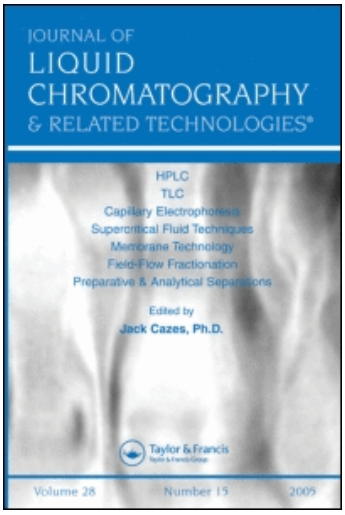

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HPLC Determination of Cilostazol in Tablets, and Its Validation

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ABSTRACT

A simple, rapid, and validated HPLC method has been developed for determination of cilostazol in tablets. A LiChrospher 100 RP-18 column was used with a mobile phase consisting of methanol–acetonitrile–water (20 : 50 : 30). Quantitative evaluation was performed at 260 nm. The HPLC method is selective, precise, and accurate, and it can be used for routine analysis of the preparations in pharmaceutical industry quality control laboratories.

Key Words: Cilostazol; HPLC; Tablets; Validation.

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INTRODUCTION

Cilostazol, 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone, is a quinolinone derivative that inhibited cellular phosphodiesterase III, and is used for inhibition of platelet aggregation and a vasodilator.[1] This drug is already marketed in Indonesia, presently.

No official method was available in the *Indonesian Pharmacopoeia*[2] and other Pharmacopoeias.[3–6] Determination of cilostazol and its metabolites in biological fluids, using HPLC methods, are reported.[7,8] Braimer et al.[9] described the quantitative analysis of cilostazol and its metabolites in human plasma using LC/MS/MS. An HPLC method for the determination of cilostazol as a bulk drug was described.[10]

The aim of this present work was to develop a simple, validated, and rapid HPLC method for routine analysis of cilostazol in tablet preparations using HPLC.

EXPERIMENTAL

Materials and Reagents

Cilostazol (Dipharma SpA, Milan, Italy, Lot 1/100-1-1-Cil, assay: 99.8%) was pharmaceutical grade. The substance was used as received for preparing laboratory-made preparations and standard solutions.

Acetonitrile, methanol, sodium hydroxide, and hydrochloric acid (E. Merck, Germany) were analytical grade reagents. Hydrogen peroxide was technical grade reagent (PT BrataChem, Surabaya, Indonesia). The solvent and reagents were used without further purification. Excipients for laboratory-made tablet preparations (lactose, corn starch, magnesium stearate, Vivapur™, Vivastar™, Aerosil™) were pharmaceutical grade substances.

Laboratory-made tablet preparations were prepared containing five different concentrations of cilostazol (80.0, 90.0, 100.0, 110.0, and 120.0 mg tablet^{-1}); these were used for accuracy determination.

Commercial tablets containing cilostazol 100 mg tablet^{-1} was purchased in March 2004 from a local pharmacy in Surabaya. The commercial pharmaceutical preparation was produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed cilostazol (20.0, 25.0, and 30.0 mg) in mobile phase (10.0 mL). For linearity study, various standard solutions were prepared from these three stock solutions by dilution with the mobile phase (70.0, 140, 210, 280, 350, 420, 490, 560, 630, and 700 μg mL^{-1}), and each of these solutions (20 μL) was injected into the HPLC. The standard solution was stable for at least

24 hr at room temperature (100.5% ± 1.3%, $n = 3$, at 24°C ± 2°C, room humidity 50% ± 10%).

Sample Extraction

Twenty tablets were each weighed, and their mean was determined. After homogenizing the powder, an equivalent weight of a 1/8 tablet (equivalent to 12.5 mg cilostazol) was transferred into a 25.0-mL-volumetric flask containing about 20 mL of the mobile phase, ultrasonicated for 15 min and diluted to 25.0 mL with the mobile phase. The solution was filtered through 0.45 μm Durapor™ membrane filters (Millipore, Ireland) before injecting into the HPLC apparatus (20 μL).

Chromatography

The HPLC systems used in this work comprised a Shimadzu HPLC-2010C system (Kyoto, Japan) equipped with auto-injector, and Chromatography Data System Shimadzu Class-VP version 6.1. A Shimadzu LC-10 AS pump, a Shimadzu UV-Vis SPD-10A detector, a Shimadzu Chromatopack CR-7A integrator, and a 20 μL Rheodyne 7125 injector was used for intra-laboratory precision studies. An Agilent 1100 HPLC Series (Waldbronn, Germany) equipped with a photo diode array detector (DAD), auto-sampler, and Agilent Chem Station Plus for LC 2002 was used for inter-laboratory precision study. A Hitachi L-6200 (Tokyo, Japan) intelligent pump equipped with a Hitachi LC organizer and dynamic mixer mode 655A, a Hitachi L-4500 DAD detector, a 20 μL Rheodyne 7125 injector, was only for selectivity studies. The analysis was carried out on a LiChrospher 100 RP-18 (5 μm; 250 mm; Cat. 1.50983, E. Merck, Darmstadt, Germany) with flow rate of 1 mL min^{-1}.

A mobile phase, a mixture of methanol–acetonitrile–water (20 : 50 : 30, v/v/v), modified from published work,[10] was used. The mobile phase was prepared daily, and filtered through 0.45 μm Ultipor N™ (Pall, Washington, USA) filters and ultrasonicated for 30 min before use.

To confirm the purity and identity of the analyte peak, the eluent was monitored using a DAD detector in the range of 210–400 nm; all qualitative data evaluation (identification, purity check) were performed by using a Hitachi model D-6500 chromatography data station software, DAD system manager. Routine quantitation was performed at its λ_{max} (260 nm) via peak areas with linear regression, using at least three-point external calibration. The quantitative calculations were performed using software Chromatography

Data System Shimadzu Class-VP version 6.1, or Shimadzu Chromatopack CR-7A integrator, or Agilent Chem Station Plus for LC 2002.

Validation

The method was validated for linearity, homogeneity, detection limit (DL), accuracy, robustness, and range by the modified method of Funk et al.,[11] USP 26,[3] and Kromidas.[12] The selectivity of the method was proven by identification and purity checks of the analyte peaks. In order to assure the selectiveness of the method, forced degradation studies using HCl, NaOH, and H_2O_2 were performed on 500 mg powdered laboratory-made tablets (equivalent to 2.5 tablets). Five-point accuracy studies (80–120% of the expected value) were performed on the laboratory-made tablet preparations. A standard addition method (30% of the label claim) accuracy study was performed on the commercial tablets. The precision (repeatability, intra- and inter-laboratories studies) was evaluated by analyzing six different extract aliquots from laboratory-made tablets containing 80.0, 100.0, and 120.0 mg cilostazol tablet^{-1} on different days by different analysts, and HPLC equipment in the two different laboratories.

RESULTS AND DISCUSSION

Extracts of the excipients of the laboratory-made tablets showed no peak. All HPLC chromatograms of the extracts of laboratory-made tablets showed a single peak of cilostazol. To confirm the identity and purity of the analyte peaks, a DAD detector was used. Figure 1 showed the typical HPLC chromatogram of cilostazol, its UV spectrum, and its contour plot. The λ_{max} of cilostazol (260 nm) was then selected for further work, and quantitative evaluation. All the UV-spectra of the analyte peaks showed good correlation to standard peaks ($r > 0.99$). Purity checks of the analyte peaks showed that all the peaks were pure ($r > 0.99$). This showed the proposed HPLC method is sufficiently selective. The relative standard deviation (RSD) of its retention time (R_t) data from this work (except of the robustness and inter-laboratory studies) were 0.10–1.30% ($n = 86$). The asymmetry factor (AF) (at 10% peak height) yielded relatively good values (0.99–1.35; $n = 126$).

Using this HPLC system, linearity of cilostazol was achieved in the range of 70.0–700 μg mL^{-1} (linear regression line equation: $Y = 223066 + 46925X$, $n = 10$, relative process standard deviation (SD) V_{XO}[11] = 1.57%, $r = 0.9996$, injection volume 20 μL). The calculated testing-value X_p (for $p = 0.05$),[11] and the RSD of response factor (RF)

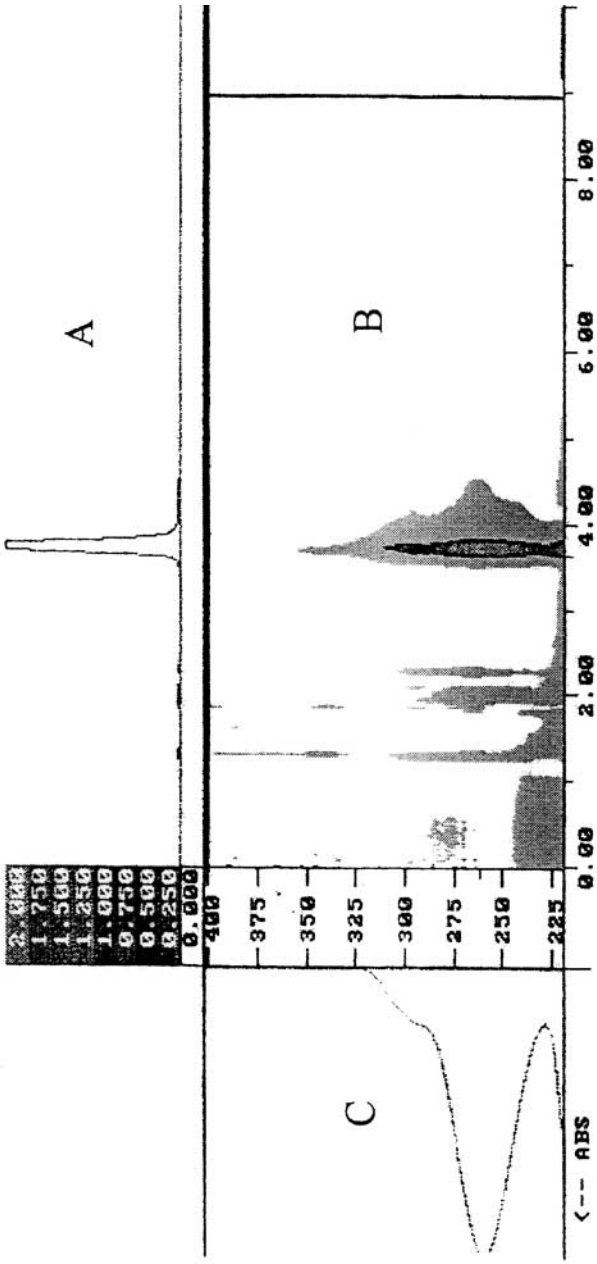

Figure 1. Typical HPLC chromatogram of cilostazol at 260 nm (A), its UV spectrum (B), and its contour plot (C). Mobile phase: a mixture of methanol–acetonitrile–water (20 : 50 : 30, v/v/v), flow rate of 1 mL min^{-1}, injection volume 20 μL, cilostazol 500 μg mL^{-1}; column: LiChrospher 100 RP-18 (5 μm; Cat. 1.50983, E. Merck, Germany); detector: DAD Hitachi L-4500.

were satisfactory (26.9 $\mu g\,mL^{-1}$ and 0.27%, respectively). The ANOVA regression-test for linearity testing of the regression line showed calculated F-value (11114.7, for $p < 0.0001$), and standard error of the intercept (S_a), and slope (S_b) were 193325 ($p = 0.282$) and 445 ($p < 0.0001$). The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graph (data not shown). The residuals were distributed at random around the regression line, neither trend nor uni-directional tendency was found. This basic linear calibration curve showed variance homogeneity over the whole range. The calculated parameter PW[11] was 3.53; this PW-value was less than the F_{table}-value (5.35; for $f_1 = 9$, $f_2 = 9$; $p = 0.01$). If the range of the analyte was increased from 70 to 800, and 1000 $\mu g\,mL^{-1}$, the calibration curve would not be linear anymore ($r = 0.972$, $V_{XO}{}^{[11]} = 14.1\%$, $n = 10$), and it would yield a quadratic or cubic curve (data not shown).

Although, the validation parameters DL and quantitation limit (QL) were not required for the assay of active ingredient(s) in tablets,[3] those parameters were also determined in this present work. These parameters may be used for other purposes (e.g., for in vitro bio-equivalence-, stability-studies, etc.). DL was determined by making a linear regression of relatively low concentration of cilostazol (2.00–63.0 $\mu g\,mL^{-1}$, injection volume 20 μL) according to the method of Funk et al.[11] The calculated equation of the regression line was $Y = 7041 + 48224X$ ($n = 10$; $V_{XO} = 1.34\%$; $r = 0.9998$; RSD of RF = 1.8%). ANOVA calculated F-value = 21750 ($p < 0.001$), $S_a = 12221$ ($p = 0.58$), $S_b = 326$ ($p < 0.001$). The calculated testing-value $X_p{}^{[11]}$ (for $p = 0.05$) was 1.83 $\mu g\,mL^{-1}$. In this case, the value of DL = X_p.[11] According to Carr and Wahlich,[13] the value of the QL could be estimated as three times of the DL-value (5.48 $\mu g\,mL^{-1}$ for injection volume 20 μL).

Table 1 demonstrates the high accuracy, as revealed by the percentage of mean recovery data (99.9% $\pm$ 1.3%, $n = 10$). To prove whether systemic errors did not occur, linear regression of recovery curve of X_f (concentration of the analyte measured by the proposed method) against X_c (nominal concentration of the analyte) of the laboratory-made tablets was constructed.[11] The confidence interval data ($p = 0.05$) of the intercept [$V_{b(a_r)}$] and slope [$V_{b(b_r)}$] from the recovery curve did not reveal the occurrence of constant- and proportional-systematic errors. The standard addition method on the commercial tablets also showed relatively good recovery results (99.5% $\pm$ 1.9%, $n = 3$).

All the RSD values of the repeatability, intermediate precision, and inter-laboratory study evaluations were less than 2% (see Table 2), and the calculation by using David-, Dixon-, and Neumann's-Test[12] showed satisfactory results (data not shown). All the SD data (not shown) of the precision studies yielded values below the permitted maximum SD as reported by

Table 1. Results from determination of accuracy using laboratory-made tablets.

Nominal concentration of cilostazol (X_c) ($\mu g\,mL^{-1}$)	Measured values (X_f) ($\mu g\,mL^{-1}$)
400	401
400	399
450	449
450	449
500	511
500	503
550	541
550	536
600	602
600	603
Mean recovery $\pm$ SD (%)	99.9 $\pm$ 1.3
Line equation of the recovery curve	$X_f = 4.89 + 0.989X_c$
$V_{b(a_f)}$[a]	4.89 $\pm$ 37.5
$V_{b(b_f)}$[a]	0.989 $\pm$ 0.07

[a]For $p = 0.05$.

Ermer[14] (2.43 for specification range 95–105%, basic lower limit 99%, $n = 6$). The measurements were performed within two laboratories on different days by different analysts, and by using different HPLC equipment. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range of 80–120% of the expected value.

Table 2. Results from evaluation of precision of laboratory-made tablets.

	RSD value (%, $n = 6$)		
Measurement	Cilostazol (80.0 mg tablet^{-1})	Cilostazol (100.0 mg tablet^{-1})	Cilostazol (120.0 mg tablet^{-1})
1[a]	0.6	1.2	0.3
2[a]	0.7	0.9	0.6
3[a]	1.4	0.4	1.7
4[b]	Nd[c]	1.8	Nd[c]

[a]Each measurement was performed by a different analyst on the different days, and equipment within one laboratory.
[b]Measurement was performed in the different laboratory.
[c]Not determined.

Table 3. Robustness evaluation of the HPLC method of cilostazol.

Mobile phase (methanol–acetonitrile–water)	R_t[a] (min)	R_f[a,b]	AF[a,c]
19 : 50 : 31	3.69	2070×10^{-8}	1.16
21 : 50 : 29	3.50	2069×10^{-8}	1.31
21 : 49 : 30	3.63	2070×10^{-8}	1.35
19 : 51 : 30	3.61	2074×10^{-8}	1.36
20 : 49 : 31	3.74	2075×10^{-8}	1.24
20 : 51 : 29	3.56	2072×10^{-8}	1.20
20 : 50 : 30[d]	3.63	2093×10^{-8}	1.09
Mean ± SD	3.55 ± 0.25	$(2074 \pm 8.4) \times 10^{-8}$	1.24 ± 0.11
Flow of mobile phase[d] ($mL\,min^{-1}$)			
0.9	4.05	2796×10^{-8}	1.11
1.0[d]	3.63	2089×10^{-8}	1.09
1.1	3.30	2264×10^{-8}	1.17
Mean ± SD	3.66 ± 0.37	$(2383 \pm 212) \times 10^{-8}$	1.12 ± 0.4

[a]The presented values were mean of four replications ($n = 4$).
[b]Response factor.
[c]AF at 10% of peak height.
[d]The mobile phase composition and flow rate that used in this present work.

In order to evaluate the robustness of the proposed method, the influence of small variations of mobile phase composition and flow rate on the values of R_t, response factor, and AF were evaluated. Table 3 indicates that small variations result in no changes of parameters.

Table 4 showed that, although the recovery of the cilostazol was reduced (86–90%) in stressed samples, purity and identity checks of the analyte peaks using DAD detector yielded good values (>0.99); this showed that all the

Table 4. Results of forced degradation studies of laboratory-made tablets.

Storage condition	Time	%Recovery of cilostazol[a] (mean ± SD, $n = 3$)
0.5 mL NaOH 1N	17 hr at 80°C	96.9 ± 0.5
0.5 mL NaOH 2N	17 hr at 80°C	86.6 ± 1.5
0.5 mL HCl 2N	17 hr at 80°C	90.5 ± 1.7
0.5 mL H_2O_2 15%	17 hr at 80°C	100.1 ± 2.0

[a]Purity and identity checks of the analyte peaks yielded good values ($r > 0.99$).

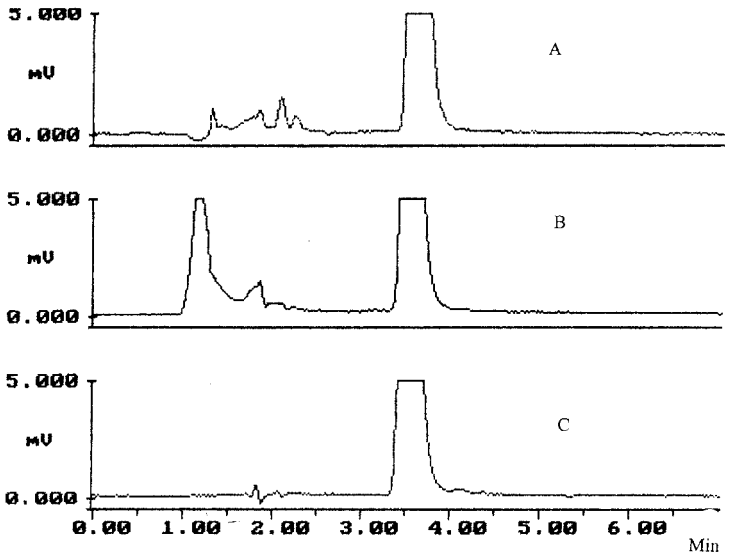

Figure 2. HPLC chromatogram (at 260 nm) of stressed samples, by using HCL 1N (A), NaOH 2N (B), H_2O_2 (C). For detailed information see text and Table 4. The degradation product(s) have R_t between 1.0 and 2.5 min.

peaks were still pure and identical with the standard. This proved that there was no interference in the analyte peaks by the degradation products (see Fig. 2).

The present work showed that the proposed HPLC method is suitable for the routine analysis of products of similar composition in the pharmaceutical industry quality control laboratories.

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