Smartphone-based thin layer chromatography for the discrimination of falsified medicines

Hojeong Yu, Huy Le, Steven Lumetta, and Brian T. Cunningham Department of Electrical and Computer Engineering UIUC Urbana, USA bcunning@illinois.edu Eliangiringa Kaale

School of Pharmacy Muhimbili University of Health and Allied Sciences Dar Es Salaam, Tanzania elia.kaale@gmail.com Thomas Layloff

Management Sciences for Health

Arlington, USA tlayloff@pfscm.org

Abstract—Identification of counterfeit and substandard drugs, which pose severe risks to patient safety is increasingly important, as inauthentic drugs become more commonplace in developing parts of the world. Though thin layer chromatography (TLC) performed with laboratory-based instruments enables accurate analysis of suspect medicines, there is tremendous interest in development of an inexpensive mobile platform that would broaden the applicability of TLC to remote pharmacies and clinics that presently do not have access to laboratory analysis. In this work, we demonstrate identification and characterization of pharmaceutical products via TLC using a custom cradle that interfaces with a smartphone. A UV lamp integrated within the cradle illuminates a TLC plate loaded with calibration standards and an aliquot of a drug of unknown concentration. Phosphorescence from the plate surface excited by UV light reveals principal spots. Two independent image processing approaches were developed to enable image processing to be performed locally with the smartphone processor, or remotely by a server running MatLab routines on uploaded images. Both approaches report the intensity and travel distance of spots within a TLC plate. The system is able to discern 5% medicine concentration differences and to deliver analytical results that are identical to those obtained by a laboratory TLC densitometer.

Keywords—thin layer chromatography, smartphone sensing, pharmaceutical compound analysis, falsified medicine detection

I. INTRODUCTION

Life-threatening counterfeit and illegally manufactured medicines are on the rise around the globe, especially thriving in developing countries, and identification of fake medicines is an imperative task for pharmaceutical safety. Falsified drugs contain inappropriate quantities of active pharmaceutical ingredients or unidentified substances that are invalid while physical characteristics like color, shape, and weight are similar to those of authentic products. According to the World Health Organization (WHO), the market share percentages of the falsified medicines range from less than 1% in advanced countries to up to 30% in developing countries [1]. It is reported that 100,000 deaths worldwide in 2010 were linked to exposure to the counterfeit and substandard medicines [2]. Thus, there is a pressing demand for a new inexpensive and efficient methodology for providing accurate analysis of medicines near the point of use and throughout the supply chain.

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Several chromatography-based methods such as thin layer chromatography (TLC), gas chromatography (GC), and liquid chromatography (LC) have been used for analysis of falsified medicines. Among them, TLC is the most simple and inexpensive approach that is adoptable even in the developing countries that are suffering from lack of medical infrastructure. However, a benchtop laboratory instrument is required to scan a TLC plate precisely, and it restricts the wide use of the TLC approach in the field.

The system is comprised of a 3D-printed plastic cradle, a Samsung Galaxy Fame smartphone, and a UV lamp, lowering the overall cost and system complexity for TLC plate analysis. The cradle holds the lamp, the phone, and the plate in a fixed orientation during a measurement. The UV illumination excites a phosphor coating on the TLC plate loaded with calibration standards (100% and 75% of full concentration; 75% is the lowest limit considered acceptable for a single tablet) and a target substance (95% of full concentration). The image of principal spots revealed from background phosphorescence is captured by the phone camera, and then two independent image processing algorithms analyze the image to extract both spot intensity and travel distance (R_t) . The performance of smartphone-based TLC is evaluated by measuring a set of TLC plates prepared with nevirapine (an anti-retroviral drug), amodiaquine (an anti-malarial drug), and paracetamol (an antiinflammatory drug) and by comparing the results with those taken from a commercially available TLC densitometer.

II. MATERIALS AND METHODS

A. Preparation of samples and densitometric scanning

The procedures described in the Global Pharma Health Fund Minilab[®] manual were utilized to prepare the TLC plate with the samples [3]. Aliquots of each drug tablet (nevirapine 200 mg, amodiaquine 200 mg, and paracetamol 500 mg) were loaded onto the TLC aluminum plates with concentrations of 100%, 95%, and 75% of full medicinal concentration. The plate was pre-coated with silica gel (60 F₂₅₄), and the principal spots run by capillary action were shown up when the plate was illuminated with UV light. The principal spots surrounded by green background were analyzed using the conventional densitometer (TLC Scanner 3; CAMAG) performed with the instrument-supplied chromatography manager software.

This work was supported by the National Science Foundation (NSF) under Award No. CBET 1264377 and by the Management Sciences for Health (MSH) with funding through the NSF Center for Innovative Instrumentation Technology (CiiT) I/UCRC (IIP 1067943).



Fig. 1. Illustration of the smartphone-based TLC scanner and photos of the system.



Fig. 2. Images of the TLC plate at different stages of measurement. (a) Under visible light. (b) With UV illumination. (c) After analysis taken by image processing algorithm. Areas surrounded by black box are magnified and compared to each other below the full-size image of (b) and (c).

B. Smartphone-base TLC scanner

A schematic of the system is depicted in Fig. 1. A custom 3D-printed cradle interfaces with the rear-facing camera of the smartphone while holding the UV lamp ($\lambda_{peak} = 254$ nm) and the TLC plate during a measurement. The cradle is designed to provide a dark environment required for improving the signalto-noise ratio (SNR) and enable the camera to capture a full-size image of the plate bounded by the origin (bottom) and solvent front (top) lines. The TLC plate is inserted into the cradle via an entrance slit, and a holder keeps the plate at the exact position to illuminate and take a picture of it. The optical axis of the UV lamp makes an angle of 39° with the longitudinal axis of the plate to expand the area of UV illumination over the surface. The issue of irregular illumination caused by the oblique-orientated lamp and by the uneven intensity distribution of UV light is resolved by image processing algorithms using image subtraction between the sample-loaded plate and a blank plate under UV light. The principal spots are not visible without UV illumination (Fig. 2(a)) while the spots emerge from the green background from the phosphorescent compound on the plate when they are excited by the UV light (Fig. 2(b)).

C. Image processing: external and internal algorithms

Two independent algorithms were developed for image processing. The "external algorithm" aims to use the computing power of an external computer that facilitates faster processing while the "internal algorithm" that forms an essential part of software application conducts the analysis locally using the phone that gathered the TLC image. The algorithms analyze an image averaged from ten plate images that are taken in a series to minimize noise and to enhance SNR [4], [5]. Both provide two crucial characteristics of the principal spots, such as the spot intensity and travel distance associated with the concentration and type of compound, respectively (Fig. 2(c)).

The external algorithm utilizes RGB (red, green, blue)-to-YIQ (luminance, in-phase, quadrature) color conversion to produce the surface luminosity levels from an original RGB image. The YIQ color model extracts information of perceptual luminosity using the following conversion equation [6]:

$$\begin{bmatrix} Y = 0.2989R + 0.5866G + 0.1144B \\ I = 0.5959R - 0.2741G - 0.3218B \\ Q = 0.2113R - 0.5227G + 0.3113B \end{bmatrix}$$

After the image subtraction using a blank TLC plate, analysis areas for the three spots are set using threshold value (T_h) , 0.45, that is determined by taking account of background noise and facilitates the formation of a rectangular region of interest. The travel distance of each spot is defined by the equation:

$$R_f = \frac{\# \text{ of pixels between the box's center and the origin line}}{\# \text{ of pixels between the solvent front line and the origin line}}$$

The spot intensity is characterized by selecting the pixels in the analysis domain whose intensity is between the T_h and a 10%-offset cutoff. The cutoff value is described by the equation:

$$10\%$$
-offset = min. $PV + (T_h - \min PV) / 10$

where the min. PV is the minimum pixel value (PV) within the domain. The spot intensity of each principal spot is defined by:

Spot intensity =
$$\sum_{j=p_{y1}}^{p_{y2}} \sum_{i=p_{x1}}^{p_{x2}} PV_{i,j}$$
 (if cutoff $\leq PV_{i,j} \leq T_h$)

where the $p_{xl}, p_{x2}/p_{yl}, p_{y2}$ are pixel indices representing the both vertical and horizontal edges of the analysis area, respectively.

The internal algorithm also starts the analysis using the RGB-to-YIQ conversion. The TLC image is blurred by 2D-Gaussian filtration with a sigma of four in order to smooth the image. Then global thresholding that transforms an image into a binary image is adopted [7]. The spots are separated from the background by multiplying the image by the inversion of a blank plate image and located by a connected component algorithm with eight connectivity [7]. Travel distance is measured by defining the center of the analysis area using a quadratic curve fitting and by calculating the distance between the spot center and the origin line. To determine the spot intensity, 400 lowest pixel intensities within the spot area are integrated.

III. RESULTS AND DISCUSSION

The performance of the smartphone-based TLC scanner was validated by measuring the normalized spot intensity and travel distance of the three different medicines (nevirapine,



Fig. 3. Spot characteristics (normalized spot intensity and travel distance) resulted from ten independent measurements of a paracetamol TLC plate performed by the external algorithm (a, b) and by the internal algorithm (c, d).



Fig. 4. Comparison of functionality between the densitometer and the smartphone-based TLC scanner using a paracetamol-loaded TLC pate.

amodiaquine, and paracetamol) prepared at three different concentrations (100%, 95%, and 75%).

Fig. 3 demonstrates the repeatability of the system, taken by removing and re-inserting the paracetamol-loaded TLC plate into the cradle. The ten overlaid data show that the measurements are highly repeatable in both algorithms. Fig. 4 compares the primary spot characteristics measured by the densitometer and the smartphone-based TLC scanner. Both instruments provide almost the same travel distance while spot intensity decreases as the medicinal concentration is reduced. The error bars represent three standard deviations resulting from ten independent experiments. The measurement and comparison of the test performed on three drugs are summarized in Table 1. The average spot intensities and travel distances given by the smartphone-based TLC scanner are similar to those from the densitometer. A test of one TLC plate requires 14s for the external algorithm running on a 3.4GHz i7-2600 processor and 30s for the internal algorithm running on a 1GHz Cortex-A9 processor. The entire TLC analysis procedure requires approximately an hour including sample preparation.

TABLE I. ANALYSIS AND COMPARISON OF TLC READING MODALITIES

Drugs	Measurements	Drug Conc.	Densito- meter	Smartphone-based TLC Scanner	
				External	Internal
Amodiaquine	Normalized Spot Intensity	100 %	1	1	1
		95 %	0.958	0.906	0.980
		75 %	0.932	0.755	0.941
	Travel Distance	100 %	0.73	0.73	0.74
		95 %	0.73	0.73	0.74
		75 %	0.73	0.73	0.73
Nevirapine	Normalized Spot Intensity	100 %	1	1	1
		95 %	0.938	0.817	0.974
		75 %	0.806	0.771	0.947
	Travel Distance	100 %	0.61	0.61	0.62
		95 %	0.61	0.61	0.62
		75 %	0.61	0.61	0.62
Paracetamol	Normalized Spot Intensity	100 %	1	1	1
		95 %	0.919	0.832	0.939
		75 %	0.831	0.579	0.921
	Travel Distance	100 %	0.40	0.40	0.41
		95 %	0.40	0.40	0.41
		75 %	0.40	0.40	0.40

IV. CONCLUSION

We demonstrated that a smartphone-based TLC scanner provides quantitative pharmaceutical product analysis. By integrating a UV lamp with a custom 3D-printed cradle that interfaces with a smartphone, the scanner serves as a portable and inexpensive tool for conducting TLC plate measurement and discriminating falsified medicines. Two independent algorithms designed for either an external computer or a smartphone processor facilitate direct reporting of the primary characteristics of the principal spots such as spot intensity and travel distance. The medicines tested were diluted to 100%, 95%, and 75% of full medicinal concentration, and it was shown that the system can discern 5% differences in concentration, representing the same performance as a benchtop laboratory densitometer. The smartphone-based TLC scanner provides an efficient solution to analyze UV-absorbing medicines, especially in resource-limited or remote areas.

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