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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR CILOSTAZOL IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise rapid and economical reverse phase high performance liquid chromatographic method has been developed for the estimation of Rasagiline in tablet dosage from, using mobile phase containing Acetonitrile and Water in volume ratio (60 : 40 v/v) at a flow rate of 1.0 ml/min. An ODS C_{18} RP column (150 x 4.5 mm, 5 µ) was used as stationary phase. The linearity of Rasagiline was in the range of 5 – 25 µg/ml, shows a regression coefficient of 0.9997, quantification was done using UV detector at 265 nm. The retention time of the drug was found to be 4.61 min. The limit of detection and limit of quantification was 10.72 µg/ml and 32.49 µg/ml respectively. The percentage assay of Cilostazol in PLETOZ and ST1LOZ were 106.54 ± 0.663 % and 103.27 ± 0.5965 %. This proposed method is precise, accurate and rapid for determination of Cilostazol.

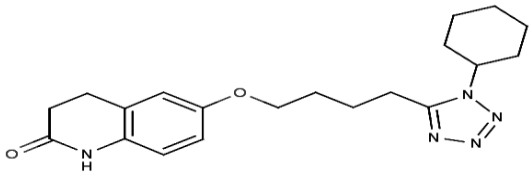
Keywords: RP-HPLC, Cilostazol

INTRODUCATION

Cilostazol is chemically known as 6 - [4 - (1-cyclohexyl -1H – tetrazol – 5 - yl) - butoxy] - 3,4dihydro – 2(1H) – quinolinone [1]. It is an antithrombic, peripheral vasodilator and used in the treatment of intermittent claudication as it is a phosphodiestrase III inhibitor [2]. Literature survey reveals HPLC and LC-MS methods in human plasma [3, 4] and a HPLC method in tablet dosage form [5].

In the present investigation a RP-HPLC method was modified to give a more economic, rapid and precise method for the estimation of Cilostazol in tablet dosage form.

Structure



MATERIALS AND METHODS

Materials

Cilostazol was a gift sample from Glenmark Pharmaceuticals, Mumbai, Different brands of Cilostazol tablets used were Pletoz (Cipla) and Stiloz (Glenmark), Methanol, Acetonitrile and water were of HPLC grade from Qualigens fine chemicals, Mumbai.

Instruments

Shimadzu AX -220 balance, Microprocessor based pH tester from Eutech and Qakton instruments, SPD – 10 Avp / 10 Avp shimadzu UV-visible detector, LC - 10 ATvp Shimadzu solvent deliver module, a ODS C_{18} RP column (150 X 4.5 mm, 5µ), 50 µl ASGE glass syringe and Ultra sonicator mod 2200 MH were used.

Preparation of Mobile Phase

The 10 mM phosphate buffer was prepared [6] and pH adjusted to 6 and filtered through cellulose acetate filter paper using vacuum filtration and mixed with acetonitrile and methanol in the volume ratio of 30:30:40 v/v and sonicated for 15 mins to degas the mobile phase.

Preparation of Standard Stock Solution

Weighed accurately 50 mg of Cilostazol and transferred into a 50 ml standard flask and dissolved with minimum quantity of methanol and the volume made up to the mark with methanol to get the concentration of 1000 µg/ml.

Preparation of Working Standard Solution

From the standard stock solution, 5 ml was transferred into a 50 ml standard flask and made up to the mark to produce 100 µg/ml with Mobile phase.

Calibration Graph and Linearity

To series of six 10 ml standard flasks added 1, 2, 4, 6, 8 and 10 ml of above solution and made up to marks with mobile phase to get the concentration of 10 - 100 µg/ml and the calibration graph was plotted between concentration and peak area.

Quantification of Formulations

Twenty tablets **(Pletoz and Stiloz)** containing 100 mg of Cilostazol was accurately weighed and powdered. The powdered tablet equivalent to 50 mg of Cilostazol was transferred into a 50 ml beaker, added 10 ml of methanol and shaken vigorously for few minutes and repeated the extraction consequently for four times

(4 x 10 ml) sonicated for 5 minutes and made up to the mark with methanol. Pipetted out 6 ml of solution into a 50 ml standard flask and the volume was made up with the mobile phase to produce 120 µg/ml and half of this solution was filtered using Whatman filter paper No 41. From the above clear solution 1 ml was pipetted and added to 10 ml standard flasks and made to produce 12 µg/ml solutions using mobile phase. The peak area measurements were done by injecting the sample containing 12 µg/ml solution under the optimum chromatographic conditions and the amount of Cilosatzol was calculated from the respective calibration curve.

Recovery Studies

From each of the preanalyzed formulation, known quantities of formulations before filtration

were taken (60 µg/ml) and raw material solution added in the increasing amount (50, 100, 150, 200, 250 and 300 µg/ml) to 10 ml standard flasks. The contents were mixed well and made up to the mark and filtered. The peaks area measurements were done by injecting the samples under the optimum chromatographic conditions and the % recovery was calculated.

Validation [7]

Precision

To study reproducibility of the method precision was carried. Exercise was initiated with running six replicates of test solution of analyte. Mean response was calculated followed by calculation of relative standard deviation. For determination of method precision, six different solutions of sample were prepared and injected into the chromatographic system.

Accuracy

To study the closeness of the results accuracy was carried. The six replicate recovery studies were performed by adding increasing concentrations of raw material solution to a fixed concentration of formulation solution, mixed well, volume made and reanalyzed.

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

Preparation of calibration curve from the several dilutions of standard was repeated for six times. The LOD and LOQ were calculated by using the average values of slope and standard deviation of intercept values and were found to be 10.7243 µg/ml and 32.4981 µg/ml.

System Suitability Studies [8]

Weighed accurately 50 mg of Cilostazol in 50 ml methanol, transferred 5 ml of the solution into a 50 ml standard flask and made the volume with mobile phase and pipetted out 1 ml of the solution into a 10 ml standard flask and volume was made with the mobile phase and the solution was injected under optimum chromatographic conditions. The chromatogram obtained was tested for its acceptance using parameters like column efficiency, tailing factor, asymmetric factor and capacity factor.

Statistical Validation

The obtained results were treated for statistical validation parameters like Standard Deviation (SD), Percentage Relative Standard Deviation (% RSD) and Confidence Interval (CI).

RESULTS AND DISCUSSION

Even though a varied number of RP-HPLC method has been reported a sincere attempt have been made to identify a simple, cost effective, rapid, reliable, specific an accurate method for the estimation of Cilostazol in tablet dosage form.

The typical chromatogram of Cilostazol was shown in Fig 1, it was found that the retention time for Cilostazol was 4.61 min in the present developed RP-HPLC method, the sample preparation required less time and no tedious extraction were involved.

As per USP-XXIV system suitability tests were carried out on freshly prepared solution of Cilostazol and the parameters found to be acceptable.

A good linear relationship (r = 0.9997) was observed between the concentration range 10 -100 µg/ml. The assay of Cilostazol in tablets Pletoz and Stiloz were found to be 106.54 ± 0.6663 % to and 103.2 ± 0.5965 % indicate that there is no interference of recipients present in the formulation. From the recovery studies it was found that about 102.08 % and 103.21 % for Pletoz and Stiloz were recovered which indicated the high accuracy of the method.

The % RSD was found to be 0.6254 and 0.5514 for Pletoz and Stiloz respectively and was satisfactorily low which indicates that the excipients in the formulation did not interfere in accurate estimation of Cilostazol in tablet dosage form.

This demonstrates that the developed RP-HPLC method is simple, linear, rapid, precise and economic compared to other reported methods. Thus, the developed method can be easily used for routine quality control purpose.

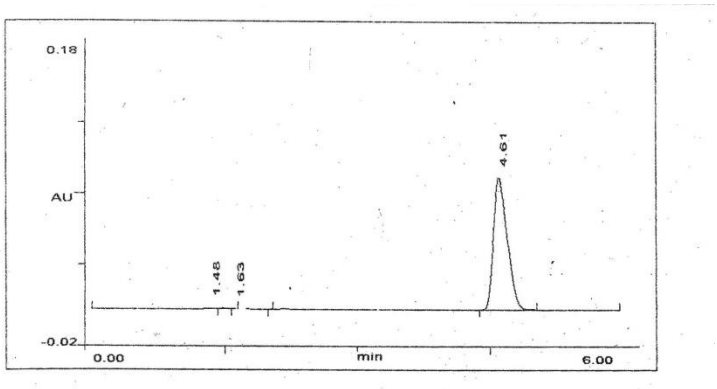
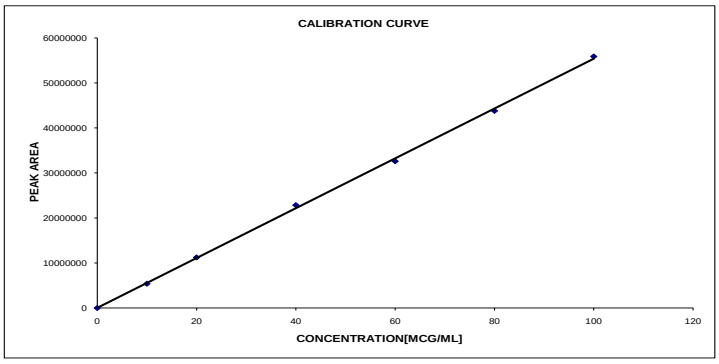


Fig 1Typical Chromatogram of Cilostazol Sample Solution



Graph A Calibration Curve

Table I: Optical Characteristics of Cilostazol

Parameters	Results
$\lambda_{max\ (nm)}$	258
Beers law limit (µg/ml)	10 – 100
Sandell's sensitivity (µg/cm^2/0.001 A.U)	1.80669 E-09
Molar extinction coefficient (l mol^{-1} cm$^{-1)}$	2.16054 E+11
Correlation coefficient (r)	0.09997
Regression equation (y = mx + c)	Y = 553496.92X + 21820.114
Slope (m)	553496.92
Intercept (c)	2182.114
LOD (µg/ml)	10.7243766
LOQ (µg/ml)	32.4981109
Standard error of mean of Regression line	53308.8454

Table II: Quantification Studies

Label claim (mg)	Amount found in (mg) ± SD		%RSD		Confidence Interval	
	PLETOZ*	STILOZ*	PLETOZ*	STILOZ*	PLETOZ*	STILOZ*
100	106.564 ± 0.6663	103.27 ± 0.5695	0.6254	0.5514	0.6993	0.5977

* Average of 6 determinations

Table III: Recovery Studies – Pletoz

Amount of drug in sample solution (mg)	Amount of standard drug added (mg)	Total amount of recovered drug (mg)	% Recovery
6.4060	5	10.6433	
6.3623	10	16.4704	
6.4309	15	22.2768	102.08 %
6.4266	20	27.1190	
6.3340	25	30.9540	
6.4084	30	36.6964	

Table IV: Recovery Studies – Stiloz

Amount of drug in sample solution (mg)	Amount of standard drug added (mg)	Total amount of recovered drug (mg)	% Recovery
6.1872	5	10.9549	
6.1702	10	16.6159	
6.1569	15	22.0040	103.21 %
6.2296	20	24.9295	
6.2453	25	30.0934	
6.1875	30	38.4682	

Table V: System Suitability Parameter and Chromatographic Conditions

Parameters	Values
Retention time	4.61 min
Flow rate	0.8 ml/min
Theoretical plates/m	3981
Tailing factor	1.35
Asymmetry factor	1.04
HETP	$2.65X10^{-2}$ mm
LOD(µg/ml)	10.72
LOQ(µg/ml)	32.49

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REFERENCES

[1]. Budavari, s., Eds., In; **The Merck Index**, Merck and co., Inc., white house station, NJ, 14, 2006, 2277-2278.
[2]. Rang HP, Dale MM, Ritter JM and Moore PK. **Pharmaology**, Elsevier science, UK, 5, 2003, 295.
[3]. Bramer S.L., et. al., Methods for the Quantative Analysis of Cilostazol and its metabolites in Human Plasma, **Journal of Biomedical Analysis**, 26(4), 2001, 637-50.
[4]. Tata P.N., et. al., Determination of Cilostazol and its metabolites in Human Urine by HPLC, **Journal of Biomedical Analysis**, 24(3), 2001, 381-9.
[5]. Basniual P.K., et. al., Hydrolytic Degradation Profile and RP-HPLC Estimation of Cilostazol in Tablet Dosage Form, **Indian Journal of Pharmaceutical science**, 24, 2008, 222-224.
[6]. **The Indian pharmacopoeia**, The Controller of Publication New Delhi, 2, A-144 - A-145.
[7]. Code Q2B, Validation of Analytical Procedures, Methodology. **ICH Harmonized Tripartite Guidelines**. Geneva, Switerland, 6, 1996, 1-8.
[8]. The United states Pharmacopoeia/ National Formulary (USP 24/MF 19), United Stated Pharmacopoeial convention Inc., Rockville, USA, Asian Edn., 2002, 1225.