

## A Comparison of Image Analysis Software for Quantitative TLC of Ceftriaxone Sodium

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### Abstract

Three image analysis software, Photoshop, Sorbfil TLC Videodensitometer software and Scion Image, were used for quantitative evaluation of TLC images of ceftriaxone sodium (CFX). Regression plots and detection sensitivity of quantification from each TLC-image analysis were determined and compared to TLC-densitometry. TLC-image analysis method using Scion Image and Sorbfil TLC Videodensitometer software and TLC-densitometry showed the polynomial regression data with good relationship ( $R^2 > 0.99$ ) over the concentration range of 1-8  $\mu\text{g}/\text{spot}$  whereas the analysis of TLC images with Photoshop showed good polynomial regression plots ( $R^2 > 0.99$ ) at higher concentration range, 4-10  $\mu\text{g}/\text{spot}$ . For detection sensitivity, LOD and LOQ determined from TLC-image analysis using Sorbfil TLC Videodensitometer were comparable to the values from TLC-densitometry. Scion Image and Photoshop was found to be less sensitive. The use of Sorbfil TLC Videodensitometer software for TLC image analysis could be further applied for rapid determination of CFX in bulk drug and dosage forms.

**Key Words:** TLC; Image analysis software; Ceftriaxone sodium

### Introduction

Ceftriaxone sodium (CFX) is a semisynthetic, third-generation cephalosporin antibiotic which has been widely used for treatment of severe bacterial infections and bacterial meningitis. For determination of CFX in bulk drugs and dosage forms, several chromatographic and spectrophotometric methods including densitometric-TLC and HPTLC (Dhanesar, 1998; Eric-Jovanovic et al., 1998; Nabi et al., 2004; Mohamed et al., 2008), HPLC (Abdel-Hamid, 1998; Xu and Trissel, 2003), ion-pair

reversed-phase liquid chromatography, fluorimetric and capillary electrophoresis methods (Gáspár et al., 2002; Bebawy et al., 2003; El-Shaboury et al., 2007) have been used.

Until recently, the use of TLC-image analysis has been applied for content determination of several compounds (Hung et al., 2001; Mustoe and McCrossen, 2001; Johnsson et al., 2007; Sotanaphun et al., 2009). The major advantages of TLC are due to its simplicity, a small quantity of solvents used, minimum sample preparation and

high sample throughput. With a combination of simple computer technology and image analysis software for evaluation of TLC chromatogram, the quantitative TLC method based on image analysis is more convenient and less expensive than other chromatographic methods.

Commercial and free web-based image software for TLC-image analysis are available in which performances are based on sensitivity of spot detection, background compensation algorithms, intensity resolution, precision and accuracy of image analysis. From our attempt to develop a simple and rapid TLC-image analysis method for determination of CFX in bulk and pharmaceutical dosage forms, chromatographic separation of CFX was achieved on RP-18 F<sub>254S</sub> TLC plates. The use of three image analysis software including Photoshop, Sorbfil TLC Videodensitometer and Scion Image for evaluation of the TLC plates were compared with TLC-densitometric method in terms of regression plots and detection sensitivity of quantification and described herein.

## Material and Methods

### Chromatographic Conditions

TLC analysis was performed on TLC silica gel 60 RP-18 F<sub>254S</sub> aluminium plates (10 cm x 8 cm with 185 µm thickness, Merck, Germany). Two µL of CFX solution were spotted manually onto a TLC plate by using a 2 µL capillary tube (Drummond Scientific Company, USA). A distance between each spot was 0.8-1.0 cm. The plate was then developed to a distance of 6.5 cm in a TLC chamber previously saturated with 15% w/v ammonium acetate buffer (pH 6.2)-methanol-acetonitrile (12:0.5:0.25, v/v/v) for 20 min. After air-drying, an image of the developed plate under a UV lamp (CAMAG UV-cabinet II, Switzerland) at 254 nm was taken by a digital camera (Ixus 860, Cannon) setting on a

tripod to a distance of 37 cm above the image. An auto mode, ISO setting at 200 and Macro (close-up) function was used and the flash was off. The shutter speed, aperture settings and zoom in were at 1/8, 5.8 and 3.8x, respectively. The image size captured by a digital camera was set to superfine (S) and large (L) and saved in a form of a joint photographic experts group (JPEG) file. Then, a JPEG image was resized and cropped according to the plate dimension at 10 cm x 8 cm and saved at a resolution of 60 pixels/cm for TLC-image analysis.

### TLC-Image Analysis Method

Quantification of CFX in the TLC image was carried out by Photoshop, Sorbfil TLC Videodensitometer software and Scion Image software.

The analysis of TLC images by Photoshop was performed by adjusting the image at threshold value of 130. The image was converted to black and white. Each band was selected by a magic wand tool and the area was determined in terms of pixel numbers. Pixel numbers in the selected area were measured by a histogram command in Photoshop software.

For image analysis by Sorbfil TLC Videodensitometer software (Sorbpolymer, Krasnodar, Russia), Background Approximation tool was used to provide the uniform illumination of the background and arrangement of track lines was made by using Regular Tracks command. The evaluation of the chosen track was processed by using Process Track command. In order to determine the background intensity of the plate, for each line method at the values of width and height at 3 and 30, respectively, was used. A chromatogram was constructed on the deviation of track intensity from background intensity. *R<sub>f</sub>*, peak area and peak height of CFX were determined and the results of the track evaluation were given in the Track Evaluation Results box.

The greyscale image of TLC chromatogram was used for analysis by Scion Image program version Alpha 4.0.3.2 (Scion Corporation, Maryland, USA). A profile plot along the chromatogram was generated using the macro Gelplot2. The peak corresponding to CFX was selected by the wand tool for measuring the area under the curve (AUC)

### TLC-Densitometry

For TLC-densitometric analysis, the developed TLC plate under the chromatographic conditions as above was scanned by using a CAMAG TLC scanner II with CAMAG CATS 3.1 software in the absorbance mode at 302 nm. The slit dimension was 5.0 mm × 5.0 mm and the scanning speed was 4 mm/s.

### Regression plots

Regression plots were performed by spotting two µL of working standard solutions of CFX (0.25-5.0 mg/mL) prepared from a stock solution of CFX (5.0 mg/mL) in distilled water on a TLC plate to give the concentrations of 0.5, 1, 2, 4, 6, 8 and 10 µg/spot of CFX. The plate was developed according to the above TLC-image analysis and TLC-densitometric methods. Plots between peak height or peak area and drug concentration in µg/spot were constructed and treated by least square regression analysis using Excel.

### Detection Sensitivity

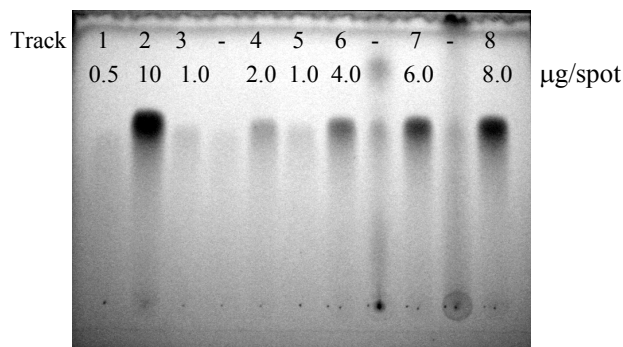
The linear regression data constructed from three concentrations at the lower range were used to determine limit of detection (LOD) and limit of quantitation (LOQ) values (Ansari et al., 2005). The LOD and LOQ were determined from the formulae  $3\text{ SD/S}$  and  $10\text{ SD/S}$  where SD was the standard deviation (SD) of the intercept and S corresponded to the slope.

## Results and Discussion

Reversed phase TLC (RP-18 F<sub>254s</sub> TLC) plates and the mobile phase consisting of 15% w/v ammonium acetate buffer (pH 6.2)-methanol-acetonitrile (12:0.5:0.25, v/v/v) was employed to analyze CFX, showing a well-defined spot of CFX at an *R<sub>f</sub>* value of 0.58. The peak area and the peak height of CFX were determined from TLC-image analysis and TLC-densitometric method and used for construction of regression plots. The study showed that the determination of peak area and peak height was available by Sorbfil TLC Videodensitometer and TLC-densitometer whereas Photoshop and Scion image could measure only peak area. For peaks without full separation at peak base, peak height could be more useful for determination of the content of substance in those peaks.

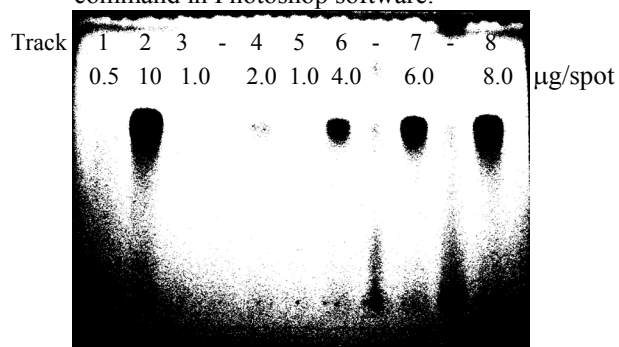
For the analysis of CFX by different TLC methods, the results showed that the peak area of the spot at concentration being less than 4 µg/spot was unable to be detected by TLC-image analysis using Photoshop whereas both TLC-densitometric and TLC-image analysis methods using Scion Image and Sorbfil TLC Videodensitometer software could detect the peak corresponding to the concentration at 0.5 µg/spot (Figure 1). However, poor precision of detection was observed at this concentration. Therefore, the regression data of the plots between peak height or peak area and concentrations over the range of 1-10 µg/spot were examined.

The data from the analysis of TLC images with Photoshop showed good polynomial regression plots ( $R^2 > 0.99$ ) over the concentration range of 4-10 µg/spot whereas the data from both TLC-densitometric and TLC-image analysis methods using Scion Image and Sorbfil TLC Videodensitometer software



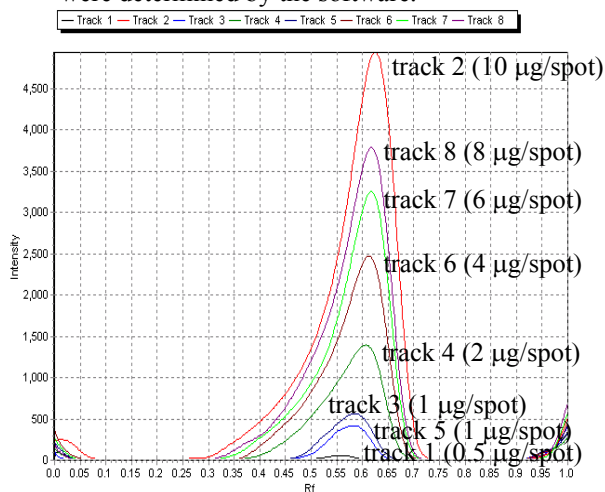
a) before evaluation

The image was converted to black and white and pixel numbers in the selected area were measured by a histogram command in Photoshop software.



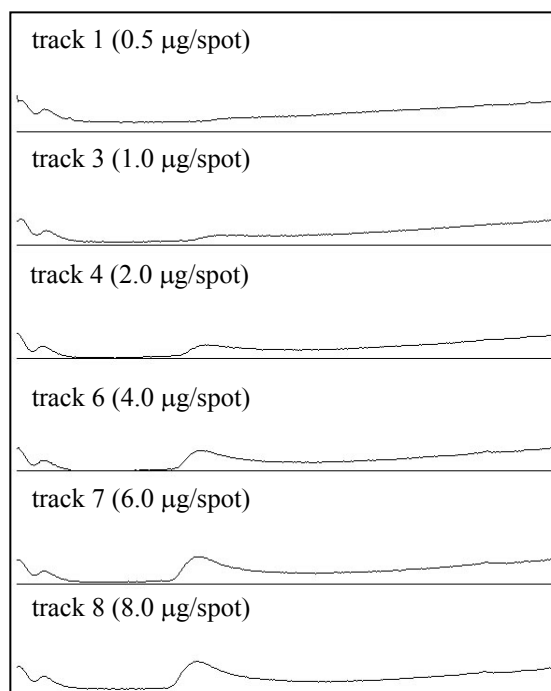
b) Photoshop

A chromatogram was constructed from each track evaluation and the peak area and height were determined by the software.



c) Sorbfil TLC Videodensitometer software

A chromatogram was constructed for each track and the peak area was selected by the wand tool and measured.



d) Scion Image

**Figure 1** TLC image a) before evaluation, and evaluation by b) Photoshop, c) Sorbfil TLC Videodensitometer software, and d) Scion Image

**Table 1** Polynomial regression data determined using TLC-densitometric and TLC-image analysis methods

Analytical technique	Concentration ( $\mu\text{g}/\text{spot}$ )	Regression between	a*	b*	c*	R <sup>2</sup>
TLC-Densitometry	1-8	A and C	-56.072	1171.3	1435.7	0.9938
		H and C	-0.7195	13.524	5.9907	0.9983
	2-10	A and C	1.1625	602.03	2624.9	0.9906
		H and C	-0.0875	7.475	17.86	0.9853
TLC-Image analysis using Photoshop	4-10	A and C	117.31	1513.2	-1013.4	0.9979
TLC-Image analysis using Sorbfil	1-8	A and C	-2872.3	51644	-22073	0.9980
		H and C	-44.705	883.99	-254.09	0.9989
	2-10	A and C	58.964	23891	31358	0.9758
		H and C	-5.2679	507.26	482.6	0.9880
TLC-Image analysis using Scion Image	1-8	A and C	-18.767	305.84	-101.98	0.9967
	2-10	A and C	0.7321	119.66	261.8	0.9654

\* polynomial regression:  $y = ax^2 + bx + c$ ; A = Area, H = Height, C = Concentration

showed the polynomial regression data with good relationship ( $R^2 > 0.99$ ) over the concentration range of 1-8  $\mu\text{g}/\text{spot}$  (Table 1). At higher concentration range, 2-10  $\mu\text{g}/\text{spot}$ , poorer correlation coefficients were obtained.

In order to assess and compare the sensitivity of detection limits obtained from TLC-densitometric and TLC-image analysis methods using Scion Image and Sorbfil TLC Videodensitometer, the linear regression data constructed from the lower concentration range (1, 2 and 4  $\mu\text{g}/\text{spot}$ ) were used to determine LOD and LOQ values (Ansari et al., 2005) (Table 2). The data showed that LOD and LOQ determined from TLC-densitometric method were comparable to the values from TLC-image analysis method using Sorbfil TLC Videodensitometer. TLC-image analysis using Scion Image was found to be less sensitive.

**Table 2** Detection limits

Analytical technique	Regression between	LOD ng/spot	LOQ ng/spot
TLC-Densitometry	A and C	169	564
	H and C	103	343
Image analysis using Sorbfil	A and C	170	568
	H and C	131	439
TLC-Image analysis using Scion	A and C	419	1397

A = Area, H = Height, C = Concentration

Better detection sensitivity was observed when TLC-image analysis was performed by Sorbfil TLC Videodensitometer. This might be due to the background approximation tool available in the software which could be used to correct the

whole image brightness, thus, providing uniform illumination of the plate before evaluation. Even though TLC-image analysis using Scion Image and Photoshop was found to be less sensitive, Scion image had an advantage of being public software and free downloaded whereas Photoshop was common commercial software and might be suitable for analysis of a TLC image with uniform illumination and spots having distinct color from a background.

### Conclusions

Three image analysis software including Photoshop, Sorbfil TLC Videodensitometer software and Scion Image software were used for quantitative evaluation of CFX from TLC images and compared to TLC densitometric method. Regression data and detection sensitivity of quantification of CFX assessed from TLC-image analysis by Sorbfil TLC Videodensitometer software were shown to be comparable to TLC-densitometry. From data above, TLC-image analysis using Sorbfil TLC Videodensitometer could be further applied for rapid determination of CFX in bulk and dosage forms and might be used as alternative to more expensive quantitative chromatographic methods, e.g., TLC densitometry and HPLC, which could not be afforded by small labs.

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