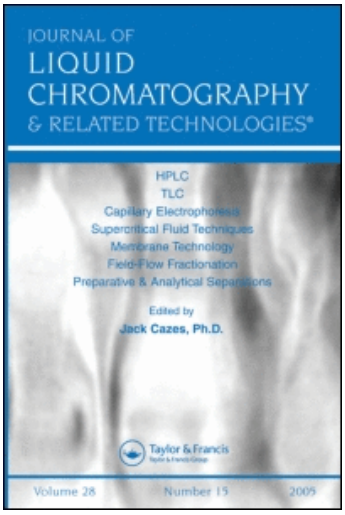


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# Densitometric Determination of Tranexamic Acid in Tablets: Validation of the Method

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# Densitometric Determination of Tranexamic Acid in Tablets: Validation of the Method

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**Abstract:** A simple and rapid densitometric method has been developed for determination of tranexamic acid in tablets and its dissolution media. After extracting the samples with a mixture of a 96% ethanol and water (1 : 1, v/v), the solutions were spotted on precoated silica gel TLC plates, which were eluted with a mixture of *n*-butanol–glacial acetic acid–water (8.0 : 2.0 : 2.0, v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the tranexamic acid spots at $\lambda = 488$ nm by using ninhydrin reagent. The TLC-densitometric method is selective, precise, and accurate, and can be used for routine analysis of tablets in the pharmaceutical industry quality control laboratories.

**Keywords:** Tranexamic acid, Densitometry, Dissolution, Tablet, TLC, Validation

## INTRODUCTION

Tranexamic acid, trans-4-(aminomethyl)cyclohexane carboxylic acid, is an antifibrinolytic drug which inhibits breakdown of fibrin clots by blocking the binding of plasminogen and plasmin to fibrin. The drug is used for haemorrhage, and prophylaxis of heredity angioedema.[1] Tranexamic acid is presently marketed in Indonesia.

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The official method for assay of tranexamic acid by using the titration method was described in British Pharmacopoeia 2003, European Pharmacopoeia 4th edition, Japanese Pharmacopoeia XIV, and Chinese Pharmacopoeia.[2–5] Lunn[6] described some high performance liquid chromatography (HPLC) methods for the determination of tranexamic acid in blood and tablets. Uehara et al.[7] reported the analysis of tranexamic acid using HPLC after reaction with 2,6-dinitro-4-trifluoromethyl-benzenesulphonate solution. The analysis of cis/trans isomer of tranexamic acid using TLC methods has been reported.[8] British Pharmacopoeia 2000[9] described a TLC method for analyzing iminodi-acid impurities in tranexamic acid tablets. To the best of our knowledge, no report is available which described the quantitative determination of tranexamic acid in tablets by using TLC and its validation.

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



## EXPERIMENTAL

### Materials and Reagents

Tranexamic acid (trans isomer; Hunan Dongting Pharmaceutical Co. Ltd., Deshan, Changde City, Hunan Province, China; Batch No. 0303012M; Assay 100.33%, Manufacturing date: March 2003; Expiration date: March 2008) was a pharmaceutical grade substance. The substance was used as received for preparing laboratory-made tablets, and standard solutions.

Glacial acetic acid, *n*-butanol, ninhydrin, 96% ethanol (E. Merck, Darmstadt, Germany); HCl (JT. Baker, Philipsburg, NJ, USA) were analytical grade reagents; the solvents and reagents were used without further purification. Excipients for laboratory made tablet preparations (Ca-diphosphate, Vivapur®, Vivastar®, lactose, corn starch, sodium starch glycolate, magnesium stearat, talc, Eudragi E100®, titanium oxide, polyethylene glycol 4000, isopropyl alcohol, and polyvinylpyrrolidone) were pharmaceutical grade substances.

Laboratory made (LM) tablets were prepared containing five different concentrations of tranexamic acid (400.0, 450.0, 500.0, 550.0, and 600.0 mg tablet$^{-1}$); these were for accuracy determination. These laboratory made tablets fulfill the requirement of the weight variation test of the Indonesian Pharmacopoeia.[10]

Two commercial tablets that contain tranexamic acid (500 mg tablet$^{-1}$; TR Batch: 0695A and KL Batch: 622113) were purchased at a local pharmacy in October 2004. The commercial tablets were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed tranexamic acid (37.5 mg) in a 25.0 mL mixture of 96% ethanol and water (1 : 1, v/v). Various standard solutions were prepared from the stock solution by dilution with a mixture of 96% ethanol and water (1 : 1, v/v). For tablet assay linearity studies, the solutions were prepared containing 375, 500, 625, 750, 876, 1000, 1250, 1380, and 1500 μg $mL^{-1}$; for dissolution studies the concentrations were 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 μg $mL^{-1}$; and 2.0 μL of these solutions was spotted on the TLC plate. The standard solutions were stable at least for 24 h at room temperature (100.81 ± 1.45%, n = 3, at 24 ± 2°C, room humidity 50 ± 10%).

## Sample Preparation

### Assay of Tablets

Twenty of tablets were each weighed, and their mean was determined. After homogenizing the powder, an equivalent weight of a 0.05 tablet (equivalent to 25.0 mg tranexamic acid) was transferred into a 25.0 mL volumetric flask containing about 20 mL of a mixture of 96% ethanol and water (1 : 1, v/v), ultrasonicated for 15 min, and diluted to 25.0 mL with a mixture of 96% ethanol and water (1 : 1, v/v). The solution was filtered through 0.45 μm Durapore®, membrane filters (Milipore, Ireland) before spotting on to TLC plates (2.0 μL), together with the standard.

### Assay of Dissolution Media

Dissolution studies were performed by the paddle method (100 rpm), using 900 mL 0.1 N HCl as the dissolution medium. Six dissolution tubes were used for each series of dissolution study. After 30 min, aliquots of the dissolution medium were filtered through 0.45 μm Durapore®, membrane filters (Milipore, Ireland) and spotted on the TLC plates (2.0 μL).

## Chromatography

Chromatography was performed on precoated silica gel 60 aluminum back sheets (E. Merck, # 1.05553, all the precoated plates were cut into 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 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*-butanol–glacial acetic acid–water (8.0 : 2.0 : 2.0, v/v).[9] 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-through

chamber (for 20 × 10 cm plates) after at least 2 h of saturation; the mobile phase migration distance in all experiments was 8.0 cm (development time ca. 1 h 45 min at 24 ± 2°C). After being air dried for 30 min at 100°C, the plates were dipped in a 0.25% solution of ninhydrin in ethanol, air dried for 20 min, then heated for 1 min (100°C), and 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 400 to 800 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at its λ maximum (488 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 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 expected value). Each extract aliquot sample 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.[11] In

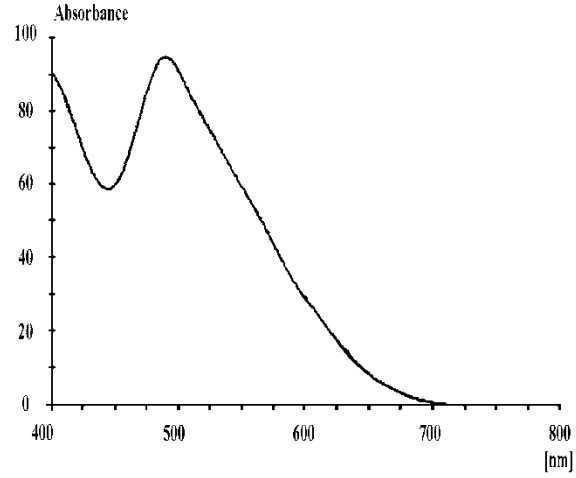


***Figure 1.*** In situ absorbance-reflectance spectrum of tranexamic acid from 400 to 800 nm, with its maximum absorption wavelengths at 488 nm. TLC conditions, stationary phase: precoated TLC plate silica gel 60 $F_{254}$ (E. Merck); mobile phase: a mixture of *n*-butanol–glacial acetic acid–water (8.0 : 2.0 : 2.0, v/v).

order to assure the selectivity of the method, forced degradation studies using HCl, NaOH, and $H_2O_2$ were performed on ca. 1600 mg powdered laboratory made tablets (equivalent to 2 tablets) in an oven (70°C). The selectivity of the method was proven by identification and purity checks of the analyte spots. In the present work, five-point accuracy studies (80, 90, 100, 110, and 120% of the expected value) were performed for LM tablets. For the dissolution studies, three point accuracy studies using solution of standard tranexamic acid in dissolution medium was evaluated (40, 70, and 100% of the targeted values in 900 mL HCl 0.1 N). For commercial preparations, an accuracy study was performed using a one and three point standard addition method (20–50% of label claim). The precision (repeatability and intermediate precision) was evaluated by analyzing six different extract aliquots from the LM tablets containing 400, 500, and 600 mg tranexamic acid tablet$^{-1}$, and from the dissolution medium those containing 40, 70, and 100% of targeted value.



## RESULTS AND DISCUSSION

After the TLC plate was eluted, the densitogram at 488 nm (Figure 2) showed a single spot of tranexamic acid ($R_f = 0.45$). This TLC system demonstrated that all analyte spots of the laboratory made tablets and commercial preparations, furnished in situ UV spectra, identical with those of 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 $\geq 0.9999$, demonstrating that the proposed TLC method is highly selective.

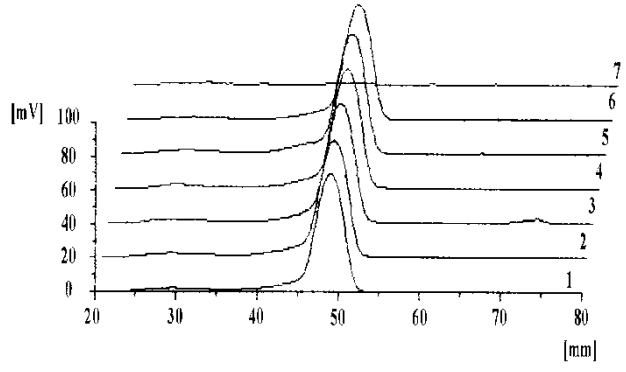


***Figure 2.*** Densitograms measured at 488 nm obtained from: (1) solution of standard tranexamic acid, (2) extract of laboratory made tablets, (3) extract of commercial tablets TR, (4) extract of stressed LM tablets using 2 N HCl, (5) extract of stressed LM tablets using 2 N NaOH, (6) extract of stressed LM tablets using $H_2O_2$, (7) extract of excipients of LM tablets. TLC conditions: see Figure 1.

The peak area was observed to be linearity dependent of the amount of tranexamic acid within the range of ca. 40 to 150% of the expected value (750 to 3000 ng $spot^{-1}$), with linear regression line $Y = 544.6 + 0.784X$ (the relative process standard deviation value $V_{XO}$[11] was 4.79%; $n = 9$; $sdv = 3.3$; $r = 0.9946$). The calculated value of test parameter $X_p$ (for $p = 0.05$) and r were satisfactory (425.47 ng $spot^{-1}$ and $\geq 0.99$, respectively).[11,12] ANOVA regression test for linearity testing of the regression line showed significant calculated F-value (648.7; $p < 0.0001$). The linearity of the basic calibration curve was also proven by the Mandel's fitting test.[11] 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 unidirectional tendency was found. The basic linear calibration curve showed variance homogeneity over the whole range. The calculated test values PW[11] were 3.6, the PW values less than the $F_{table}$-value (5.35; for $f_1 = 9$, $f_2 = 9$; $p = 0.01$).

For the dissolution study, the calibration range should be $\pm 20\%$ of the targeted value, so the lower linear range should be made smaller (ca. 300 ng $spot^{-1}$), unfortunately, in this case the values of sdv were not satisfactory (9.2, $n = 12$, range of 300 to 3000 ng $spot^{-1}$); lowering the upper limit to 2400 ng $spot^{-1}$ could not make the sdv value better (300 to 2400 ng $spot^{-1}$, $n = 15$, $sdv = 11.8$). If the upper limit was lowered to 1200 ng $spot^{-1}$, an acceptable basic linear curve was obtained. In this case, the relative process standard deviation value $V_{XO}$[11] was 2.962% (linear regression line equation was $Y = 290.3 + 1.74X$; $n = 10$; $sdv = 2.6$; $r = 0.9976$, for calibration range 300 to 1200 ng $spot^{-1}$). The calculated value of test parameter $X_p$ (for $p = 0.05$) and r were satisfactory (107.286 ng $spot^{-1}$ and $\geq 0.99$, respectively).[11,12] The ANOVA regression test for linearity testing of the regression line showed significant calculated F-value (1671.96; $p < 0.0001$). The calculated test values PW[10] were 0.42; the PW values less than the $F_{table}$-value (5.35; for $f_1 = 9$, $f_2 = 9$; $p = 0.01$).

Examples of the linear regression calibration curve parameters used in the accuracy and precision studies for LM tablets were presented in Table 1. All values of the correlation coefficient, r, in this present work are $>0.99$; and the values of other parameters such as, $X_p$ (should be less than lower limit in the calibration range), sdv ($<5$), $V_{xo}$ ($<5\%$), and p ($<0.05$) for the ANOVA linear test are also satisfactory.

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 bioequivalence and stability studies, etc.). DL was determined by making a linear regression of relatively low concentration of tranexamic acid (100 to 1000 ng $spot^{-1}$) according to the method of Funk et al.[11] The calculated equation of the regression line was $Y = 50.5 + 2.50X$ ($n = 9$; $V_{XO} = 3.78\%$; $r = 0.9982$; $sdv = 3.5$; $F_{calculated}$-value = 1967.4 for

***Table 1.*** Examples of linear regression data of the calibration curves used for evaluation of LM tablets

| No. | Intercept (standard error) | Slope (standard error) | r | p (ANOVA linear testing) | sdv | $V_{xo}$ | $X_p$ (ng spot$^{-1}$) | Range (ng spot$^{-1}$; n = 4) |
|---|---|---|---|---|---|---|---|---|
| 1[a] | 487.6 (107.9; p = 0.046) | 0.787 (0.049; p = 0.003) | 0.9961 | 0.003 | 2.7 | 3.54 | 774 | 1200–2800 |
| 2[a] | 908.6 (109.0; p = 0.015) | 0.664 (0.0486; p = 0.005) | 0.9947 | 0.005 | 2.7 | 4.44 | 923 | 1200–3000 |
| 3[b] | 706.1 (101.5; p = 0.045) | 0.793 (0.045; p = 0.003) | 0.9968 | 0.003 | 2.5 | 3.46 | 750 | 1200–3000 |
| 4[b] | 767.2 (80.6; p = 0.011) | 0.681 (0.035; p = 0.002) | 0.9972 | 0.002 | 2.1 | 3.20 | 697 | 1200–3000 |
| 5[b] | 910.0 (40.4; p = 0.002) | 0.636 (0.018; p = 0.000) | 0.9992 | 0.000 | 0.7 | 1.15 | 369 | 1600–2800 |
| 6[b] | 579.7 (50.9; p = 0.007) | 0.802 (0.022; p = 0.000) | 0.9992 | 0.001 | 1.3 | 1.71 | 401 | 1200–3000 |
| 7[b] | 943.2 (115.3; p = 0.014) | 0.734 (0.051; p = 0.004) | 0.9951 | 0.004 | 1.8 | 2.85 | 808 | 1600–2800 |
| 8[b] | 845.6 (18.9; p = 0.000) | 0.763 (0.011; p = 0.000) | 0.9998 | 0.000 | 0.4 | 0.66 | 158 | 1200–2400 |
| 9[b] | 844.9 (176.9; p = 0.041) | 0.960 (0.095; p = 0.009) | 0.9902 | 0.009 | 3.3 | 4.93 | 907 | 1200–2400 |
| 10[b] | 503.1 (125.7; p = 0.057) | 0.743 (0.055; p = 0.005) | 0.9945 | 0.007 | 3.5 | 4.54 | 949 | 1200–3000 |

[a]Accuracy studies.

[b]Precision studies.

***Table 2.*** Results from determination of accuracy using laboratory-made tablets

| Nominal concentration of tranexamic acid ($X_c$) (ng spot$^{-1}$) | Measured values ($X_f$) (ng spot$^{-1}$) |
|---|---|
| 1601 | 1587 |
| 1601 | 1602 |
| 1801 | 1795 |
| 1801 | 1802 |
| 2001 | 2001 |
| 2001 | 2009 |
| 2201 | 2221 |
| 2200 | 2175 |
| 2401 | 2419 |
| 2401 | 2409 |
| Mean recovery $\pm$ SD (%): | $100 \pm 0.1$ |
| Line equation of the recovery curve: | $X_f = -37.5 + 1.019X_c$ |
| $V_{b(af)}$[a]: | $-37.5 \pm 70.28$ |
| $V_{b(bf)}$[a]: | $1.019 \pm 0.034$ |

[a]For $p = 0.05$.

$p < 0.0001$). The calculated value of test parameter $X_p$ (for $p = 0.05$)[10] was 90.7 ng spot$^{-1}$. In this case, the value of DL = $X_p$.[11] According to Carr and Wahlich,[13] the value of the QL could be estimated at 3 times of the DL value (272.02 ng spot$^{-1}$).

***Table 3.*** Results from determination of accuracy using dissolution medium

| Nominal concentration of tranexamic acid ($X_c$) (ng spot$^{-1}$) | Measured values ($X_f$) (ng spot$^{-1}$) |
|---|---|
| 444 | 447 |
| 444 | 440 |
| 444 | 441 |
| 778 | 774 |
| 778 | 786 |
| 778 | 789 |
| 1111 | 1109 |
| 1111 | 1128 |
| 1111 | 1138 |
| Mean recovery $\pm$ SD (%): | $100.5 \pm 1.16$ |
| Line equation of the recovery curve: | $X_f = -11.98 + 1.02X_c$ |
| $V_{b(af)}$[a]: | $-11.98 \pm 21.94$ |
| $V_{b(bf)}$[a]: | $1.02 \pm 0.03$ |

[a]For $p = 0.05$.

***Table 4.*** Results of the accuracy evaluation from the commercial preparations

| Sample | TR | KL |
|---|---|---|
| Amount found[a] (Mean ± SD)[d] | 100.1 ± 0.89 | 102.3 ± 0.36 |
| Amount added[a] | 30[d] | 20, 33, 47[d] |
| Recovery % (Mean ± SD) | 101.3 ± 0.24[d] | 99.3 ± 1.29[e] |
| Recovery curve[b] | — | $X_f = -108.9 + 1.04X_c$ |
| $V_{b(af)}$[c] | — | −108.9 ± 1112 |
| $V_{b(bf)}$[c] | — | 1.04 ± 0.51 |

[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]$n = 3 \times 3 = 9$.



Tables 2 and 3 demonstrated good accuracy, as revealed by the percentage of mean recovery data of the assay of LM tablets, and for dissolution study. Accuracy study of dissolution media was performed by analyzing three levels of solutions of the analyte in the dissolution medium and calculating their recovery. To prove whether systematic errors did not occur, linear regression of the 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.[11] Good mean recovery data using the standard addition method was also observed in the commercial preparations (see Table 4).

***Table 5.*** Results from evaluation of precision of LM tablets and dissolution media

| | RSD values (%, n = 6) | | | | | |
|---|---|---|---|---|---|---|
| | LM tablets | | | Dissolution media | | |
| Measurement | 80% | 100% | 120% | 40% | 70% | 100% |
| 1[a] | 0.57 | 0.61 | 1.00 | 0.82 | 0.96 | 0.96 |
| 2[a] | 0.68 | 0.78 | 0.64 | nd | nd | nd |
| 3[a] | 0.49 | 0.92 | 1.15 | nd | nd | nd |

[a]Each measurement was performed by a different analyst on the different days, and plates within one laboratory.

nd: Not determined.

***Table 6.*** Results of forced degradation studies of laboratory-made tablets

| Storage condition | Time | Recovery of tranexamic acid [a] (Mean ± SD, n = 3) (%) |
|---|---|---|
| 3 drops of 2 N NaOH | 16 hours at 70°C | 88.3 ± 1.80 |
| 3 drops of 2 N HCl | 16 hours at 70°C | 86.4 ± 0.88 |
| 3 drops of 15% $H_2O_2$ | 16 hours at 70°C | 94.1 ± 0.99 |

[a]Purity and identity checks of tranexamic acid spots using CATS software yielded good values ($r > 0.999$).

All the relative standard deviations (RSD) of the repeatability and intermediate precession evaluations have values less than 2% (see Table 5), and the calculation by using David, Dixon, and Neumann Test[14] 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[15] (2.43 for specification range 95–105%, basic lower limit 99%, n = 6). 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 40 to 100% of the targeted concentrations in the dissolution media.

Table 6 showed that although the recovery of the tranexamic acid was reduced by NaOH (12%), $H_2O_2$ (6%), and HCl 0.1 N (14%) in stressed samples, the purity and identity check of the analyte spots using CATS software yielded good values (>0.999), this showed that all the analyte

***Table 7.*** Influence of the mobile-phase composition on the $R_f$ and T values[a]

| Mobile-phase composition (v/v) | | | | T | |
|---|---|---|---|---|---|
| *n*-Butanol | Acetic acid | Water | $R_f$ | Standard | Samples |
| 8.0 | 2.0 | 2.0 | 0.45 | 0.92 | 0.93 |
| 8.5 | 2.0 | 2.0 | 0.54 | 1.00 | 0.92 |
| 7.5 | 2.0 | 2.0 | 0.58 | 0.98 | 0.93 |
| 8.0 | 2.5 | 2.0 | 0.58 | 0.92 | 1.05 |
| 8.0 | 1.5 | 2.0 | 0.52 | 0.95 | 1.02 |
| 8.0 | 2.0 | 2.5 | 0.56 | 0.91 | 0.99 |
| 8.0 | 2.0 | 1.5 | 0.52 | 0.92 | 0.93 |
| Mean ± RSD (%) | | | 0.54 ± 0.017 | 0.94 ± 0.01 | 0.97 ± 0.02 |

[a]Data are presented as the mean value (n = 3).

spots were still pure and identical with the standard. This proved that the analyte peaks were not interfered by the degradation products (see Figure 2). It seemed that the degradation product(s) were not detected clearly in the stressed samples measured at 488 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 variation on mobile phase composition on the values of $R_f$ and tailing factor (T) were evaluated. Table 7 indicated that the small variations shown above generally did not affect the selected parameters. All the $R_f$ and T values were within the acceptance criteria.[16]

The present work showed that the proposed densitometric method is suitable for the routine analysis of products of similar composition in the pharmaceutical industry quality control laboratories. Our experiences showed that the TLC methods are cheaper, compared to the HPLC methods, especially for developing countries in which the price of HPLC grade solvents and column are very expensive.



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