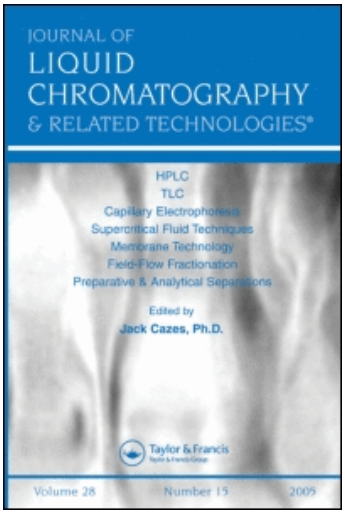

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Densitometric Determination of Tadalafil Citrate in Tablets: Validation of the Method

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Densitometric Determination of Tadalafil Citrate in Tablets: Validation of the Method

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Abstract: A simple and rapid densitometric method has been developed for determination of tadalafil citrate in tablets and its dissolution media. After extracting the samples with acetone, the solutions were spotted onto pre-coated silica gel TLC plates which were eluted with a mixture of *n*-hexane-ethyl acetate-methanol (8.0:6.0:2.0, v/v). quantitative evaluation was performed by measuring the absorbance reflectance of the tadalafil citrate spots at $\lambda = 285$ nm. The TLC-densitometric method is cheap, selective, precise, and accurate and can be used for routine analysis of tablets in pharmaceutical industry quality control laboratories.

Keywords: Tadalafil citrate, Densitometry, Dissolution, Tablet, TLC, Validation

INTRODUCTION

Tadalafil, which is chemically known as (6R,12aR)-2,3,6,7,12a-Hexahydro-2-methyl-6-[3,4-(methylenedioxy)phenyl]pyrazino-[1′,2′:1,6] pyrido[3,4-b] ind- ole-1,4 dione, is a phosphodiesterase type-5 inhibitor with actions and

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uses similar to sildenafil. Both drugs are used in management of erectile dysfunction.[1] Tadalafil and its citrate salt is already marketed in Indonesia.

No official method for assay of tadalafil (citrate) is described in the Indonesian Pharmacopoeia,[2] USP 28,[3] and BP 20.[4] The determination of tadalafil in plasma using HPLC-UV and LC-MS was reported by Cheng and Chau[5] and Ramakrishna et al.[6] The analysis of tadalafil as a synthetic adulterant in herbal medicine using LC-MS was also reported.[7–10] Ali and Aboul-Enein reported a validated method for analysis tadalafil in pharmaceutical preparations using capillary electrophoresis,[11] and HPLC.[12] No report on the determination of tadalafil by TLC/HPTLC is available in the CBS TLC data base 2005 from Camag.[13] To the best of our knowledge, no publication is available at the present time which describes the quantitative determination and validation of tadalafil citrate in tablets by TLC.

The objective of the present work is to develop a cheap, rapid, and simple validated TLC densitometry method for determining tadalafil citrate in tablets for use in pharmaceutical quality control laboratories.

EXPERIMENTAL

Materials and Reagents

Tadalafil citrate (Siris Impex, Brindavan Colony, Vijayawada, India; Batch 040904;Assay 99.81%, Manufacturing date: September 2004; Expiration date: October 2008) was pharmaceutical grade substance. The substance was used as received for preparing laboratory-made tablets, and standard solutions.

Acetone, n-hexane, ethyl acetate, methanol (J. T. Baker, Philipsburg, NJ, USA), $Na_2HPO_4 \cdot H_2O$, and H_3PO_4 (E. Merck, Darmstadt, Germany) were analytical grade reagents; the solvents and reagents were used without further purification. Excipients for laboratory-made (LM) tablets (lactose, magnesium stearate, polysorbate 80, talc, Pharmacoat 606®, titanium dioxide, polyethylene glycol 4000, and simethicone) were pharmaceutical grade substances.

For performing accuracy and precision studies, LM tablets containing five different concentration levels of tadalafil citrate (80, 90, 100, 110, and 120% of label claim) were prepared. The label claims were 16 mg (LM-1) and 32 mg (LM-2) tadalafil citrate $tablet^{-1}$, respectively.

Commercial tablets (CT) containing tadalafil (10 mg $tablet^{-1}$; Batch: 065336) were purchased at a local Pharmacy at Jakarta in November 2005. The commercial tablets were produced in England.

Dissolution medium comprised a solution mixture of phosphate buffer, pH 6.8, with the addition of polysorbate 80 (3%).

Stock standard solutions were prepared daily by dissolving accurately weighed tadalafil citrate (20.0, 25.0, and 35.0 mg) in 50.0 mL acetone.

Various standard solutions were prepared from the stock solution by dilution with acetone. For tablet assay linearity studies, the solutions were prepared containing 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 μg mL^{-1}; for dissolution studies, the concentrations were 25.0, 40.0, 50.0, 80.0, 100, 150, 200, 250, and 300 μg mL^{-1}, and 2.0 μL of these solutions was spotted onto the TLC plate. The standard solutions were stable at least for 12 hours at room temperature (99.43 ± 0.78%, n = 3, at 24 ± 2°C, room humidity 50 ± 10 %).

Sample Preparation

Assay of Tablets

Twenty tablets were each weighed, and their mean was determined. After homogenizing the powder, an equivalent weight of 0.25 or 0.125 tablets (equivalent to 4.0 mg tadalafil citrate) was transferred into a 25.0 mL volumetric flask containing about 20 mL of acetone, ultrasonicated for 15 min, mixed with a vortex-mixer for 5 min, and diluted to 25.0 mL with acetone. The solution was filtered through 0.45 μm Durapore®, membrane filters (Millipore, Ireland) before spotting onto TLC plates (5.0 μL), together with the standard.

For performing the content uniformity testing, tablets were each weighed, powdered, and homogenized individually, and were processed as described above. Content of the uniformity test of the LM tablets was performed using 10 tablets.[2]

Assay of Dissolution Media

Dissolution studies were performed using paddle-method (75 rpm; 37 ± 2°C), using 900 mL of the dissolution-medium. Six dissolution tubes were used for each series of dissolution studies. After 45 min, aliquots of the dissolution medium were filtered through 0.45 μm Durapor® membrane filters (Millipore, Ireland) and spotted onto the TLC plates (2 × 5.0 μL). The targeted concentration of [Q + 5][2] was 85% in 45 min (ca. 15.1 μg mL^{-1}, for LM-1 tablets).

Chromatography

Chromatography was performed on pre-coated silica gel F254 aluminum-back sheets (E. Merck. #1.05554; all the pre-coated plates were cut to 10 × 20 cm before used). The plates were used as obtained from the manufacturer without any pretreatment; a Nanomat III (Camag, Muttenz, Switzerland) equipped with a dispenser magazine containing 2.0, or 5.0 μL and glass capillaries

(Camag) was used for sample application (as spot with diameter *ca.* 1−2 mm). The mobile phase used in this experiment is *n*-hexane-ethyl acetate-methanol (8.0: 6.0: 2.0, v/v). The distance from the lower edge was 10 mm; distance from the side was 15 mm, and track distance was 10 mm. Ascending development was performed in a Camag twin-trough chamber (for 20 × 10 cm plates) after at least 1 h of saturation; the mobile phase migration distance in all experiments was 8.0 cm. (development time *ca.* 10 min at 24 ± 2°C). After being air dried for 30 min, the plates were scanned in the TLC scanner.

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning the absorbance-reflectance mode from 200 to 400 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at its λ maximum (285 nm) (See Figure 1). The densitometric scanning parameters were: bandwidth 10 nm, slit width 4, slit length 6 and scanning speed 4 mm s^{-1}. Calculations for identity, purity checks ($r_{S,M}$ and $r_{M,E}$ where S = start, M = center, E = end spectrum), *sdv* (relative standard deviation) of the linear/calibration curve, and quantification of the analyte

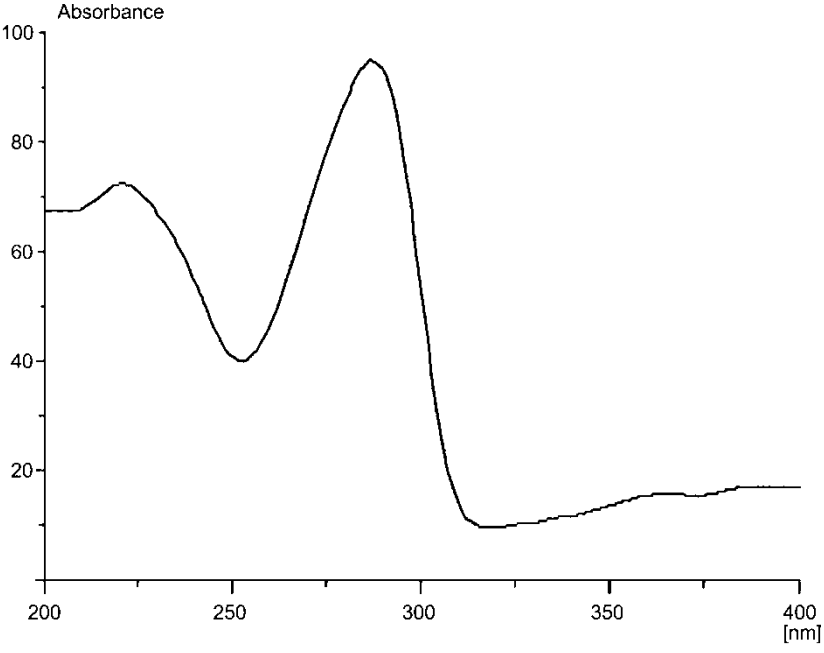

Figure 1. *In situ* absorbance-reflectance UV-spectrum of tadalafil citrate spot. (from 200 to 400 nm; maximum absorption wavelengths at 285 nm). TLC conditions: stationary phase: pre-coated TLC plate silica gel 60 F_{254} (E. Merck); mobile phase: *n*-hexane-ethyl acetate-methanol (8.0 : 6.0 : 2.0, v/v).

spots were performed by CATS version 3.17 (1995) software (Camag). Routine quantitative evaluations were performed *via* peak areas with linear regression, using 4–5 points' external calibration on each plate (80 to 120% of the targeted value). Each of the extract aliquot samples was spotted at least in duplicate.

Validation

The method was validated for linearity, detection limit (DL), quantitation limit (QL), accuracy, and range by the modified published methods.[14] In order to assure the selectivity of the method, forced degradation studies using HCl, NaOH, and H_2O_2 were performed on *ca.* 100 mg powdered laboratory-made tablets (equivalent to 4.0 mg of tadalafil citrate) in an oven (50°C for 15 hours). The selectivity of the method was proved by identification and purity checks of the analyte spots. In the present work, five-points accuracy studies (80, 90, 100, 110, and 120% of the expected value) were performed for the LM Tablets. For the dissolution studies, a three-point accuracy study using a solution of tadalafil citrate in dissolution medium was evaluated (60, 90, and 120% of [Q + 5]% value of LM-1 tablets). For commercial preparations, an accuracy study was performed using a two-point standard addition method (30 and 40% of label claim). The precision (repeatability and intermediate precision) was evaluated by analyzing six different extract aliquots from the LM tablets (80, 100, and 120% of label claim) and, from the dissolution-medium, those containing 60, 90, and 120% of targeted value. Robustness evaluation was performed using full factorial design. In this case, the influence of small variations of the mobile phase composition on the % recovery of LM- tablets was evaluated. Design and analysis of effect of the robustness data were performed and calculated by using Unscramble 9.5™ (2006) software from CAMO (Bangalore, India).

RESULTS AND DISCUSSION

After the TLC-plate was eluted, the densitogram at 285 nm (Figure 2) showed a single spot of tadalafil citrate or tadalafil (R_f ca. 0.35). This TLC system demonstrated that all analyte spots of the laboratory-made tablets and commercial preparation, the furnished *in situ* UV spectra were identical with those of the standards ($r \geq 0.9999$). Purity check of the analyte spots, using CATS software, also showed that all analyte spots of the extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were ≥ 0.9999, demonstrating that the proposed TLC method is highly selective. Figure 2 also showed that tadalafil citrate and tadalafil yielded identical Rf values.

The peak area was observed to be linearity dependent on the amount of tadalafil citrate within the range of *ca.* 40 to 150% of the expected value

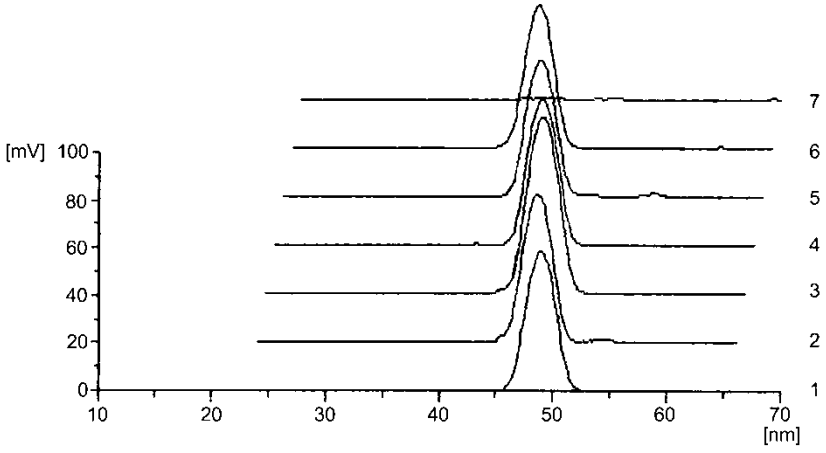

Figure 2. Densitograms measured at 285 nm, obtained from: (1) solution of standard tadalafil citrate, (2) extract of laboratory-made tablets, (3) extract of commercial tablets CT which contains tadalafil, (4) extract of stressed LM tablets using 1 N HCl, (5) extract of stressed LM tablets using 1 N NaOH, (6) extract of stressed LM tablets using H_2O_2, (7) extract of excipients of LM tablets. TLC conditions: see Figure 1.

(300 to 1,200 ng spot^{-1}), with linear regression line $Y = 398.4 + 1.18\ X$ (the relative process standard deviation value V_{XO}[14] was 3.09 %; $n = 10$; $sdv = 2.5$; $r = 0.9974$). The calculated value of test parameter X_p (for $p = 0.05$) and r were satisfactory (111 ng spot^{-1} and ≥ 0.99, respectively).[14,15] ANOVA regression-test for linearity testing of the regression line showed significant calculated F-value (1,532; $p < 0.0001$). The linearity of the basic calibration curve was also proved by the Mandel's fitting test.[14] 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 lines; neither trend nor uni-directional tendency was found. The basic linear calibration curve showed variance homogeneity over the whole range. The calculated test values PW[14] was 5.76, The PW values less than the F_{table}-value (6.99 for $f_1 = 8$, $f_2 = 8$; $p = 0.01$).

For the dissolution study, the calibration range should be at least $\pm 20\%$ of the targeted value, so the lower linear range should be made smaller (ca. 50.0 to 300 ng spot^{-1}), In this case, the relative process standard deviation value V_{XO}[14] was 2.28% (linear regression line equation was $Y = 5.02 + 2.604\ X$ ($n = 9$; $sdv = 2.9$; $r = 0.9996$). The calculated value of test parameter X_p (for $p = 0.05$) and r were satisfactory (25.2 ng spot^{-1} and ≥ 0.99, respectively).[14,15] ANOVA regression-test for linearity testing of the regression line showed significant calculated F-value (9,242.5; $p < 0.0001$). The calculated test values PW[14] was 3.76. The PW values less than the F_{table}-value (6.03; for $f_1 = 9$, $f_2 = 9$; $p = 0.01$).

All the linear regression calibration curve parameters used in this present work showed satisfactory results (data not shown). All values of the correlation coefficient r in this present work are >0.99; and the values of other parameters such as Xp (less than lower limit in the calibration range), sdv (<5), Vxo ($<5\%$), and p (<0.05) for ANOVA linear-test also showed satisfactory results.[15,16]

Although the validation parameters DL and QL were not required for the assay of active ingredient(s) in tablets, those parameters were also determined in this present work. These parameters may be used for other purposes (e.g., for bio-equivalence studies, limit test for adulterants detection, stability testing, etc.). DL was determined by making a linear regression of relatively low concentrations of tadalafil citrate (25.0 to 300 ng $spot^{-1}$) according to the method of Funk et al.[14] The calculated equation of the regression line was $Y = -51.74 + 5.46\ X$ ($n = 8$; $V_{XO} = 3.64\ \%$; $r = 0.9987$; $sdv = 3.5$; $F_{calculated}$-value $= 2466.4$ for $p < 0.0001$). The calculated value of test parameter X_p (for $p = 0.05$)[14] was 24.03 ng $spot^{-1}$. In this case, the value of $DL = X_p$.[14] According to Carr and Wahlich,[17] the value of the QL could be estimated 3 times of the DL-value (72.09 ng $spot^{-1}$).

Table 1 demonstrated good accuracy, as revealed by the percentage of mean recovery data of the assay of LM, CT tablets, and for dissolution media. An accuracy study of dissolution media was performed by analyzing three levels of solutions of the analyte in the dissolution-medium and calculating their recoveries. To prove that systematic errors did not occur, linear regression of recovery curve of X_f (concentration of the analyte measured by the propose method) against X_c (nominal concentration of the analyte) was constructed. The confidence interval data ($p = 0.05$) of the intercept $\{VB(a_f)\}$ and slope $\{VB(b_f)\}$ from the recovery curves did not reveal the occurrence of constant- and proportional-systematic errors.[14] Good mean recovery data, using standard addition method, were also observed for the commercial preparations. Content uniformity test which was performed with the LM tablets also yielded good results. The percent of recovery of LM-1 was $100.82 \pm 0.43\%$ ($n = 10$; Mean $\pm$ RSD), whilst for LM-2 was $101.48 \pm 1.38\%$ ($n = 10$; Mean $\pm$ RSD). The results fulfilled the requirement of content uniformity test of the Indonesian Pharmacopoeia.[2]

All the relative standard deviations (RSD) of the repeatability and intermediate precession evaluations have values less than 2% (see Table 2), and the calculation by using David-, Dixon-, and Neumann-Tests[18] showed satisfactory results (data not shown). All the standard deviations (SD) (data not shown) of the precision studies yielded values below the permitted maximum standard deviation as reported by Ermer (2.43 for specification range 95-105%, basic lower limit 99%, $n = 6$).[19] The measurements were performed in one laboratory by different analysts, on different plates and days, on the three different concentrations of the analytes in the laboratory-made tablets. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range of 80 to 120% of the expected concentration in LM tablets, and 60 to 120% of the targeted concentrations in the dissolution media.

Table 1. Results of the accuracy evaluation of Tadalafil citrate

Sample	Amount found[a] (Mean ± SD)[d]	Amount added[a]	%Recovery (Mean ± SD)	Recovery curve[b]	$V_{B(af)}$[c]	$V_{B(bf)}$[c]
LM-1	–	–	100.2 ± 1.22[e]	$X_f = 16.52 + 0.98\ X_c$	16.52 ± 50.67	0.98 ± 0.06
LM-2	–	–	100.51 ± 1.34[e]	$X_f = 3.19 + 1.00\ X_c$	3.19 ± 62.02	1.00 ± 0.07
Dissolution media	–	–	100.33 ± 0.75[f]	$X_f = -0.53 \pm 1.01\ X_c$	−0.53 ± 4.68	1.01 ± 0.06
CT	99.03 ± 0.72[d]	30	100.79 ± 1.63[d]	–	–	–
CT	99.03 ± 0.72[d]	40	101.04 ± 1.14[d]	–	–	–

[a]% of label claim.
[b]X_f and X_c are, respectively, the measured and nominal amount of the analyte spotted (ng spot^{-1}).
[c]For $p = 0.05$.
[d]$n = 3$.
[e]$n = 2 \times 5$ levels $= 10$.
[f]$n = 3 \times 3$ levels $= 9$.
– Not determined.

Table 2. Results from evaluation of precision of LM tablets and dissolution media

Measurement	LM-1 (80%)[a]	LM-1 (100%)[a]	LM-1 (120%)[a]	LM-2 (80%)[a]	LM-2 (100%)[a]	LM-2 (120%)[a]	Dissolution media (60%)[b]	Dissolution media (90%)[b]	Dissolution media (120%)[b]
RSD values (%, n = 6)									
1[c]	1.17	1.58	1.01	0.95	0.67	0.90	1.08	0.98	0.80
2[c]	0.81	0.55	0.86	1.23	1.24	0.58	Nd	Nd	Nd
3[c]	1.20	0.70	0.88	1.19	0.86	0.67	Nd	Nd	Nd

[a]% of label claim.
[b]% of targeted concentration (Q + 5).
[c]Each measurement was performed by a different analyst on the different days, and plates within one laboratory.
Nd: not determined.

Table 3. Results of forced degradation studies of laboratory-made tablets

Storage condition	Time	%Recovery of (Mean ± SD, n = 3)[a]
5 Drops of 1 N NaOH	15 hours at 50°C	100.19 ± 0.62
5 Drops of 1 N HCl	15 hours at 50°C	101.23 ±0.39
5 Drops of 15% H_2O_2	15 hours at 50°C	75.38 ± 0.88

[a]Purity and identity checks of tadalafil citrate spots using CATS software (Camag) yielded relatively good values ($r > 0.999$).

Table 3 shows that the recovery of tadalafil citrate was reduced only by 15% H_2O_2 stressed samples. It seemed that the analyte was relatively stable if incubated using 1 N HCl and 1 N NaOH. The purity and identity check of the analyte spots using CATS software yielded good values (>0.999); this showed that all the analyte spots were still pure and identical with the standard. This proved that the analyte peaks were not contaminated with the

Table 4. Effect of the mobile phase compositions on the Rf, TF and %recovery of LM tablets values

n-Hexane	Ethyl acetate	Methanol	Rf	TF	% Recovery[a]
LM-1 tablets					
7.5	5.5	1.5	0.23	1.0	101.21
8.5	5.5	1.5	0.20	1.0	100.39
7.5	6.5	1.5	0.28	1.0	101.11
8.5	6.5	1.5	0.21	1.0	100.98
7.5	5.5	2.5	0.31	1.0	102.34
8.5	5.5	2.5	0.32	1.0	100.22
7.5	6.5	2.5	0.44	1.0	100.75
8.5	6.5	2.5	0.32	1.0	100.70
8.0	6.0	2.0	0.31	1.0	99.67
LM-2 tablets					
7.5	5.5	1.5	0.22	1.1	100.74
8.5	5.5	1.5	0.20	1.0	100.73
7.5	6.5	1.5	0.26	1.1	101.34
8.5	6.5	1.5	0.21	1.0	101.99
7.5	5.5	2.5	0.39	1.0	100.94
8.5	5.5	2.5	0.39	1.0	100.62
7.5	6.5	2.5	0.40	1.0	100.45
8.5	6.5	2.5	0.40	1.0	101.54
8.0	6.0	2.0	0.34	1.0	101.52

[a]Mean value of duplicate determinations.

Table 5. Analysis of effect of the robustness data (HOIE method)[a,b]

Variable (Mobile phase)	p[c] value of Rf		p[c] value of TF		p[c] value of R[d]	
	LM-1	LM-2	LM-1	LM-2	LM-1	LM-2
n-Hexane	0.1135	0.1836	1.0000	0.1161	0.1636	0.3382
Ethyl acetate	0.1243	0.1836	1.0000	1.0000	0.7556	0.1531
Methanol	0.0095[e]	0.0001[e]	1.0000	0.1161	0.8697	0.3894

[a]Calculated from the data which presented on Table 4.
[b]Calculation was performed by using Unscrambler 9.5™ software (CAMO).
[c]probability value.
[d]%Recovery.
[e]Significant for $p = 0.05$.

degradation products (see Figure 2). It seems that the degradation product(s) were not detected clearly in the stressed samples measured at 285 nm. Therefore, the proposed TLC method is suitable for the routine analysis of products of similar composition in pharmaceutical industry quality control laboratories.

In order to evaluate the robustness of the proposed method, the influence of small variations of the mobile phase composition on percent recovery of the LM-1 and LM-2 tablets were evaluated (Table 4). Analysis of the effect of the data was performed by using Unscrambler 9.5™ software. Higher order interaction effect (HOIE) method showed that the percent recovery, also tailing factor (TF), were not affected by these small variations (Table 5). The Rf values were affected only by the concentration of the methanol. This data proved that the proposed method was robust.

The present work showed that the proposed TLC- densitometric method is suitable for the routine analysis of products of similar composition in the pharmaceutical industry quality control laboratories, especially for developing countries like Indonesia. Our experiences showed that the TLC methods are very low cost compared to the LC-MS method, and even with HPLC equipped with a DAD/UV detector. To the best of our knowledge, no Indonesian pharmaceutical companies have LC-MS in their QC/R&D laboratories at the present time. For developing countries in which the prices of HPLC grade solvents and columns are relatively very expensive, the availability of a alternative low cost method is important.

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