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RESEARCH ARTICLE

HPTLC DETERMINATION OF CILOSTAZOL IN PHARMACEUTICAL DOSAGE FORMS.

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Abstract

A simple, selective and precise high performance thin layer chromatographic method was developed and validated for the determination of Cilostazol in bulk drug and in formulation. The method uses aluminium plates pre-coated with silica gel 60_{F-254} as the stationary phase and hexane:acetone:chloroform (5:2:3, v/v/v) as solvent system. This system gave compact spot for Cilostazol (R_f: 0.15 ± 0.01). Densitometric analysis of Cilostazol was performed in the absorbance mode at 254nm. The linear regression analysis data for the calibration plot showed good linear relationship over a concentration range of 1 – 10 µg $spot^{-1}$. The values of correlation coefficient, slope and intercept were 0.998, 1489 and 4915 respectively. The method was validated for precision, robustness and recovery. The limit of detection and limit of quantification were 0.089 and 0.269µg $spot^{-1}$, respectively.

INTRODUCTION:

Cilostazol is chemically 6-[4-1(-cyclohexyl-1H-tetrazol-5-yl-butoxyl] 3-4-dihydro-2(1H)- quinolinone[1]. Cilostazol and its metabolites are cyclic adenosine monophosphate (cAMP) phosphodiesterase III inhibitors, inhibiting phosphodiesterase activity and suppressing cAMP degradation with a resultant increase in cAMP in platelets and blood vessels, leading to inhibition of platelet aggregation and vasodilation[2]. Therefore, Cilostazol is used for the treatment of intermittent claudication resulting from peripheral arterial disease. Cilostazol is commercially available as single dosage forms and combined dosage forms.

Cilostazol is official in United States Pharmacopoeia 2009. USP describes HPLC method for the assay of Cilostazol and its tablets, using a column packed with octadecylsilanized silica gel with a mobile phase of water, acetonitrile and methanol (10:7:30) equipped with a 254nm detector and a flow rate of 1ml/min.

The Literature survey reveals few studies regarding determination of Cilostazol in pharmaceutical dosage forms and biological fluids. These works include HPLC, UV spectrophotometric and potentiometric methods to determine Cilostazol in pharmaceutical dosage forms[3-4]. The assay of Cilostazol in the human plasma and mouse serum are also reported by HPLC methods.[5-12]

Today TLC is rapidly becoming a routine analytical technique due to its low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase and thus reducing the analysis time and cost per sample as compared to HPLC. The aim of the present study was to develop a simple, validated and rapid HPTLC method for routine analysis of Cilostazol in tablets. The HPTLC method was studied following official guidelines, evaluating the main parameters and the procedures and validated according to ICH guidelines.

MATERIALS AND METHODS:

Chemicals and Reagents

Cilostazol Reference Standard was supplied by Glenmark Pharmaceuticals Ltd, Mumbai, India. Cilostazol Tablets STILOZ-50, Glenmark Pharmaceuticals Ltd, Mumbai was procured from the market. Hexane, chloroform, acetone and methanol HPLC grade were procured from Merck, Germany.

HPTLC Instrumentation

The samples were spotted in the form of 6mm width with a Camag microlitre syringe on precoated silica gel aluminium plates 60 F_{254} (10 × 10 cm with 250 mm thickness, E. Merck), using a Camag Linomat 5 applicator . The plates were pre-washed with methanol and activated at 60^{o}C for 5 min prior to chromatography. The slit dimension was kept at 4.00 × 0.30 mm(micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of hexane:acetone:chloroform (5:2:3, (v/v/v), and 10 ml of mobile phase was used. Linear ascending development was carried out in a 10×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25^{o}C±2). The length of the chromatogram run was approximately 8 cm, subsequent to development; the TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by WINCats software.

Preparation of standard solution and linearity study:

An accurately weighed quantity of 10 mg of Cilostazol was transferred to 10 ml volumetric flask, dissolved in methanol and made up to mark with the same solvent to obtain concentration 1µg/ µl. Standard solutions of 0.5,1, 2, 5, 10, and 15 µl of Cilostazol was applied on TLC plate with the help of microlitre syringe, using Linomat 5 sample applicator to obtain the concentration of 0.5,1, 2, 5, 10 and 15 µg spot^{-1}. The standard curves were evaluated for within day and day-to-day reproducibility. Each experiment was repeated six times.

Method validation:

Precision:

Repeatability of sample application and measurement of peak area were carried out using six replicates of the same spot (5µg spot^{-1} of Cilostazol). The intra and inter-day variation for the determination of Cilostazol was carried out at three different concentration levels of 2, 5 and 10 µg per spot.

Limit of detection (LOD) and limit of quantification (LOQ):

In order to determine detection and quantification limit, Cilostazol concentrations in the lower part of the linear range of the calibration curve were used. Cilostazol solutions of 0.5, 1, 2, 5, 10 and 15 were prepared and applied in triplicate. The LOQ and LOD were calculated using equation LOD=3.3 × N/B and LOQ=10×N/B, where, N is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and B is the slope of the corresponding calibration curve.

Specificity:

The specificity of the method was ascertained by analyzing standard drug and sample. The spot of Cilostazol in sample was confirmed by comparing the R_f values and spectra of the spot with that of standard. The peak purity of Cilostazol was assessed by comparing the spectra at three levels, i.e., peak start(S), peak apex(M) and peak end(E) positions of the spot.

Ruggedness:

Ruggedness of the method was performed by spotting 5µg spot^{-1} of Cilostazol by two different analyst keeping same experimental and environmental conditions.

Accuracy:

The analyzed samples were spiked with extra 80, 100 and 120% of the standard Cilostazol and the mixtures were analyzed by the proposed method. At each level of the amount, three determinations were performed. This was done to check the recovery of the drug at different levels in the formulations.

Robustness:

By introducing small changes in the mobile phase composition, the effects of the results were examined. Mobile phases having different compositions of hexane:acetone:chloroform was tried and chromatograms were run. The amount of mobile phase, temperature and relative humidity was varied in the range of ±5%. The plates were

prewashed by methanol and activated at 60±5°C for 2, 5 and 7 min prior to chromatography. Time from spotting to chromatography and from chromatography to scanning was varied from 0, 20 and 40 min.

Application of proposed method to Tablet formulation:

Twenty tablets of Cilostazol (STILOZ-50) were accurately weighed and powdered. Average weight of a tablet was determined. A quantity of tablet powder equivalent to 10 mg of Cilostazol was dissolved in methanol, sonicated for 20 minutes and made up to volume in a 10 ml volumetric flask. After filtration through 0.41 µm filter (millifilter, Milford, MA), 5µl of the solution was spotted followed by development and scanning as described in standard preparation. The analysis was repeated in triplicate.

RESULTS AND DISCUSSION:

Development of optimum mobile phase:

TLC procedure was optimized with a view to develop a sensitive and reproducible assay method for Cilostazol. Different mobile phases were tried by trial and error method. But, hexane:methanol 1:4, (v/v) gave good resolution for Cilostazol, but typical peak nature was missing. Finally, the mobile phase consisting of hexane:acetone:chloroform (5:2:3, (v/v/v) gave a sharp and well defined peak at R_f value of 0.15. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature. This system was selected for the study.

Calibration curve:

The linear regression data for the calibration curves showed good linear relationship over the concentration range 1-10µg $spot^{-1}$. Linear regression equation was found to be Y=1489x+4915 (r^2=0.998).

Validation of method:

Precision

The precision of the developed HPTLC method was expressed in terms of % relative standard deviation (%RSD). The results depicted revealed high precision of the method presented in Table I.

LOD and LOQ:

Detection limit and quantification limit was calculated by the method described above. The LOQ and LOD were found to be 0.089 and 0.269µg $spot^{-1}$ respectively. This indicates the adequate sensitivity of the method.

Recovery studies:

The proposed method when used for extraction and subsequent estimation of Cilostazol from the pharmaceutical dosage form after spotting with 80, 100 and 120% of additional drug; afforded good recovery of Cilostazol. The amount of drug added and the % recovery are listed in Table II.

Specificity:

The peak purity of Cilostazol was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot, i.e., r^2 (S,M)=0.999 and r^2 (M,E)=0.9998. Good correlation (r^2=0.99) was also obtained between standard and sample spectra of Cilostazol.

Robustness of the method:

The standard deviation of peak areas was calculated for each parameter and %RSD was found to be less than 2%. The low values of %RSD values as shown in Table III. indicated robustness of the method.

Analysis of the marketed formulation:

A single spot at R_f 0.15 was observed in the chromatogram of the drug samples applied from the tablets. There was no interference from excipients. The % drug content and %RSD were calculated (Table IV). The low %RSD value indicated the suitability of this method for the routine analysis of Cilostazol in pharmaceutical dosage forms.

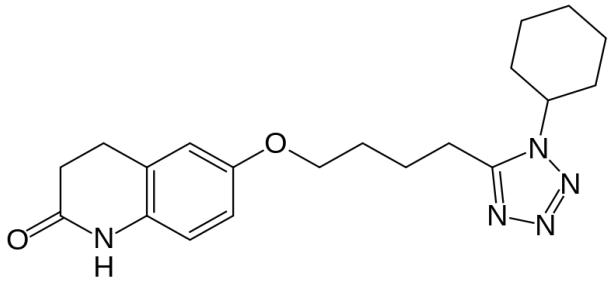

Figure I. Structure of Cilostazol

Table I. Intraday and inter-day precision studies

Drug	Conc. µg/spot	Intra-day		Inter-day	
		%Amount found*	%RSD	%Amount found*	%RSD
Cilostazol	2	102.50	0.27	102.56	0.11
	5	99.20	0.22	99.80	0.65
	10	99.49	0.08	99.55	0.09

*mean of three estimations

Table II. Recovery Studies of Cilostazol

Label claim of Cilostazol in STILOZ-50 (mg/tablet)	**Amount of Standard drug added (%)**	**Drug recovered (%)**	**%RSD**
50	0	99.18	1.02
50	80	100.20	1.80
50	100	99.80	0.98
50	120	100.60	1.34

*mean of three estimations at each level

Table III. Robustness of the method*

Parameter	**S.D. of peak area**	**% RSD**
Mobile phase composition	62.07	0.50
Mobile phase volume	47.64	0.39
Development distance	40.07	0.32
Activation of TLC plate	58.62	0.47
Duration of saturation	55.02	0.45
Time from spotting to chromatography	62.19	0.50
Time from chromatography to scanning	63.96	0.52

*n=6

Table IV. Results of analysis of Cilostazol Tablet (STILOZ-50) by proposed method*

Label Claim	**Amount found ± SD**	**% of Label claim ± SD**
50mg	49.97mg ± 0.08	99.95 ± 0.16

*n=3

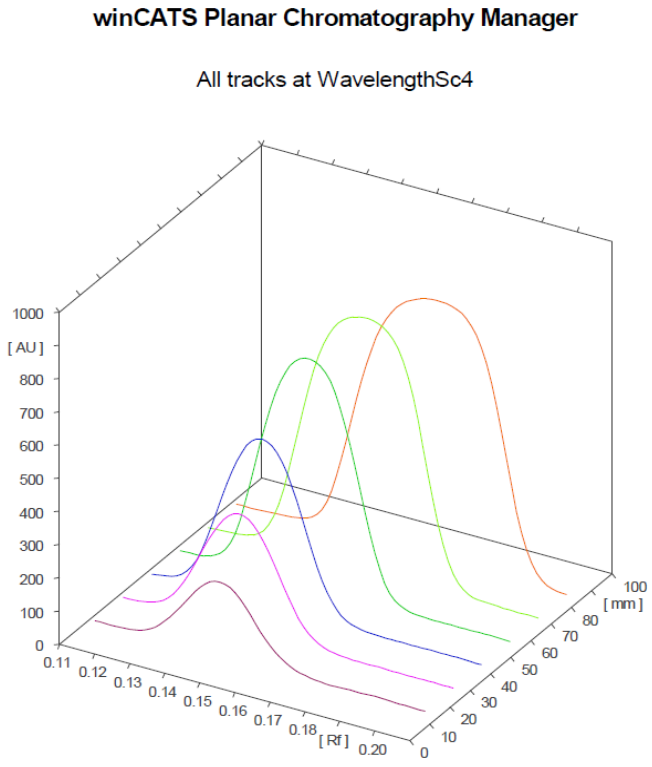

Fig II. Scan of all tracks

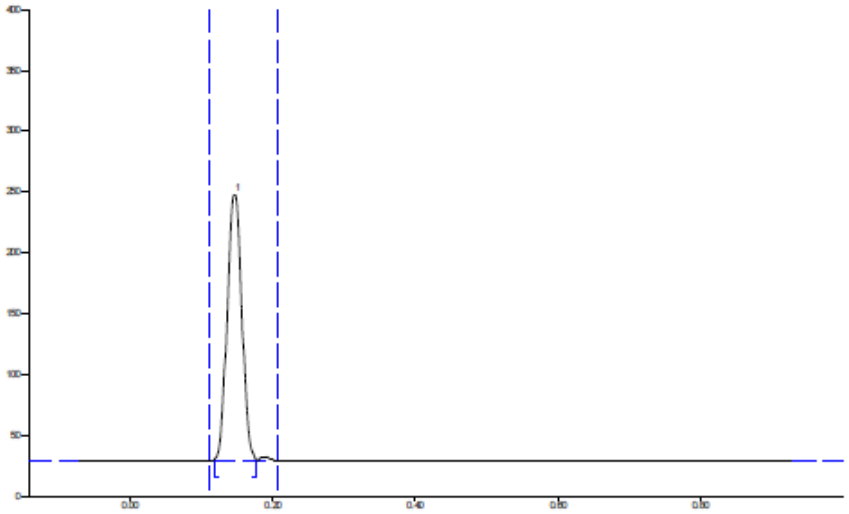

Fig III. Densitogram of Standard Cilostazol (Rf: 0.15±0.01) measured at 254nm

CONCLUSION

The developed HPTLC technique was simple, specific, accurate, economical and validated based on ICH guidelines. Statistical analysis proves that the method is reproducible and selective for the analysis of Cilostazol as bulk drug and in pharmaceutical dosage forms. The method can be used to determine the purity of the drug available from various sources.

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