chem.* 2006;384:780-90.
- [66] Varanasi VVS, Sridhar V, Potharaju S, Shraddha R, Sivakumar SPN, Sabapathi KS. Development and validation of a liquid chromatography/tandem mass spectrometry assay for the simultaneous determination of nateglinide, cilostazol and its active metabolite 3, 4-dehydro cilostazol in Wistar rat plasma and its application to pharmacokinetic study. *J Chromatogr B.* 2008;865:91-8.
- [67] Wang J, Wang Z, Chen C, Wang Z, Liu N, Ma J. Gradient elution LC-ESI-MS determination of cilostazol in rat plasma and its application. *Lat Am J Pharm.* 2012;31(2):240-4.
- [68] Manikandan TRS, Sridhar V, Kanthikiran VVS, Swaroopkumar VVS, Mukkanti K. Liquid Chromatography – Tandem Mass Spectrometry for the Simultaneous Quantitation of Glipizide, Cilostazol and its Active Metabolite 3, 4-dehydro-cilostazol in Rat Plasma: Application for a Pharmacokinetic Study. *Arznei-Forschung.* 2012;62:425-32.
- [69] Taleuzzaman M, Ali S, Gilani SJ, Imam SS, Hafeez A. Ultra Performance Liquid Chromatography (UPLC) - A Review. *Austin J Anal Pharm Chem.* 2015;2(6):1056.
- [70] Bhatt NM, Chavada VD, Patel DP, Sharma P, Sanya M, Shrivastav PS. Determination of cilostazol and its active metabolite 3, 4-dehydro cilostazol from small plasma volume by UPLC-MS/MS. *J Pharm Anal.* 2015;5(1):1-11.