Developed of a Cost-Affordable Thin-Layer Chromatography Testing Kit for Falsified and Substandard Pharmaceuticals in Tanzania

Eleanor Hatcher
elhatch@clemson.edu

Follow this and additional works at: https://tigerprints.clemson.edu/all_theses

Recommended Citation
https://tigerprints.clemson.edu/all_theses/4297

This Thesis is brought to you for free and open access by the Theses at TigerPrints. It has been accepted for inclusion in All Theses by an authorized administrator of TigerPrints. For more information, please contact kokeefe@clemson.edu.
DEVELOPMENT OF A COST-AFFORDABLE THIN-LAYER CHROMATOGRAPHY TESTING KIT FOR FALSIFIED AND SUBSTANDARD PHARMACEUTICALS IN TANZANIA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Bioengineering

by
Eleanor Lynn Hatcher
May 2024

Accepted by:
Dr. Delphine Dean, Committee Chair
Dr. Jordon Gilmore
Dr. Renée Lyons
ABSTRACT

A significant number of pharmaceuticals supplied to Tanzania are falsified or substandard. A majority of these inadequate pharmaceuticals are medications to treat life-threatening conditions. This problem not only causes mistrust in the healthcare system but also prolonged illness and potentially death for the patients. This calls for the need for the development of a cost-affordable testing kit for falsified and substandard pharmaceuticals in Tanzania.

Thin-layer chromatography (TLC) was used as the testing method since it is inexpensive and requires a low skill level to complete. Malaria, human immunodeficiency virus, hypertension, tuberculosis, and diabetes are five of the most prevalent diseases in Tanzania and medications to treat these diseases are commonly falsified or substandard. Doxycycline hyclate, tenofovir disoproxil fumarate, lisinopril dihydrate, pyrazinamide, and dapagliflozin are treatments for the diseases listed above that were used for testing in this project. Five rounds of baseline TLC testing were conducted to determine baseline retention factor (Rf) values for each of the pharmaceuticals. Five rounds of humidity and temperature-controlled TLC testing were conducted for each of the pharmaceuticals to determine if extreme environmental conditions would affect the results.

A graphical user interface was developed using MATLAB that contains a library of known Rf values that are referenced when a user enters their experimental values to determine the validity of the pharmaceutical. The user interface, alongside the developed TLC-based testing kit, will reduce overall costs while also effectively determining the
integrity of a drug. User testing will be conducted, and the kit will be taken to Arusha, Tanzania, to test in the target environment and obtain feedback from scientists at Arusha Technical College.
ACKNOWLEDGMENTS

I want to acknowledge my committee, Dr. Delphine Dean, Dr. Jordon Gilmore, and Dr. Renée Lyons, for guiding and supporting me through this degree. I also want to acknowledge my undergraduate assistants, Jade Bowers, Rachel Hillman, Sydney Lundeen, and Nicole Souza, for working with me in the laboratory to obtain results for this project. I could not have made the progress I did without their help. Additionally, I would like to acknowledge my fellow Multiscale Bioelectromechanics Lab members, Jeremiah Carpenter, Arianna Csiszer, Azrin Jamison, Andrew Landefeld, Diego Nigoa, and Calvin Paulsen. Their advice and guidance were invaluable to my work. I would finally like to acknowledge Arusha Technical College in Tanzania for their guidance and feedback throughout this project.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>CHAPTER</td>
</tr>
<tr>
<td>I. Introduction</td>
</tr>
<tr>
<td>The Problem</td>
</tr>
<tr>
<td>Project Overview</td>
</tr>
<tr>
<td>II. Literature Review</td>
</tr>
<tr>
<td>Origin of Falsified and Substandard Pharmaceuticals</td>
</tr>
<tr>
<td>Active Pharmaceutical Ingredients (APIs)</td>
</tr>
<tr>
<td>Current Solutions</td>
</tr>
<tr>
<td>Thin Layer Chromatography (TLC)</td>
</tr>
<tr>
<td>III. Methodology</td>
</tr>
<tr>
<td>Pharmaceutical Selection</td>
</tr>
<tr>
<td>Mobile Phase and Universal Solvent Selection</td>
</tr>
<tr>
<td>Mobile Phase Selection</td>
</tr>
<tr>
<td>Universal Solvent Selection</td>
</tr>
<tr>
<td>Baseline Testing</td>
</tr>
<tr>
<td>Humidity Testing</td>
</tr>
<tr>
<td>Temperature Testing</td>
</tr>
<tr>
<td>Development of User Interface</td>
</tr>
<tr>
<td>Steps for Adding a New Pharmaceutical to the Process</td>
</tr>
<tr>
<td>User Testing</td>
</tr>
<tr>
<td>Preparation for Testing in Tanzania</td>
</tr>
<tr>
<td>IV. Results and Discussion</td>
</tr>
</tbody>
</table>
Table of Contents (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Testing</td>
<td>29</td>
</tr>
<tr>
<td>Humidity Testing</td>
<td>30</td>
</tr>
<tr>
<td>Temperature Testing</td>
<td>34</td>
</tr>
<tr>
<td>Development of User Interface</td>
<td>35</td>
</tr>
</tbody>
</table>

V. Conclusion .................................................. 39

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing Kit Breakdown</td>
<td>39</td>
</tr>
<tr>
<td>Conclusions</td>
<td>40</td>
</tr>
<tr>
<td>Testing in Tanzania</td>
<td>40</td>
</tr>
<tr>
<td>Future Directions</td>
<td>41</td>
</tr>
</tbody>
</table>

APPENDICES .................................................................. 43

A: MATLAB Code for Graphical User Interface ................. 44

REFERENCES .................................................................... 47
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Medications were chosen to test based on prevalent diseases in Tanzania.</td>
<td>13</td>
</tr>
<tr>
<td>3.2</td>
<td>The mobile phase compositions were used for each drug that was tested.</td>
<td>16</td>
</tr>
<tr>
<td>4.1</td>
<td>Baseline testing results with their standard deviation represented.</td>
<td>30</td>
</tr>
<tr>
<td>4.2</td>
<td>Results of humidity testing at 90% humidity compared to the baseline results.</td>
<td>31</td>
</tr>
<tr>
<td>4.3</td>
<td>Results of humidity testing at 85% humidity compared to the baseline results.</td>
<td>33</td>
</tr>
<tr>
<td>4.4</td>
<td>Results of temperature testing at 38°C compared to the baseline results.</td>
<td>34</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The printing on a fake blister pack is less crisp at 32x magnification</td>
</tr>
<tr>
<td>2.2</td>
<td>Use of spectroscopy to determine drug composition</td>
</tr>
<tr>
<td>2.3</td>
<td>Visual representation of the TLC process</td>
</tr>
<tr>
<td>2.4</td>
<td>Global Pharma Health Fund’s Minilab testing kit</td>
</tr>
<tr>
<td>3.1</td>
<td>Representation of a TLC Test with the Mobile Phase</td>
</tr>
<tr>
<td>3.2</td>
<td>The chemical structure of acetone</td>
</tr>
<tr>
<td>3.3</td>
<td>Examples of TLC tests where the mobile phase was not the right match for the pharmaceutical</td>
</tr>
<tr>
<td>3.4</td>
<td>TLC tests where the mobile phase was the right match for the pharmaceutical</td>
</tr>
<tr>
<td>3.5</td>
<td>The chemical structure of ethanol</td>
</tr>
<tr>
<td>3.6</td>
<td>Protocol for TLC testing conducted in this paper</td>
</tr>
<tr>
<td>3.7</td>
<td>TLC plate with labels demonstrating what each marking represents</td>
</tr>
<tr>
<td>3.8</td>
<td>Humidity chamber set-up for humidity control testing</td>
</tr>
<tr>
<td>3.9</td>
<td>ProJet Finishing Oven used for temperature testing</td>
</tr>
<tr>
<td>3.10</td>
<td>Initial GUI screen prior to user entry</td>
</tr>
<tr>
<td>4.1</td>
<td>Baseline testing results with their standard deviation represented</td>
</tr>
<tr>
<td>4.2</td>
<td>Results of humidity testing at 90% humidity compared to the baseline results</td>
</tr>
</tbody>
</table>
List of Figures (Continued)

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>Results of humidity testing at 85% humidity compared to the baseline results</td>
<td>32</td>
</tr>
<tr>
<td>4.4</td>
<td>Results of temperature testing at 38°C compared to the baseline results</td>
<td>34</td>
</tr>
<tr>
<td>4.5</td>
<td>GUI screen when user values reflect the results of a legitimate drug</td>
<td>36</td>
</tr>
<tr>
<td>4.6</td>
<td>GUI screen when user values reflect the results of a falsified drug</td>
<td>37</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

The Problem

Over thirty percent of pharmaceuticals supplied to African countries are substandard or falsified. Some reasons for this include online business, light sanctions for drug infringers, ignorance, and lack of consumer education. [1] Substandard drugs occur due to insufficient expert knowledge, poor manufacturing processes, or insufficient infrastructure. [1,2] The World Health Organization (WHO) has defined counterfeit drugs as “one which is deliberately and fraudulently mislabeled with respect to identity and/or source.” [1,3] Counterfeit pharmaceuticals are produced by criminals who purposefully mislabel the drug’s identity. A specific example demonstrating the need for a way to detect falsified and substandard pharmaceuticals is the fight against malaria. The most common treatment for malaria is artemisinin-based combination therapy (ACT). Studies have shown that of the millions of doses of ACT medicines in Asia and sub-Saharan Africa, one-third are falsified or substandard. [4] Antimalarials are one of the most consumed drugs in many developing countries, and of the 12 main antimalarial drugs, there have been reports of at least eight of them being counterfeit. [5] The increase in falsified and substandard pharmaceuticals creates a hazard to the already crippling problem that developing countries face with the following prevalent diseases, malaria, Human Immunodeficiency Virus (HIV), hypertension, diabetes, and tuberculosis. One-fourth of generic hypertensive drugs were found to be of poor quality, and similar results were found in anti-tuberculosis medications. [6] According to the
National Academies of Science, Engineering, and Medicine, falsified pharmaceuticals can be classified into five categories based on the sophistication of the counterfeit. [7]

(1) The product is completely fraudulent, has unknown contents and effects, and significantly differs from the expected drug.

(2) Has a similar appearance to the expected drug, but the composition is unknown.

(3) Almost identical appearance to the expected drug but has a completely different composition.

(4) Almost identical appearance but contains an alternative drug with a therapeutic value like the expected product.

(5) Identical appearance and similar makeup that cannot be detected using most field and laboratory methods.

The WHO has established that the distribution of these illegal pharmaceuticals is a major problem that needs attention in developing countries. [8,9] For many patients with the aforementioned diseases, if they do not receive the proper medication, they will most likely die or suffer prolonged illness. Further, microbial resistance to the drug may develop if the medicine they are taking has subtherapeutic doses or lacks the active pharmaceutical ingredient. [10] Finally, the consistency at which drugs are inadequate and still distributed to patients has caused a large mistrust between the healthcare system and consumers in developing countries.
**Project Overview**

This paper summarizes the steps taken to design a cost-affordable and effective testing kit for falsified or substandard pharmaceuticals in developing countries, with an emphasis on Tanzania. A small selection of pharmaceuticals was chosen to be analyzed and tested to design protocols and factors to improve testing effectiveness while reducing cost. The pharmaceuticals utilized in this paper include Doxycycline hyclate, Tenofovir disoproxil fumarate, Lisinopril dihydrate, Pyrazinamide, and Dapagliflozin. Thin-layer chromatography (TLC) testing was utilized to determine a pharmaceutical's integrity, and baseline testing was conducted to obtain retention factor (Rf) values for each pharmaceutical. Environmental testing on humidity and temperature was also completed because many pharmacies in Tanzania lack air conditioning and, as a result, keep their windows and doors open, exposing their supplies to the external environment. The environmental testing was included to determine if extreme humidities or temperatures would alter the testing results. A graphical user interface was developed to eliminate cost and increase convenience by storing a library of known values so that the user can instantly compare their results to determine the validity of the sample being analyzed. Finally, the usability of the kit was tested by scientists not affiliated with the project to determine if the process was achievable by someone conducting the testing with no prior knowledge of the steps.
CHAPTER TWO
LITERATURE REVIEW

Origin of Falsified and Substandard Pharmaceuticals

The main reason for substandard pharmaceuticals is poor manufacturing practices. Poor manufacturing can stem from a lack of funding to pay for proper equipment or staff necessary to meet the good manufacturing processes, poor infrastructure, inconsistent regulatory checks, and the regulatory authority being completely unaware of the problem. [11]

Falsified pharmaceuticals are much more common than substandard pharmaceuticals and can occur anywhere in the process, from the production to the distribution of the drug. The falsification of pharmaceuticals is extremely easy and inexpensive, and the use of unregulated markets makes it easy to get away with the crime. It is also hard to convince law enforcement to investigate pharmaceutical crime when there is immediate pressure for more violent crimes. [11] Falsification of pharmaceuticals can come in many forms; including a perfect imitation of the expected pharmaceutical with the same active pharmaceutical ingredients (APIs) in the same packaging, medicine in the correct packaging but contains no APIs at all, medicine that contains ingredients different from what is labeled on the packaging, or the packaging is falsified. [12] The complexity of global importing and exporting of goods, as well as that of the supply chain and storage of medications, contributes greatly to the falsification of pharmaceuticals.
Active Pharmaceutical Ingredients (APIs)

Active pharmaceutical ingredients (APIs) are chemical-based compounds that serve as the main ingredient in diagnosing, curing, mitigating, or treating a specific disease. Every medication is composed of the API, the active component that accomplishes the desired function of the medication, and excipients, the inactive component that provides a function separate from actually treating the disease, such as API release time, color, or flavor. [13] They are often made of carbohydrates such as starch or lactose. Excipients' main function is to aid in the release of the API into the body as well as protect the API from degradation prior to the medication being taken. [14]

Many falsified pharmaceuticals contain either very little or no amount of API at all. In these cases, criminals will increase the amount of excipient used to make up for the lack of mass to mimic the expected weight of the drug. [15] Therefore, many falsified pharmaceuticals are purely made of excipients, meaning that patients are essentially being given a placebo or sugar pill. Given that the most falsified pharmaceuticals in Tanzania are those designed to treat severe illnesses, patients are receiving placebo pills instead of the medication required to preserve their lives.

Current Solutions

The main methods of pharmaceutical analysis can be categorized as visual inspection, testing physical properties, chemical tests, chromatography, spectroscopic testing, and mass spectrometry. [7] These categories contain techniques that vary in the
amount of training required, cost, effectiveness, and level of laboratory equipment required.

(1) Visual Inspection

Differences from the actual drug in color, size, shape, tablet quantity, and packaging can indicate a falsified or substandard medicine. Depending on the quality of the fake, an educated consumer could identify poor quality frauds; however, some falsified pharmaceuticals are hard to visually detect by trained experts.[16] The inspection of the packaging can often be a place to check for falsification. The packaging may have missing expiry dates, lack instructions, have poorly written instructions, and have spelling errors.[17] Figure 2.1 shows an example of a very fine difference in lettering between a legitimate and fake blister pack using high-magnification analysis.

Figure 2.1. The printing on a fake blister pack is less crisp at 32x magnification. [7]

Visual inspections are low-cost and low-level laboratory equipment required for detecting falsified pharmaceuticals; however, they are unreliable as many criminals have mastered creating identical copies of the product and packaging.
(2) Testing Physical Properties

Active ingredients in medicine are the most expensive part of the drug. Because of this, criminals will use dilute or impure active ingredients to decrease manufacturing costs and increase their profit. [7] Physical Properties such as density, solubility, reflectance spectra, and pH are all ways to test drugs to identify active ingredients. Testing of these properties can be used to distinguish authentic from fake drugs. However, not all drugs contain these distinctive properties, and criminals have found innovative ways to use certain impure ingredients that display the same physical properties as the expected active ingredient.

(3) Chemical Testing

Similar to testing physical properties, chemical reactions can test for the presence of active ingredients. The most common technique is colorimetry, or the process by which chemicals undergo color changes when reacted with certain compounds. [7] Colorimetry is low cost and requires very little training; however, it provides very limited information and often needs to be paired with another form of testing, usually with quantitative results, to confirm falsification. [18]

(4) Chromatography

Chromatography is the most common method of analyzing pharmaceuticals. [16] The chromatography process separates mixtures into their constituent parts and can provide both qualitative and quantitative
information about the drug's active ingredients. [9] Chromatography techniques come in a range of complexity and equipment required. Thin layer chromatography (TLC) is a simple planar chromatographic technique with visual inspection. This technique can determine both the identity of the sample as well as the amount of drug present. [17] High-performance liquid chromatography (HPLC) is a technique coupled with sensitive detectors that can identify and measure active ingredients and impurities. HPLC is known to be a rapid and highly accurate process. [19] Unfortunately, HPLC is expensive and requires skilled operators and reliable electrical power.

(5) Spectroscopic Testing

Spectroscopy is the process of measuring the interaction between matter and radiation to provide information on the chemical makeup of a sample. Spectroscopy can provide the “chemical fingerprints” of drugs and give insight into the drug composition that can be used to identify falsified and substandard pharmaceuticals. [7] Spectroscopic testing is moderately expensive and requires a moderate range of equipment and training.
(6) **Mass Spectrometry**

Mass spectrometry is an advanced technique that finds the precise molecular weight of a compound and provides structural information on the sample. Mass spectrometry can also compare fragmentation patterns to help distinguish compounds that differ by only one or two atoms. [21] This is one of the most precise and specific techniques for testing pharmaceuticals, as it can detect some of the most sophisticated drug copies resistant to identification from any other technique. This method of analysis requires extensive training and expertise and is quite expensive. [22,23]

**Thin Layer Chromatography (TLC)**

TLC is performed on a sheet of glass, plastic, or aluminum foil coated with a thin layer of adsorbent material known as the stationary phase. A liquid version of the sample is applied to the plate, and then the plate is placed into a solvent or solvent mixture known as the mobile phase. The mobile phase is drawn up the plate through capillary...
action, and separation is achieved since different analytes ascend at different rates. [24] The separation occurs based on the competition between the solute and the mobile phase in finding a place to bind on the stationary phase. Factors such as polarity and acidity affect how far compounds move before binding to the stationary phase. The mobile phase solvent is chosen based on the properties of the sample. Following the rules of solubility, like dissolves like, the mobile phase will carry the most soluble compounds the furthest up the TLC plate, while compounds that are less soluble in the mobile phase will remain at the bottom. [24,25] The components usually cannot be seen with visible light, so fluorescence from a UV light is used to illuminate the testing results.

Figure 2.3. Visual representation of the TLC process. [26,27,28]

Of all the analysis methods mentioned in the previous section, TLC offers low expense, training requirements, and material acquisition while providing quality assessments suitable for developing countries. TLC can offer “versatile and robust” drug testing in these developing countries by simplifying qualitative and quantitative assessments. [9,29] Users can determine the results of a TLC test using qualitative analysis by simply visualizing the plate under UV light to see how far the sample moved.
up the stationary phase. Quantitative analysis can be used to obtain more specific results by measuring exactly how far the sample traveled in comparison to how far the mobile phase traveled along the stationary phase. The WHO has determined that most of the invalid pharmaceuticals have either no drug at all or the wrong drug altogether, so TLC would easily discover these distinctions. [9]

There are TLC-based testing kits available for the detection of falsified drugs currently available. The Global Pharma Health Fund developed a TLC-based testing kit called the Minilab, which contains all necessary reagents and supplies to perform more than 1,000 TLC-based pharmaceutical tests. The Minilab requires minimal training, and the solvents used are low toxicity. However, the Minilab costs approximately 4,000 US dollars. [9] The excessive cost stems from the kit containing samples of every drug that the kit can analyze. Their kit is set up so that the user can run two TLC tests side by side, one with the suspect drug and the other with the known sample of the drug provided by the kit. Then, the user can compare the results of the two tests to see if they match to determine validity. While this testing kit is easy to use, effective, and efficient, the price of the kit is quite high and impractical for the targeted audience of developing countries.
Figure 2.4. Global Pharma Health Fund’s Minilab testing kit. [30]
CHAPTER THREE
METHODOLOGY

Pharmaceutical Selection

As mentioned in the introduction, falsified and substandard pharmaceuticals have become common among treatments for some of the most prevalent diseases in Tanzania, including malaria, hypertension, tuberculosis, diabetes, and HIV. Therefore, a medication for each of these diseases was chosen to be tested and used as the sample for this study. The following medications were found to be commonly used to treat or prevent the above diseases in Tanzania.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Medication for Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria</td>
<td>Doxycycline hyclate, 97%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Lisinopril dihydrate, 97%</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>Pyrazinamide, 98%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Dapagliflozin, 98%</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus (HIV)</td>
<td>Tenofovir disoproxil fumarate, 98%</td>
</tr>
</tbody>
</table>

Table 3.1. Medications were chosen to test based on prevalent diseases in Tanzania.

Mobile Phase and Universal Solvent Selection

The original goal for the kit was to be able to use a singular universal solvent and mobile phase. Using only one chemical as a solvent for all drugs and only one chemical as a mobile phase for each drug would eliminate extra costs accompanied by having unique solvents and mobile phases.
Mobile Phase Selection

A mobile phase that will cause the separation of different pharmaceutical compounds with varying properties would need to separate mixtures of high polarity, cause solvation with different compound properties, and have a strong hydrogen bonding ability to inhibit the binding of the compound to the plate surface. [31]

Figure 3.1. Representation of a TLC Test with the Mobile Phase. [32]

Acetone has high solubility and volatility and is less toxic than other common solvents, such as methanol. Articles have shown that the properties of acetone make it an ideal single, universal solvent mobile phase for TLC. [33,34,35,36,37] Each of the five pharmaceuticals were tested using pure acetone as the mobile phase, and despite acetone’s promising properties, none of the compounds moved from the baseline during the test. The exact same results occurred when using pure methanol as the mobile phase for each drug.
Using a singular, universal mobile phase would have been both convenient and more cost-affordable; however, with such different properties present in each drug, it was determined that each pharmaceutical would need a unique mobile phase tailored to its properties. Examples of testing done with mobile phases that did not work can be seen in the figure below, where streaking occurred, spots stayed at the bottom of the plate, or spots ran all the way up the plate with the mobile phase.

Figure 3.3. Examples of TLC tests where the mobile phase was not the right match for the pharmaceutical.
Examples of testing completed where the mobile phase was tailored to the pharmaceutical and produced expected results can be seen in the figure below.

Figure 3.4. TLC tests where the mobile phase was the right match for the pharmaceutical.

By referencing other articles that tested similar drugs and through much trial and error, the following mobile phases were determined to work for each drug.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Mobile Phase Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline hyclate</td>
<td>0.75:8:1.5 Diisopropylethylamine: Methanol: Ethanol</td>
</tr>
<tr>
<td>Lisinopril dihydrate</td>
<td>5:2 methanol: DI water</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>9:1 chloroform: methanol</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>9:1 chloroform: methanol</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate</td>
<td>9:1 chloroform: methanol</td>
</tr>
</tbody>
</table>

Table 3.2. The mobile phase compositions were used for each drug that was tested.
Universal Solvent Selection

A universal solvent must not react chemically with the sample and can fully dissolve compounds of different properties without heat. [31] Ethanol is considered a universal solvent because of its capability to dissolve polar hydrophilic and non-polar hydrophobic structures. Ethanol is commonly used as a singular solvent of compounds used for TLC testing, so each of the five drugs was dissolved using ethanol, and then testing was conducted. Ethanol successfully dissolved each of the drugs and allowed for the successful completion of testing.

![Figure 3.5. The chemical structure of ethanol. [39]](image)

Baseline Testing

100 mg of each pharmaceutical (in powder form) was dissolved in 10 mL of pure ethanol. Glass TLC plates with silica gel 60, cut at the size of five by ten centimeters, were marked using a pencil to create a baseline one centimeter from the bottom of the plate with a tick mark to indicate where to place the sample. Using a micropipette, three microliters of the dissolved pharmaceutical were placed on the tick at the baseline. Chemicals to create the respective mobile phase were placed in a beaker using a micropipette, and the ratio of mobile phase used was determined so that the height of liquid in the beaker would not be taller than the one-centimeter baseline of the TLC plate.
Once the spot had dried, the TLC plate was placed in the beaker containing the respective mobile phase, and a watch glass was placed on top. The TLC test was run for ten minutes. After ten minutes, the plate was removed, and the height at which the mobile phase traveled was marked. After the plate was completely dry, it was observed under UV light using the MilliporeSigma handheld 254 nm UV lamp containing both UVB and UVC rays, and the compound spot seen was marked with a pencil. The retention factor (Rf) value was calculated for each test using the following equation. Each pharmaceutical had five baseline tests completed.

\[ Rf = \frac{\text{distance from baseline to middle of point of interest}}{\text{distance mobile phase traveled from baseline}} \]

The exact protocol followed can be seen below.
TLC Testing for Falsified Pharmaceuticals Protocol

1. If pharmaceutical is in pill form, crush it into a fine powder using a mortar and pestle. If pharmaceuticals are in a capsule, open the capsule and collect the powder.
2. Measure 100 mg of the pharmaceutical in powder form.
   a. Place a weighing boat on a scale and tare the scale (make it zero with the weighing boat) then add the pharmaceutical powder to the weighing boat until it reaches 100 mg.
3. Mix the 100 mg of the pharmaceutical with 10 mL pure ethanol and swirl or vortex until dissolved.
4. Obtain a 250 mL beaker, or container of a similar size and fill it with the chemical composition to create the mobile phase for the respective pharmaceutical you are testing.
   a. A micropipette set to 1000 µL can be used as equivalent to 1 mL.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Mobile Phase Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline Hyclate</td>
<td>0.75 mL Diisopropylethylamine: 8 mL Methanol: 1.5 mL Ethanol</td>
</tr>
<tr>
<td>Lisinopril dihydrate</td>
<td>10 mL methanol: 4 mL DI water</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>9 mL chloroform: 1 mL methanol</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>9 mL chloroform: 1 mL methanol</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate</td>
<td>9 mL chloroform: 1 mL methanol</td>
</tr>
</tbody>
</table>

5. Place a watch glass over the beaker once you have created the mobile phase.
6. Obtain a TLC plate and using a pencil, create a baseline approximately 1 cm from the bottom of the plate. Create a tick mark on the baseline to indicate where to place the drug sample. (see example below)

Figure 3.6. Protocol for TLC testing conducted in this paper.
7. Using a micropipette, place 3 µL of the drug/ethanol mixture onto the drawn tick mark. Let this spot dry.

8. After the spot is dry, place the TLC plate into the beaker with the mobile phase, baseline side of the TLC plate at the bottom of the beaker. Place the watch glass back on top after and start a 10 minute timer.

9. After the 10 minutes is done, remove the TLC plate and mark where the mobile phase traveled to (where the TLC plate stops being wet) using a pencil. Then let the plate dry.

10. Once the plate is dry, observe the plate using the UV light.
11. Using a pencil, circle the spot you see under the UV light.
12. Measure the distance from the baseline to the line where the mobile phase traveled to.
13. Measure the distance from the baseline to the center of the sample spot.
14. Calculate the Rf value using the equation below.

\[
R_f = \frac{\text{distance from baseline to center of sample}}{\text{distance from baseline to where the mobile phase traveled}}
\]

15. Using the user interface app, enter the drug you tested, the current humidity, and the Rf value calculated to obtain the result of your testing.

Figure 3.6 (Continued). Protocol for TLC testing conducted in this paper.
The figure below demonstrates how a TLC plate should be properly marked.

![Figure 3.7. TLC plate with labels demonstrating what each marking represents.](image)

**Humidity Testing**

Humidity testing was conducted by creating a self-made humidity chamber using a large Tupperware container (9.8” x 7.1” x 5.9”), a humidity sensor (Goabroa Mini Hygrometer Thermometer Digital Indoor Gauge Monitor), and a mini humidifier (Portable Mini 300 mL Humidifier 4.96” x 3.31” x 3.31”) as can be seen in figure 3.8 below.

![Figure 3.8. Humidity chamber set-up for humidity control testing.](image)
Humidity testing was conducted by creating a model environment with a humidity of 90 percent, as Tanzania’s humidity levels often range between 58 percent and 87 percent. [40] A large Tupperware was placed upside down with a humidity sensor and a mini humidifier inside it. The humidifier ran until the sensor reached 90 percent humidity. Then, the TLC test was started and placed inside the Tupperware while the humidifier was removed. The sensor remained inside the Tupperware throughout the test to monitor the humidity levels and ensure the environment remained at approximately 90 percent humidity. After ten minutes, the test was completed, the TLC test was removed, and Rf values were calculated. This test was completed five times for each of the pharmaceuticals.

Any pharmaceutical that showed deviation in Rf values compared to the baseline results went through another round of humidity testing at 85 percent humidity to determine the percentage of humidity the results began to deviate.

**Temperature Testing**

Temperature testing was conducted using a ProJet Finishing Oven to create a model environment with a set temperature.
Temperature testing was conducted at 38°C to model an extreme temperature scenario. The oven was set up and reached the desired temperature of 38°C before testing was conducted. Once the desired temperature was reached, the TLC test was started, and the beakers were placed inside the oven for the remainder of the ten-minute test. After ten minutes, the test was completed, the TLC test was removed, and Rf values were calculated. This test was completed five times for each of the pharmaceuticals.

**Development of User Interface**

An application was created to allow for user interface and reference of a library of known Rf values. The purpose of the application is to easily check the validity of the drug the user is testing by comparing the Rf value collected to a library of known values incorporated into the application. The application was created using the MATLAB software through MathWorks. Code was written to create a graphical user interface
(GUI) that can be used on computers, tablets, or smartphones. The GUI asks users to select what pharmaceutical is being tested and to enter the current humidity and the Rf value that was observed. The GUI, when it first pulls up, is demonstrated in the figure below.

![Figure 3.10. Initial GUI screen prior to user entry.](image)

**Steps for Adding a New Pharmaceutical to the Process**

The first step in adding a new pharmaceutical to the process is to analyze its’ properties to determine what mobile phase to use. Factors such as polarity, solubility, and viscosity of your pharmaceutical need to be considered when determining a mobile phase for the pharmaceutical. Optimal interaction needs to be met for the mobile phase to properly separate the pharmaceutical and move up the TLC plate. After determining these properties, looking into previous work and literature to see what others have used for a mobile phase can be helpful in determining what chemical mixtures would work
best as a mobile phase. Finally, trial and error can be used to test the mobile phases and
determine which chemical makeup works best for the pharmaceutical.

Once the mobile phase has been determined, baseline testing should be conducted
to determine the standard Rf value range for comparison to other tests for this drug.
Furthermore, environmental testing should be conducted to determine if high levels of
humidity or temperature will affect the results of the tests.

Finally, once testing has been completed, the pharmaceutical would need to be
added to the coding of the user interface. This will allow for the results of a TLC test of
this pharmaceutical to be analyzed by the user interface to determine the integrity of the
drug.

**User Testing**

The goal of conducting user testing was to confirm that the process of using the
kit and following the procedures given can be completed successfully and easily by
anyone with a laboratory background. Clemson bioengineering students without
background knowledge of the project will be recruited to complete the user testing as
they would have laboratory skill levels that can reflect our target users, pharmacists.

Since user testing is a research activity involving human subjects, a review from
the Institutional Review Board was required prior to conducting any testing. Since this
form of human testing presents no more than minimal risk and protects the anonymity of
participants, the research qualified for expedited review.
After receiving informed consent from participants, ten Clemson Bioengineering undergraduate students with no prior knowledge of the research will complete user testing while being observed by two researchers. Five of the ten participants will have experience with using a micropipette, while the other five will not have experience using a micropipette. Pharmacists have varying experience with different laboratory equipment, so using participants without micropipette experience will reflect pharmacists who have little to no experience with micropipettes. Participants will be given step-by-step instructions on how to run the TLC test and will be provided with the equipment to conduct a TLC test of ibuprofen. After completing the test, they will be asked to use the GUI and present their findings to the researchers. Following this, they will be asked to complete a short survey about their experience and confidence during the test. These are the questions that each participant will be asked to answer.

1. On a scale of 1 to 5, how confident were you in conducting this test (1 being it was hard, and I did not know what I was doing, 5 being I was confident I was doing the right thing during the test)?

2. On a scale of 1 to 5, how easy was it to follow the protocol (1 being I was confused and not sure if I was doing the right thing, 5 being I knew what I was expected to do and felt confident following the steps)?
3. On a scale of 1 to 5, how do you feel your laboratory background prepared you for this test (1 being I was not familiar with the equipment used, 5 being I knew exactly how to use the equipment)?

4. Would you change anything about the test? (protocol, user interface, etc.)

While observing the participants, the researchers will also fill out a short survey about their observations as well as documenting if the participant obtained the expected result. These are the questions that the observers will answer for each participant.

1. Did the participant ask any questions? If so, what did they ask? Did you respond? If so, what was the response?

2. Did the participant receive the expected result?

3. If not, where do you believe the error could have occurred?

4. While observing this participant, is there anything you would change about the test? (protocol, user interface, etc.)

5. How long did the test take from when they began making the mobile phase to when they got their result on the user interface?

The data collected from the participants and researchers will be analyzed to determine how user-friendly the process is for someone using the kit for the first time.
Preparation for Testing in Tanzania

The TLC-based testing kit developed from this research is being taken to Arusha, Tanzania, in May 2024 to test the usability of the kit in the target environment. Partners at Arusha Technical College will assist us in conducting this testing in a pharmacy or laboratory setting. However, when taking the kit to Tanzania, the drugs that have been tested cannot be transported internationally. For the ability to use the kit in Tanzania, baseline, humidity, and temperature tests were conducted with the use of ibuprofen, as this can be taken to Tanzania. The same protocol was used for the baseline, humidity, and temperature testing of the ibuprofen as was used for the other pharmaceuticals. The ibuprofen tablets were crushed, and 100 mg of the powder was dissolved in 10 mL of methanol. The mobile phase used for the ibuprofen was a 10 to 1 ratio of chloroform to methanol, respectively. Five rounds of baseline testing were completed, followed by five rounds of humidity testing and then five rounds of temperature testing.
CHAPTER FOUR
RESULTS AND DISCUSSION

Baseline Testing

Five baseline tests were completed for each of the five pharmaceuticals being analyzed. The results fell within the expected ranges and are represented in the following graph and table.

![Baseline Testing Results]

Figure 4.1. Baseline testing results with their standard deviation represented.
The results of each drug tested were consistent enough that the standard deviation was 0.02 or 0.03, which shows that the mobile phase used for each pharmaceutical properly separated the sample and moved it along the TLC plate. It was decided that the consistency of the results for each drug indicated that they were enough to represent the standard baseline for each drug to compare all other tests.

### Humidity Testing

Five humidity tests were completed at 90 percent humidity for each of the five analyzed pharmaceuticals. The results are represented in the following graph and table.
Figure 4.2. Results of humidity testing at 90% humidity compared to the baseline results.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Baseline Rf Value</th>
<th>Rf Value with 90% Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline hyclate</td>
<td>0.54 ± 0.02</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>Lisinopril dihydrate</td>
<td>0.48 ± 0.02</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>0.55 ± 0.03</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>0.26 ± 0.03</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate</td>
<td>0.50 ± 0.02</td>
<td>0.50 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4.2. Results of humidity testing at 90% humidity compared to the baseline results.

Based on these results, even at 90 percent humidity, the results of Lisinopril dihydrate and Tenofovir disoproxil fumarate are not affected. This indicates that even at the highest expected humidity in Tanzania of 87 percent, the results of these two drugs will not be affected.
Meanwhile, Doxycycline hyclate, Pyrazinamide, and Dapagliflozin showed deviations in their Rf values when exposed to 90 percent humidity compared to the baseline results collected. The deviation was significant enough that these results would normally suggest that the drug was illegitimate, as several of the results for each drug fell outside of the baseline range. However, the results for each test run at 90 percent humidity proved consistent, indicating that this range of values could be a good representation of what to expect in these environmental conditions.

Further testing was conducted to determine at what humidity level deviation begins for each pharmaceutical. Five humidity tests at 85 percent humidity were completed for Doxycycline hyclate, Pyrazinamide, and Dapagliflozin. The results are represented in the following graph and table.

![Graph showing 85% Humidity Results vs. Baseline Results](image)

Figure 4.3. Results of humidity testing at 85% humidity compared to the baseline results.
<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Baseline Rf Value</th>
<th>Rf Value with 85% Humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline Hyclate</td>
<td>0.54 ± 0.02</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>0.55 ± 0.03</td>
<td>0.56 ± 0.02</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>0.26 ± 0.03</td>
<td>0.25 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4.3. Results of humidity testing at 85% humidity compared to the baseline results.

Based on the results from humidity testing at 85 percent humidity, all drugs tested fall within the expected Rf range compared to the baseline results. Therefore, it was determined that at humidity of 85 percent or less, TLC testing of any of the five drugs will not be affected by humidity as an environmental factor.

Based on these results, it was determined that if users report their current humidity as at or below 85 percent, they can compare their results to those found from baseline testing. However, if they report their humidity to be above 85 percent and are testing for Doxycycline hyclate, Pyrazinamide, or Dapagliflozin, they can compare their results to those found from the humidity testing at 90 percent.

These findings are significant since many pharmacies in Tanzania do not have air conditioning. This lack of air conditioning leads to many pharmacies keeping their doors and windows open, causing the conditions of the pharmacy to reflect the external environment. Therefore, determining how different levels of humidity affect the results was imperative to this work.
**Temperature Testing**

Five temperature tests were completed at 38°C for the five analyzed pharmaceuticals. The results are represented in the following graph and table.

![Figure 4.4. Results of temperature testing at 38°C compared to the baseline results.](image)

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Baseline Rf Value</th>
<th>Rf Value at 38°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline hyclate</td>
<td>0.54 ± 0.02</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>Lisinopril dihydrate</td>
<td>0.48 ± 0.02</td>
<td>0.50 ± 0.01</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>0.55 ± 0.03</td>
<td>0.56 ± 0.01</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>0.26 ± 0.03</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate</td>
<td>0.50 ± 0.02</td>
<td>0.50 ± 0.02</td>
</tr>
</tbody>
</table>

Table 4.4. Results of temperature testing at 38°C compared to the baseline results.
Based on these results, it was determined that the variance in Rf values was minor, no more than 0.01 difference, compared to the baseline values, that at 38°C or less, temperature will not affect the results of the TLC testing. As mentioned previously, one of the highest reported temperatures in Tanzania was 36°C, so the determination that temperatures as high as 38°C would not affect the testing results was significant. Since pharmacies in Tanzania often keep their windows and doors open, the heat from warm days will not affect the results obtained from the kit.

Other TLC-based testing methods, including Global Pharma Health Fund’s Minilab, run two TLC tests simultaneously, one of the suspicious sample and one of the known sample provided by the Minilab, to compare the results of these tests to determine the integrity of the drug. By running both tests in the same environment, the effects of environmental factors were of no concern as they would experience the same conditions. With the given results of the humidity and temperature testing, it can be determined that the need to run a known test in the same conditions is not necessary, and excluding this process from the kit developed in this project is acceptable.

**Development of User Interface**

The MATLAB graphical user interface was designed to take into consideration the user’s drug, humidity, and calculated Rf value to determine if the drug under investigation is either legitimate or falsified. The software first reads what drug is being tested. Once this is determined, it determines if the current humidity is greater than 85 percent or at 85 percent or less. The software will compare the Rf value entered to the
values associated with the appropriate drug and humidity range. If the Rf value falls within the expected range based on all of the above criteria, then the graphical user interface will display the word “Legitimate,” as can be seen below.

![GUI screen when user values reflect the results of a legitimate drug.](image)

Figure 4.5. GUI screen when user values reflect the results of a legitimate drug.

If the Rf value does not fall within the expected range based on all of the above criteria, then the graphical user interface will display the word “Falsified,” as can be seen below.
Figure 4.6. GUI screen when user values reflect the results of a falsified drug.

This GUI is the preliminary version of a user interface that will eventually be converted into a smartphone app that any user can download for free. The use of the app is imperative to this project, as it is the main source of eliminating the cost of the kit. By including a library of known Rf values that the app references after analyzing the user inputs, the app determines if the drug is falsified or legitimate, assuming the user follows the same parameters explained in the kit for testing. The Global Pharma Health Fund’s Minilab testing kit includes samples of every drug that can be tested by the kit so that the user can run the known sample provided at the same time as the suspect sample and then compare the results at the end to determine legitimacy. The inclusion of the drug samples and extra materials is the reason for the Minilab being as expensive as it is. By eliminating the need for these drug samples, the cost of the kit becomes significantly more affordable.
The use of a GUI, or eventually a smartphone app, simplifies the process of comparing experimental results to known values as it eliminates the potential for human error and expedites the process to obtain a result.
CHAPTER FIVE

CONCLUSION

Testing Kit Breakdown

Based on communication with partners at Arusha Technical College in Tanzania, it was determined that pharmacists and technicians should have access to ethanol, diisopropylethylamine, methanol, DI water, chloroform, pipettes, pencils, rulers, beakers or jars, watch glass or lid, micropipettes, and a smartphone. Considering this, the testing kit will only need to consist of a handheld UV light and TLC plates. Plastic transfer pipettes can also be included in case micropipettes are not available in the pharmacy. The kit will also have the option to add vials of desired chemicals at an extra cost in case a pharmacy does not have a particular chemical needed for the mobile phase of a certain pharmaceutical.

The MilliporeSigma 254nm handheld UV lamp used in the testing conducted in this paper costs 313 US dollars. A pack of 50 glass TLC plates with silica gel 60 costs 42 US dollars. A pack of 100 1.5 mL plastic, non-sterile transfer pipettes costs 8.75 US dollars. The Goabroa mini hydrometer used in the testing conducted costs 7.90 US dollars. The smartphone app with a library of known Rf values would be free of charge. Therefore, a kit with the capability to conduct 50 tests would have an initial cost of 371.65 US dollars. The price of the UV light is a one-time cost, so after the initial purchase of the testing kit, users can order TLC plates separately at a lower cost. Additional costs would be added to users who request the extra vials of chemicals, and the price would be subject to the specific chemical.
Conclusions

In conclusion, the use of TLC is an effective and cost-affordable method for testing the integrity of pharmaceuticals in developing countries such as Tanzania. It was determined that environmental factors, specifically high humidity, can alter the results of TLC tests. This finding was considered in the development of the user interface by incorporating the assessment of the humidity conditions during the test and referencing the appropriate set of Rf values for said conditions. The use of a user interface that references a library of known Rf values further reduces the cost of testing by eliminating the need for samples of every pharmaceutical in the testing kit. Finally, the development of this cost-affordable TLC-based testing kit for falsified and substandard pharmaceuticals in Tanzania can reduce the risk of further illness, death, and mistrust in the healthcare system.

Testing in Tanzania

In May 2024, the TLC-based testing kit developed from this research will be taken to Arusha, Tanzania, to show the research and findings of this project to partners at Arusha Technical College. By taking the kit to Tanzania, testing can be conducted in the target environment, and the usability of the kit in the target setting can be analyzed. Our partners at Arusha Technical College will assist us in conducting this testing in a pharmacy or laboratory setting. Results and feedback obtained from this trip will be used to further enhance the research and development of the TLC-based testing kit.
Future Directions

The findings of this project serve as preliminary data for proof of concept of a cost-affordable, TLC-based testing kit for falsified and substandard pharmaceuticals in Tanzania. Future directions for this project include incorporating more pharmaceuticals into the testing pool, considering more environmental factors, and further developing the app.

Only five pharmaceuticals were originally chosen to begin with so that the procedure and kit parameters could be developed. These five pharmaceuticals served as the initial sample group to determine if the kit and testing methods would work. Next steps would include the incorporation of more drugs that are also commonly falsified to add that data into the library of known values.

Humidity and temperature testing was conducted to determine if the external environmental factors would affect the results of the test. However, these environmental factors were only applied to the drugs while they were being tested. The next step would include testing to see if the storage of these pharmaceuticals in these conditions would affect the integrity of the drug and cause different results from the testing because of the storage conditions.

A final future direction is to further develop the app to make it a full-functioning smartphone app accessible to anyone. Another consideration for the app is to develop it so that it can use the smartphone’s camera to scan the TLC plate and measure the Rf value from the image captured. This would eliminate a step for the user and cut down on the potential for human error.
The preliminary results in this paper have determined that the development of a cost-affordable, TLC-based testing kit for falsified and substandard pharmaceuticals in Tanzania can be completed using the protocol and considerations discussed in this paper. By using the findings from this project as the baseline of testing and the skeleton of the kit, further work can be completed to develop a full-functioning product.
Appendix A

MATLAB Code for Graphical User Interface

function DrugTestWorks()

    % Create a figure
    fig = figure('Position', [100, 100, 400, 300], 'Name', 'Drug Validation');

    % Create User Interface components
    drugLabel = uicontrol('Style', 'text', 'String', 'Select Drug:', ...
                            'Position', [50, 250, 100, 20]);
    drugPopup = uicontrol('Style', 'popupmenu', 'String', {'Doxycycline hyclate', 'Lisinopril dihydrate', 'Pyrazinamide', 'Dapagliflozin', 'Tenofovir disoproxil fumarate'}, ...
                           'Position', [150, 250, 200, 20]);

    humidityLabel = uicontrol('Style', 'text', 'String', 'Humidity (%):', ...
                           'Position', [50, 200, 100, 20]);
    humidityEdit = uicontrol('Style', 'edit', ...
                           'Position', [150, 200, 200, 20]);

    rfLabel = uicontrol('Style', 'text', 'String', 'Rf Value:', ...
                           'Position', [50, 150, 100, 20]);
    rfEdit = uicontrol('Style', 'edit', ...
                           'Position', [150, 150, 200, 20]);

    validateButton = uicontrol('Style', 'pushbutton', 'String', 'Validate', ...
                           'Position', [150, 100, 100, 30], 'Callback', @validateCallback);

    resultLabel = uicontrol('Style', 'text', 'String', '', ...
                           'Position', [150, 50, 200, 20]);

    % Callback function for the validate button
    function validateCallback(~, ~)
        % Get selected drug, humidity, and Rf value
        selectedDrugIndex = get(drugPopup, 'Value');
        selectedDrug = get(drugPopup, 'String');

Figure A-1: MATLAB code created to develop the graphical user interface.
selectedDrug = selectedDrug{selectedDrugIndex};
humidity = str2double(get(humidityEdit, 'String'));
Rf = str2double(get(rfEdit, 'String'));

% Check validity based on drug and conditions
if strcmp(selectedDrug, 'Doxycycline hyclate')
    if humidity <= 85
        if Rf >= 0.52 && Rf <= 0.56
            result = 'Legitimate';
        else
            result = 'Falsified';
        end
    else
        if Rf >= 0.55 && Rf <= 0.63
            result = 'Legitimate';
        else
            result = 'Falsified';
        end
    end
elseif strcmp(selectedDrug, 'Lisinopril dihydrate')
    if Rf >= 0.46 && Rf <= 0.50
        result = 'Legitimate';
    else
        result = 'Falsified';
    end
elseif strcmp(selectedDrug, 'Pyrazinamide')
    if humidity <= 85
        if Rf >= 0.52 && Rf <= 0.58
            result = 'Legitimate';
        else
            result = 'Falsified';
        end
    else
        if Rf >= 0.55 && Rf <= 0.61
            result = 'Legitimate';
        else
            result = 'Falsified';
        end
    end
elseif strcmp(selectedDrug, 'Dapagliflozin')
    if humidity <= 85
        if Rf >= 0.23 && Rf <= 0.29
            result = 'Legitimate';
        else
            result = 'Falsified';
        end
    end
end

Figure A-1 (Continued): MATLAB code created to develop the graphical user interface.
result = 'Falsified';
    end
else
    if Rf >= 0.21 && Rf <= 0.25
        result = 'Legitimate';
    else
        result = 'Falsified';
    end
elseif strcmp(selectedDrug, 'Tenofovir disoproxil fumarate')
    if Rf >= 0.48 && Rf <= 0.52
        result = 'Legitimate';
    else
        result = 'Falsified';
    else
        result = 'Invalid drug selection';
end

% Display the result
set(resultLabel, 'String', result);
end
REFERENCES

11. Institute of Medicine (U.S.). Committee on Understanding the Global Public Health Implications of Substandard F, Buckley GJ, Gostin LO (Lawrence O. Countering the problem of falsified and substandard drugs. .


26. Chavarrria J. Pipette Icon. FLATICON.


