RAPID SMARTPHONE-BASED IMAGE READING FOR ASSESSING QUALITY OF ACTIVE PHARMACEUTICAL INGREDIENTS OF COMMONLY PRESCRIBED DRUGS-LUSAKA ZAMBIA

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ABSTRACT

Third world countries suffer from supply of substandard therapeutic drugs and due to lack of robust quality testing facilities, many of these drugs find themselves in the dispensaries and various outlets for over-the-counter purchase. In order to ensure quality, simple yet informative tests are available and one of them is high performance thin layer chromatography. High Performance Thin Layer Chromatography (HPTLC) coupled with image acquisition by TLC software application on a smart phone and analysis by freeware Digitally enhanced Thin Layer Chromatography (DeTLC) respectively was performed using the minilab protocols for assessing quality of drugs dispensed at the University of Zambia clinic as well as drugs randomly bought from different pharmacies around Lusaka, Zambia. The quality of drugs was assessed by comparing retardation factors with standards and approximate percent active ingredient amounts were compared using the maximal K intensities derived by the DeTLC software for image analysis. Normalizing the spots to the 100% spot, mean values with respective standard deviations were: atenelol (85.6±0.5), ciprofloxacin (95.6±1.1), ketoconazole (78.2±1.1), mebendazole (95.5±1.1), nevirapine (96.7±1.1), penicillin v (100.5±1.1), quinine (92.9±1.1), rifampicin (99.3±0.7) and isoniazid (72.0±4.1). All the drugs tested were within the acceptable range of concentrations of the active pharmaceutical ingredient (0 to 5.3% R_f error). Thus, the simple robust HPTLC method coupled with simple to use digital imaging software provides a quick means to check on quality of the drugs dispensed.

Keywords: *Smartphone, Imaging, Thin layer Chromatography, Pharmaceutical drugs*

INTRODUCTION

Thin-layer chromatography (TLC) and High performance thin-layer chromatography (HPTLC) are closely related planar chromatography technologies that are increasingly used in pharmaceutical analysis(Kaale, Risha, Layloff, & Sherma, 2014). Striegel and Hill have described the advantages and disadvantages of TLC in their book (Striegel & Hill, 1997). With advancements in the stationary phases and the introduction of automatic sample application devices and densitometers, the techniques can achieve a precision and accuracy comparable to those obtained with HPLC(Kaale, Risha, Reich, & Layloff, 2010). Thin-layer chromatography (TLC) is considered a sustainable analytical technique, and is the method of choice in many laboratories with a limited budget. Its simplistic setup and low cost with no maintenance requirements are the main advantages over other formats of chromatographic techniques such as high-pressure liquid chromatography (HPLC) and gas chromatography (GC) (Kerr, West, & Kradtap Hartwell, 2016). A more advanced form of TLC is available with very good resolution.

High performance thin-layer chromatography (HPTLC) has been used with very good resolution in the study of propolis extracts from honey bee wax. (Ristivojević et al., 2014) This type of TLC is gaining more grounds especially with the development of free imaging software to capture high resolution images on high resolving fluorescent silica gels (Kaale, Risha, & Layloff, 2011; Sun et al., 2019). The sale of falsified and substandard drugs is currently a multi-billion dollar industry, which thrives especially in developing countries. According to the World Health Organization, 1 in 10 of the pharmaceuticals products are falsified and substandard in developing markets (WHO, 2018). The executive summary of the WHO study estimates the observed failure rates of substandard and falsified medical products in low- and middle-income countries at approximately 10.5%. If this is applied to unweighted estimates of market size in low- and middle-income countries, the estimated spend is in the order of US$ 30 billion (WHO, 2017). Such products often are produced by highly organized unscrupulous manufacturers who undertake efforts to replicate the physical characteristics such as shape, color, and packaging of authentic drug products, but with the active ingredient either missing, diluted or completely replaced with another active pharmaceutical ingredient (API). These substandard or falsified products pose severe health risks for patients who receive doses of an API that are lower than that required for efficacy, or receive a substitute API that has no therapeutic indication for the disease of concern. The most common incidences of falsified critical drugs are encountered in the treatment of malaria, HIV/AIDS, tuberculosis, and cancer; thus, these products pose an extreme health risk to the patients who take them. An estimated 100,000 deaths per year have been linked to the falsified and substandard drug trade in Africa (Yu et al., 2016). The Lancet's Commission on Essential Medicines Policies (Wirtz et al., 2017) identified five areas that are crucial to essential medicines policies: paying for a basket of essential medicines, making essential medicines affordable, assuring the quality and safety of medicines, promoting quality use of medicines, and developing missing essential medicines. With these areas in mind, a robust, quick and cheap method of assuring quality of essential drugs is a must for resource limited countries(Höllein, Kaale, Mwalwisi, Schulze, & Holzgrabe, 2016).

MATERIALS AND METHODS

Standard drugs were obtained from the Minilab supplied by Global Health Supply Chain project. The sample drugs, atenolol, ciprofloxacin, nevirapine Isoniazid-rifampicin combination, quinine sulfate, were a kind donation from the University of Zambia Clinic. Mebendazole, ketoconozale and penicillin-V (pen-V) were purchased from a local pharmacy.

All reagents for the mobile phases and development and High-performance thin layer chromatographic plates (HPTLC F_{254}) plates were purchased from Merck Biosciences.

UV lamps were procured with the help of the PFSCM-USAID –GHSC-QA Funded Project for the establishment of the Pharmaceutical Analysis Laboratory at the University of Zambia, Department of Chemistry.

Minilab procedures for running the TLC were used except for ketoconazole.

EXPERIMENTAL

This was an experimental design study and involved no animals or any material of biological origin. However, many organic solvents were utilized as mobile phases. Convenient sampling of drugs for rapid quality assessment was done and the results are reported herein.

Methods

Preparation of the Stock and Working Standard Solutions

Methods provided for in the minilab protocols were followed (Jähnke & Dwornik, 2008) . See supplementary material for details. 2 mg of neat ketoconazole was weighed and dissolved in 1 mL methanol. This represented the 100% stock standard solution. Appropriate dilutions were made to make the 100% and 80% solutions.

Preparation of the Stock Sample Solutions

Briefly, whole tablets from appropriate drug product were wrapped in aluminum foil and crushed to fine powder. The powder was transferred to an appropriate flask and methanol solvent added. The flask was shaken for 3 minutes until most of the solids dissolved. The solutions were allowed to sit for an additional 5 minutes until all undissolved residues settled below the supernatant liquid. The clear solution was carefully transferred to a clean stock sample bottle for further dilution.

Preparation of Working Sample Solutions

Appropriate milliliters of the stock sample solution were pipetted and diluted to the expected concentration of the higher working standard solution in 3.1.2 (supplementary material).

Spotting and development

All the standards and samples were spotted on 5.0 cm x 10.0 cm Merck aluminium plates coated with silica gel 60 F_{254} using disposable micro capillary pipettes of 2 µl volume (minilab stock), with all spots equally spaced and with uniform diameter. After drying, the plates were then placed in the development tank containing appropriate development solvent, as a mobile phase (Nyirenda, Mwanza, & Lengwe, 2020).Analytical grade solvents were used for the preparation of development solvent systems. Atenelol was developed by use of methanol and concentrated ammonia solution in a 20:0.2 v/v ratio. Ciprofloxacin was developed effectively by methanol, acetone,concentrated ammonia and toluene in a ratio of 10:5:5:2.5. for ketoconazole, hexane, chloroform, methanol and diethylamine in the ratio 50:40:10:1 solvent system was employed. Mebendazole developed well in toluene, ethylacetate, glacial acetic acid in the ratio 14:4:4 v/v. Nevirapine on the other hand was highly mobile in ethylacetate, methanol and toluene in the ratio of 11:5:4. Penicillin V was developed in ethylacetate, glacial acetic acid and water in a ratio of 15:5:5. For the combination drug rifamate, methanol, toluene and concentrated ammonia solution in the ratio 12:10:0.5 was used and for quinine sulfate, methanol and concentrated ammonia solution was used in the ratio of 20:0.5 v/v. Results are shown in figure 1.

Sample Detection

After drying off all solvent, the chromatoplates were first observed under white light followed by an inspection with UV-light in the dark at 254 nm for ciprofloxacin, isoniazid, mebendazole, nevirapine, ketaconazole, rimfapicin. However, pen- V spots were detected after exposing to iodine crystals for staining. For quinine detection, UV-light at 254 nm and 366 nm was used. Staining with iodine staining was done for atenolol after UV-light at 254 nm. However we report only the UV 254 nm detection.

RESULTS AND DISCUSSION

HPTLC chromatogram Development Results

The plates were developed according to minilab protocols (Figure 1) and image reading was done using a Samsung smart phone mounted on the 3D printed box Figure 2.

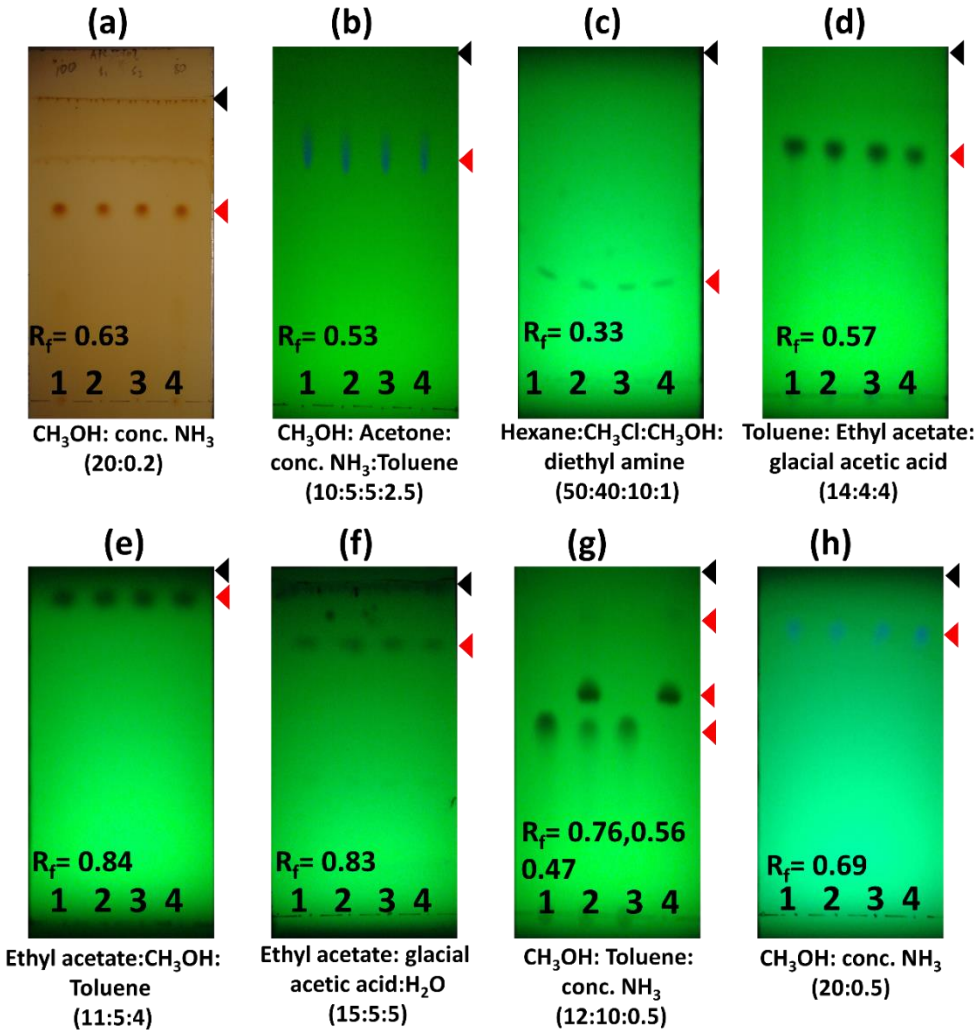

Figure 1: Shows the HPTLC F254, Aluminum support, Silica gels irradiated at 254nm UV. Panel **a**;Atenelol-100 mg, **b**; Ciprofloxacin-500 mg, **c**; Ketoconazole-2%, **d**;Mebendazole-500 mg, **e**;Nevirapine-600 mg, **f**;Penicillin V-250 mg, **g**;Rifamate (Isoniazid-75 mg. Rifampicin-150 mg combination),**h**;Quinine sulfate-300 mg.

Each of the HPTLC plates was developed in appropriate solvent mixture (figure 1). Lanes 1 and 4 for each TLC plate are for the 100% and 80% standard. Lanes 2 and 3 are for 100% sample except for panel **g**; where lanes 1 and 4 were 100% isoniazid and 100% rifampicin respectively. Lane 2 was the combination therapy Rifamate (Isoniazid-Rifampicin combination) while lane 3 was single therapy Isoniazid. The red triangles shows the API bands and the black triangles shows the solvent front. All standards used were of analytical grade (GHFP Minilab). The Atenolol plate was developed using solid iodine crystals as a source of iodine vapor. Images were obtained by use of a Samsung Galaxy FAME GT-S6810P smartphone (5 Megapixels camera) mounted on the 3D printed blackbox (Figure 2).The TLC Analyzer software developed

by Yu et al (Yu et al., 2016) was used for image capture only. Courtesy of Nyirenda et al, 2020 (Nyirenda et al., 2020)

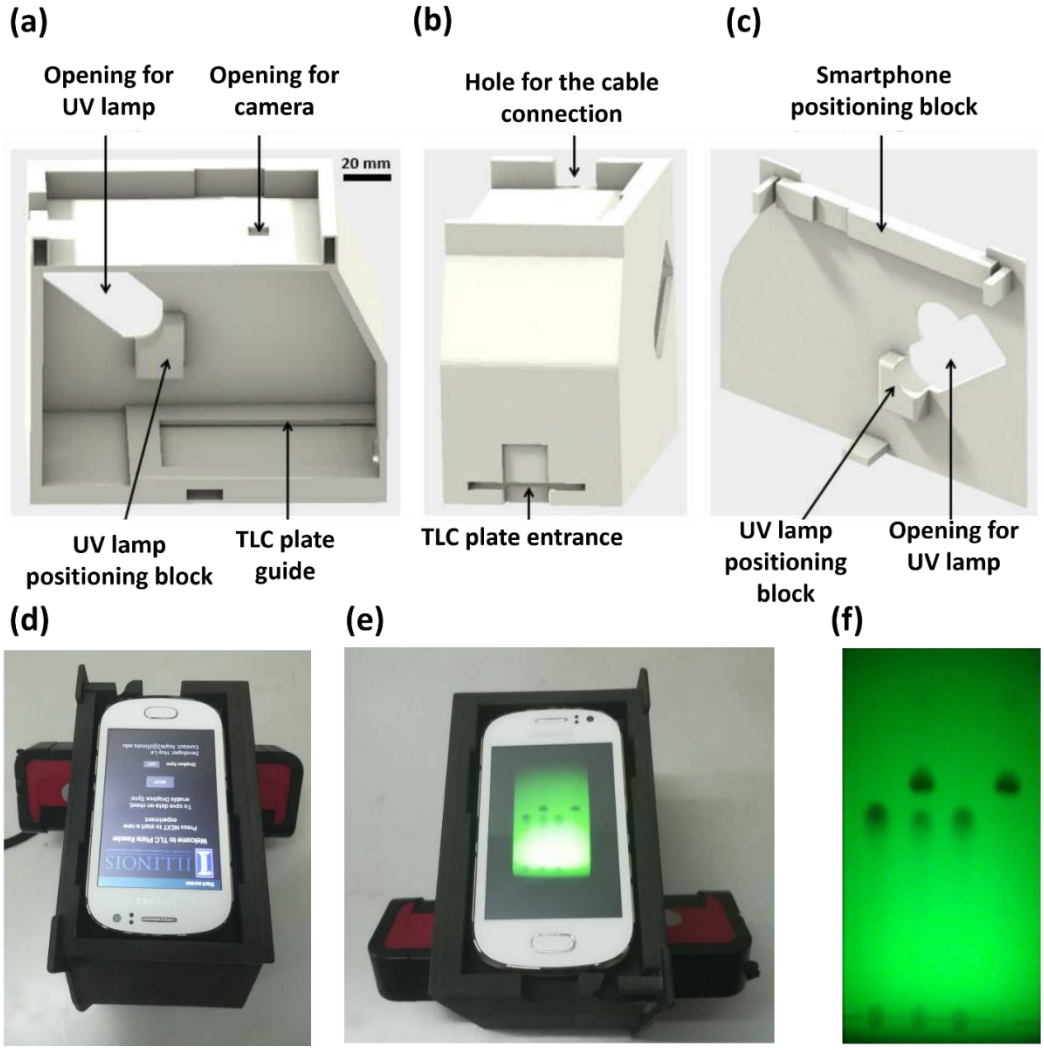

Figure 2: Illustration of the smartphone TLC analyzer and photographs of the system. (a, b) Illustration of the cradle that holds a smartphone, a UV lamp, and a TLC plate. (c) Illustration of the cover provides a dark environment and that mechanically supports the lamp. (d) Photo of the assembled smartphone TLC analyzer. (e) Photo of the TLC plate analysis (image capturing/processing). (f) Captured full image of the TLC plate- Isoniazid and Rifampicin. Figures a-c were adopted from Yu et al (Yu et al., 2016).

Retardation factors (R_f) were calculated by use of equation 1.

R_f = distance moved by spot (mm) / distance moved by solvent front (mm) 1

Retardation factor Sample Errors

The sample R_f values were normalized with standard R_f values using equation 2 (Jähnke & Dwornik, 2008) to calculate percent retardation factor error.

R_f *sample error* = [(R_f *std* - R_f *sample*) / (R_f *std*)] ×100% 2

Equation 2 was used to calculate the percent acceptability or fail in the range -10% to 10% deviation, 10% being the threshold for rejecting the sample.

Table 1: Test Drugs and appearance

Drug	**Appearance/Dosage form**	**R_f error (%)**
Atenolol -100 mg		2.1
Ciprofloxacin-500 mg		2.5
Isoniazid -100 mg		2.8
Ketoconazole-2%		5.3
Mebendazole-500 mg		0.0
Nevirapine-600 mg		0.0
Penicillin V-250 mg		0.0
Rifamate (75 mg Isoniazid, 150 mg Rifampicin)		2.8
Quinine sulfate-300 mg		0.0

Table 1: shows the drugs used in this study, the appearance and retardation factors normalized as percent error.

The DeTLC software is easy to download(Hess, 2007), install and use (http://www.sciencebuddies.org/science-research-papers/tlc_analyzer.shtml). Figure 3 shows the image of isoniazid, rifampicin and the combination therapy. The first lane is 100% isoniazid standard, second is the combination therapy, third is the isoniazid single dose and fourth is the rifampicin 100% standard.

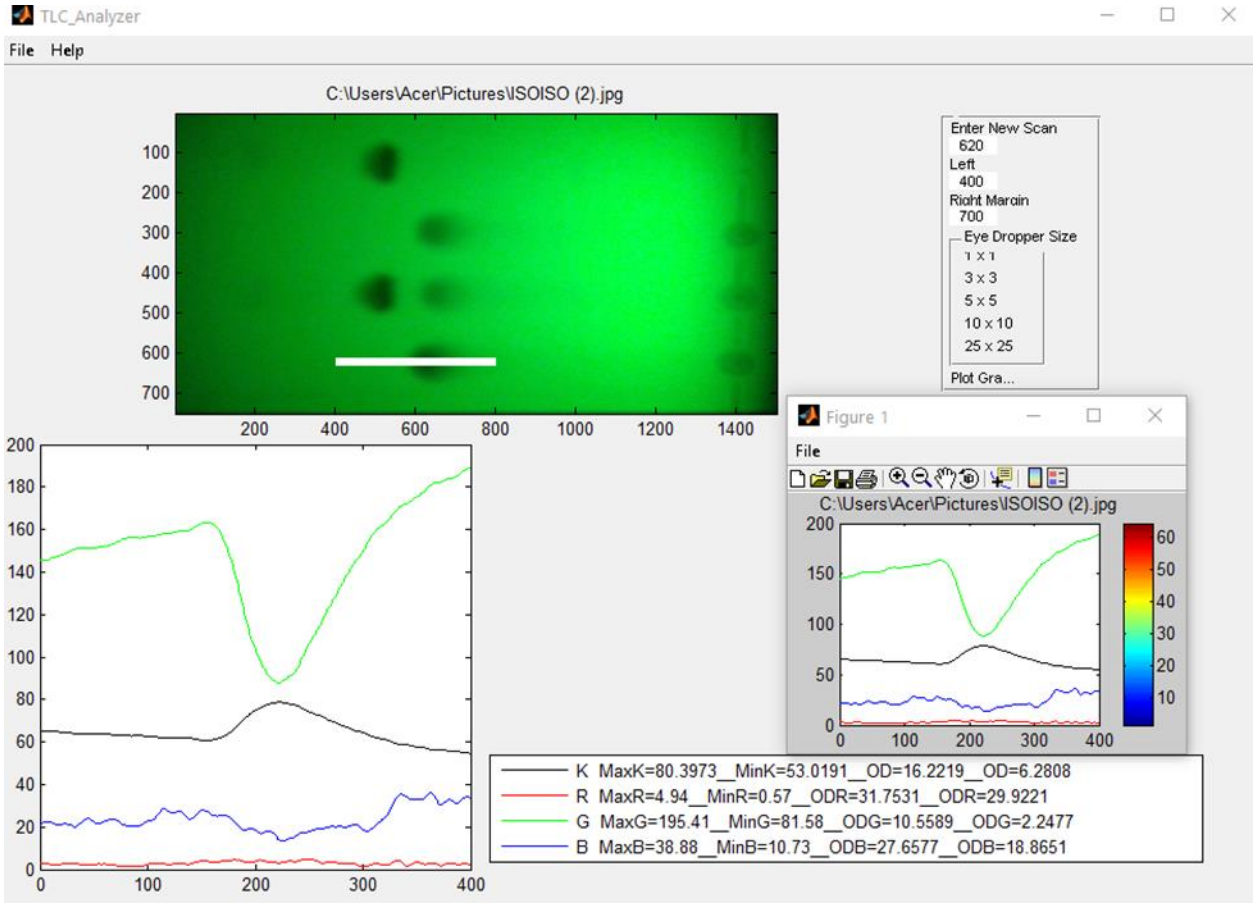

Figure 3: Typical image and intensity results of DeTLC. The figure shows lane 1 of the isoniazid standard at 100%.

Table 2. Intensities at Maximal K Values

Standard/Sample	Lane 1	Lane 2	Lane 3	Lane 4	Mean ±SD (%)
Atenelol	14.2002	12.0863	12.2329	11.5398	85.6±0.5
Ciprofloxacin	15.1742	14.6690	14.3380	15.2390	95.6±1.1
Ketoconazole	6.7114	5.3265	5.1716	5.2940	78.2±1.1
Mebendazole	18.8132	18.3301	17.6169	16.3065	95.5±1.1
Nevirapine	21.0038	20.3688	20.2483	22.0823	96.7±1.1
Penicillin V	16.1106	15.9516	16.4427	17.0149	100.5±1.1
Quinine	11.2511	10.5738	10.3273	10.9449	92.9±1.1
Rifampicin		19.0003		19.2805	99.3±0.7
Isoniazid	16.0194	10.8848	12.1985		72.0±4.1

Table 2: Shows the mean maximal intensities expressed as K values as reported by Hess et al(Hess, 2007)

DISCUSSION

Results for all drugs analyzed were within the threshold range for acceptability and were deemed as having appreciable levels of the active pharmaceutical ingredient at or about 80%

threshold for acceptability. The R_f percent error ranged from zero (mebendazole, penicillin V, nevirapine and quinine sulfate) to 5.3 % (ketoconazole). Other studies done on quantitative TLC(Kerr et al., 2016) have opined that the green bright value offers more reliability. Others like (Hess, 2007) have opined the K variable encompassing black and white intensities. Hess and others suggested that black and white calibration curves are composed of an "average" of the red, green, and blue components. Sometimes one monochromatic curve is better than another monochromatic curve, or even the composite (black and white) curve. The development of smartphones with high pixel back cameras makes them to be very versatile tools for sensing processors(Huang et al., 2018). As the capabilities of smartphones are becoming more and more powerful, researchers only need to design certain accessory attachments on smartphones for analytical biosensing, which can provide illumination sources, signal detectors, or just instrumental interfaces that supply power and process data. Considering such facts of rapid development, mass production, and pervasive distribution for smartphones in recent years, they have provided people with portable, cost-effective, and easy-to-operate platforms to integrate with microfluidics and lab-on chip (LOC) to build analytical biosensors for point of care (POC) applications and mobile health. So far, the effectiveness of smartphone based analytical biosensors has already been demonstrated in numerous applications such as healthcare diagnostics, environment monitoring, and food toxin screening (Huang et al., 2018; Shahvar, Saraji, & Shamsaei, 2018; Xu, Huang, Guo, & Ma, 2018). Low to middle income countries (LMICs) are usually flooded with a myriad of counterfeit or substandard drugs, hence the need for a robust, cost effective detection system with easy technology transfer as Popovic and Sherma opined (Popovic & Sherma, 2014). In this rapidly changing field of detection and quantification, pattern detection and alienation are of key importance in order to produce reliable and reproducible results (Ristivojević et al., 2014). From the results in this research, smartphone technology is offering a very promising multifaceted gadget which is likely to be an essential commodity in every household for day to day operations in drug quality analysis. Using Microsoft Power Point "color" function located under the Format tab in Picture Tools, Kerr et al, (Kerr et al., 2016) could quantify creatinine amounts after iodine staining. This shows yet another instance of image acquisition by domestic cameras which can be coupled to cheap quantification software. Imaging software for such works however needs to be improved to increase the signal to noise ratio by ensuring that background noise is efficiently removed to increase the signal. Also, if multi-colour substances are involved, there is need to couple integration of different colours to improve signals. Currently, most imaging software's usually convert the image to black and white and coloured spots usually lose some pixels for efficient reading.

CONCLUSION

There are a number of software's available for analyzing spot intensities on TLC plates. Some are free and others are subscription based. Results from this study indicate that software for integrating peak intensities can be highly informative about the quality of drugs. Thin layer chromatography is more affordable in resource limited countries like Zambia. With unstable power supply, it becomes very difficult to rely on sophisticated electric powered instruments such as high performance liquid chromatographs or automated ultra violet scanning densitometers. Coupled with a simple smartphone for image capturing and reliable spot intensity quantifying software loaded on a phone is a very promising venture for product and

method development. We showed within experimental limits that quality check of drugs can be done cheaply.

DECLARATIONS

Competing interests

The author declares that he has no competing interests.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTION

JN conceptualized the study and wrote the paper. JN proofread and verified the manuscript.

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SUPPLEMENTARY INFORMATION

Preparation of stock and working solutions

Concentration of standards were made according to the methods of analysis in the user manual for the minilab. The stock standard solutions were prepared by dissolving an authentic standard tablet containing appropriate milligrams of the drug. The standard reference tablet was wrapped in aluminum foil and crushed into fine powder using a pestle. The powder was then transferred quantitatively into the flasks and appropriate amount of extraction solvent added and shaken carefully to allow the solids to dissolve.

The contents of the flasks were allowed to sit for additional 5 minutes until the undissolved residues settled below the supernatant liquid.

The concentration of the solution obtained was then calculated and labeled as such.

Atenolol (50 mg) stock standard solution 5 mg/mL, 100% (5 mg/mL) and 80% (4 mg/mL). Ciprofloxacin (250 mg) stock standard solution 5 mg/mL, 100% (0.625 mg/mL and 80% (0.5 mg/mL). Isoniazid (100 mg) stock standard solution 5 mg/mL, 100% (2.5 mg/mL) and 80% (2.0 mg/mL) (Jähnke & Dwornik, 2008; Nyirenda, Mwanza, & Lengwe, 2020). Ketoconazole (neat) stock standard, 100% on a w/v basis (2mg/100 mL). Mebendazole (100 mg) stock standard solution 5 mg/mL, 100 % (2.5 mg/mL) and 80% (2 mg/mL).

Nevirapine (200 mg) stock standard solution 5 mg/mL, 100% (1.25 mg/mL) and 80% (1.0 mg/mL). Penicillin-V (250 mg) stock standard solution 5 mg/mL, 100% (0.625 mg/mL and 80% (0.5 mg/mL). Quinine (300 mg) stock standard solution 10 mg/mL, 100% (1.25 mg/mL) and 80% (1.0 mg/mL) and Rifampicin (150 mg) stock standard solution 10 mg/mL, 100% (2.0 mg/mL) and 80% (1.6 mg/mL).

Materials for TLC analysis.

Analytical grade methanol, toluene, ethyl acetate, hexane, acetone, glacial acetic acid, diethyl amine, concentrated ammonia and chloroform were purchased from Merck Biosciences. DeTLC software was used for image spot quantification(Hess, 2007)

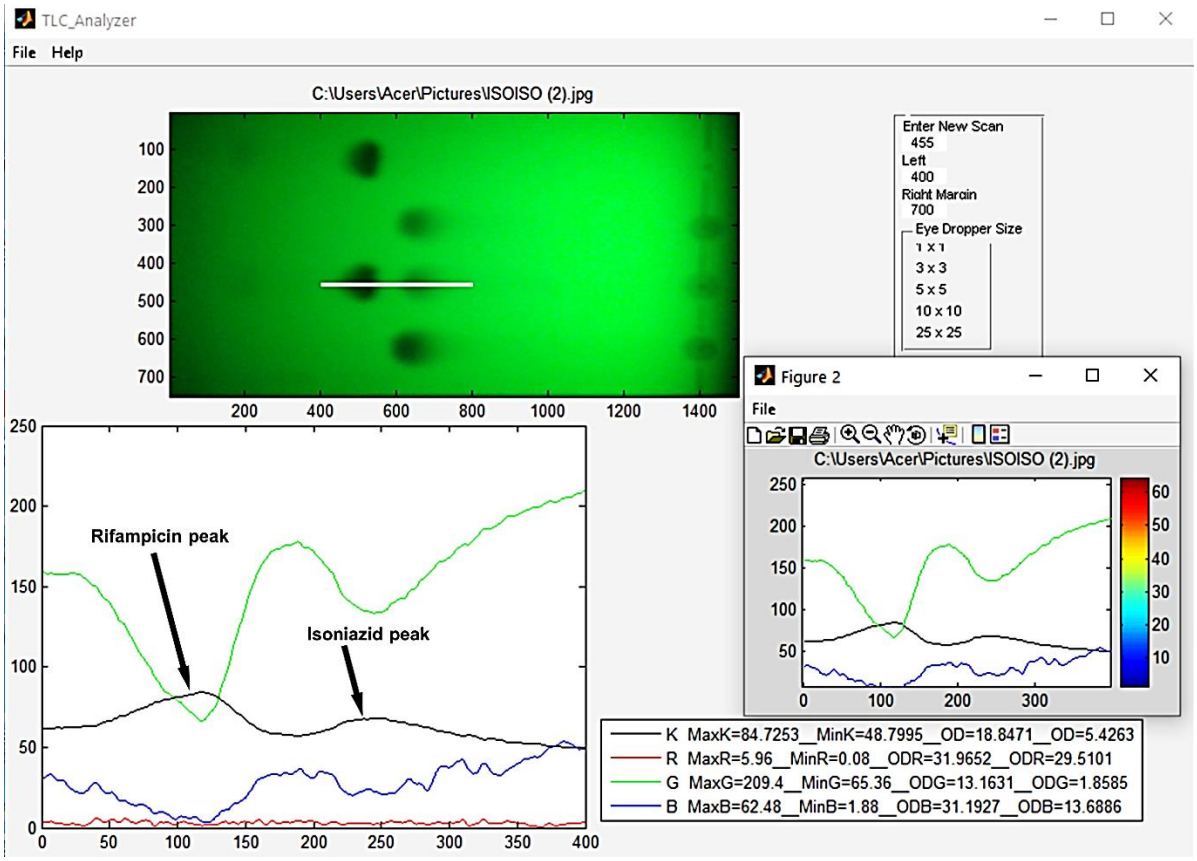

Figure S 1 DeTLC Scans for Isoniazid, rifampicin and combination dose tablets

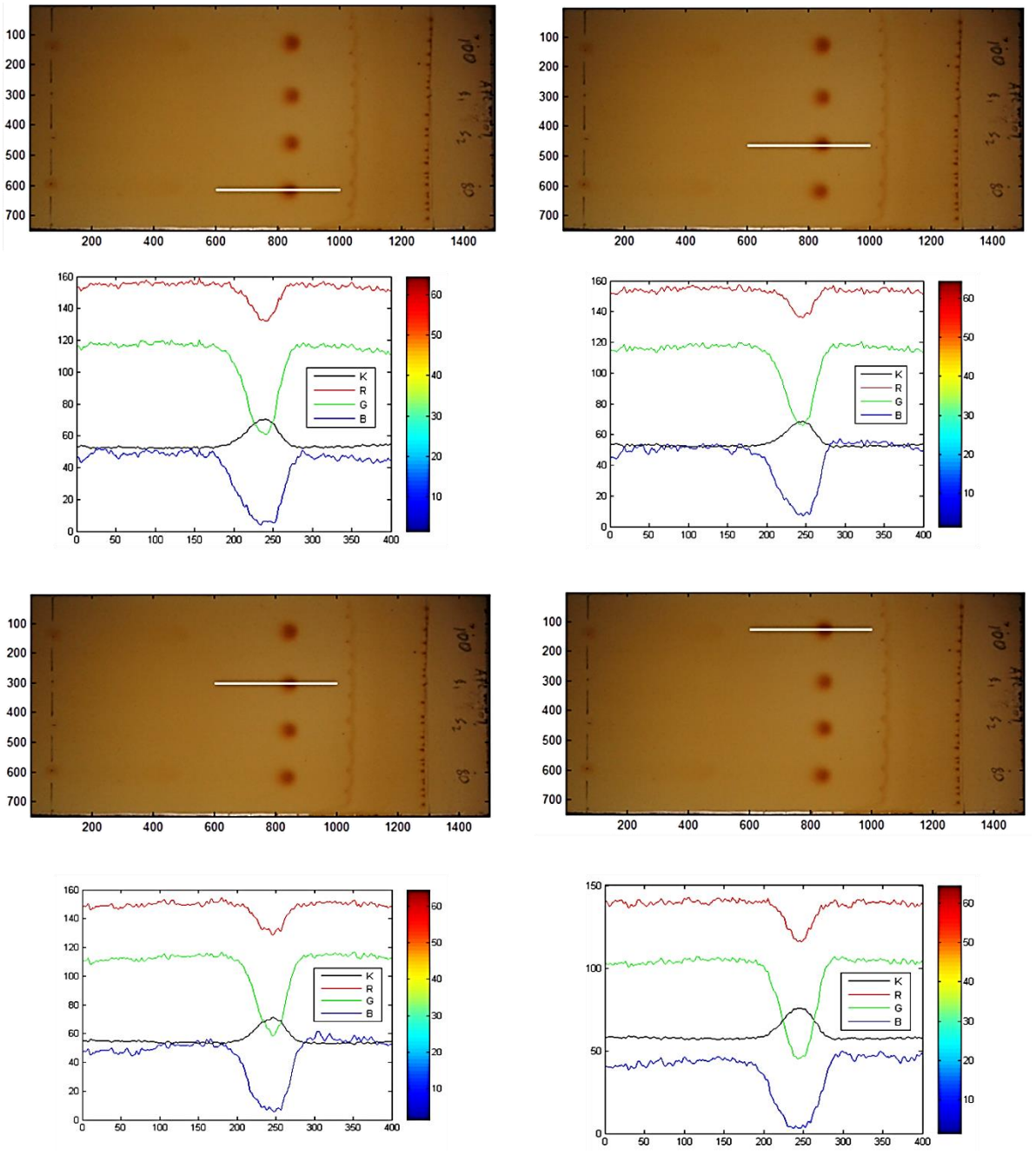

Figure S 2 DeTLC Scans for Atenolol lanes 1 to 4

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