Student Authors

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**METHOD TO IMPROVE DRUG SAFETY IN AFRICA:**

A Pharmaceutical Learning Module

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Abstract

Substandard, spurious, falsely labeled, falsified, and counterfeit (SSFFC) medications are a major concern in countries around the world, especially in the developing world. Forged and substandard medications pose major threats to the well-being and economic status of citizens in developing countries. This research focuses on designing an educational module and laboratory exercise that will teach students how to detect SSFFC medications with a universal method using high-performance liquid chromatography (HPLC). This research is also focused on the implementation of the module at the Kilimanjaro School of Pharmacy (KSP) located in Tanzania. Acetaminophen tablets from four countries were purchased in Tanzania and tested at Purdue University and KSP yielding an adequate sample of HPLC data to establish the purity of the drug product. Utilizing the approach developed here, additional medications can be analyzed to combat concerns about SSFFC medications in Tanzania and throughout the developing world. Furthermore, we describe the implementation of this method in Tanzania at KSP.

INTRODUCTION

Substandard, spurious, falsely labeled, falsified, and counterfeit (SSFFC) medications are a global problem with their prevalence being more apparent in countries in the developing world including Tanzania. SSFFC drugs pose major problems to the individuals who take them including the emergence of antimicrobial resistance and increased morbidity and mortality (Mwambete, 2014). In addition, overuse and misuse of antibiotic drugs is frequently reported (Viberg, Kalala, Mujinja, Tomson, & Lundborg, 2010). It is common for SSFFC drugs to be sold by vendors in markets throughout Tanzania. These drugs mimic the size, shape, and...
Additionally, this paper describes a universal method that can be ensured using laboratory assays and methods. Indirectly, this module also could be used outside the developing world to illustrate the principles of high-performance liquid chromatography (HPLC) and drug quality.

Since the Pharmacy Education Action Plan described that a “one size fits all” educational model is not a practical solution for providing pharmaceutical education to all areas of the world and because some individuals lack sufficient knowledge in certain pharmaceutical practices (Anderson et al., 2008), the Global Development Team of Purdue University developed a local pharmaceutical education model that will help train the future pharmacists of Tanzania. This module contains an initial video on the problems of counterfeiting, a video on the utilization of HPLC to detect SSFFC medications, and a laboratory module utilizing the universal method to detect SSFFC drugs. We believe these modules can be further used in the United States to educate pharmacists.

**METHODS**

An Agilent 1100 HPLC equipped with ChemStation software was used for the study. Standard HPLC solvents were used. In Tanzania, HPLC solvents can be obtained from Kenya and other major HPLC suppliers. Proper laboratory personal protective equipment (PPE) is required, including gloves, long pants, closed toed shoes, safety glasses, and lab coats. All glassware is cleaned and prewashed with methanol.

An Agilent Zorbax 4.6 cm C-18 column with 5-micron particle size is used. The column is pre-equilibrated with 50:50 ACN (Acetonitrile): H₂O (water).

Agilent ChemStation software is used to analyze the data. The Data Analysis tab allows easy determination of peak areas and percentages.

Typically, an average of three injections are used for determination of percentages. In some cases, three separate preparations with three injections each are used.

As an example, for acetaminophen, the contents of ten 500 mg tablets are added to a 200 milliliter (mL) volumetric flask and diluted with 200 mL of buffer.
The solution is stirred, filtered, and tested in a 3:1 methanol to water gradient over 10 minutes. The wavelength of the detector is set a 243 nanometers (nm).

An autonomous laboratory model using acetaminophen as the testing material, below, can provide an understanding of the applicability of this process to both the analysis of a medication for SSFFC properties and the use of HPLC units by students to understand the quality of a drug.

The procedure outlined below constitutes our current universal method. Furthermore, the universal method was applied to Amoxicillin, Quinine, Coartem, Hydrochlorothiazide and Mefloquine tablets. Our preliminary studies suggest it can be used for these drug products. Initial studies of Ciprofloxacin, Dexamethasone, Metformin, Carvedilol, Amitriptyline and Haloperidol based on overlap between the Walmart $4 list and the WHO Essential Medicines list have been conducted.

EDUCATIONAL MODULE 1: ACETAMINOPHEN

Purpose

The United States Pharmacopeia (USP) is responsible for setting and publishing the standards for testing the quality of all medications. The USP contains verified methods for analyzing drugs, including the quantified best HPLC procedures for Acetaminophen.

In this lab, we will separate and quantify the amount of acetaminophen in various products. Parameters set forth in Table 1 will be utilized.

We also utilized acetaminophen USP-level standard from Purdue University Industrial and Physical Pharmacy Department and Methanol purchased from Sigma-Aldrich. Fresh ultrapure water was obtained from a Milli-Q Integral system.

Preparation of Standard Solutions

First, 100 mg of acetaminophen was placed in a 200 mL volumetric flask. Then, 100 mL of mobile phase was added to the flask and it was stirred and diluted to a volume of approximately 200 mL with mobile phase. Using a funnel and an empty flask, the solution was passed through filter paper. Finally, 1.5 mL of solution was placed into an HPLC vial.

Solvents

The universal method makes use of four solvents: water, methanol, acetonitrile, and 0.5M potassium phosphate. For acetaminophen, only methanol and water were utilized.

Preparation of Drug Tablet Assay Samples

Table extraction was completed for Equate brand 500 mg acetaminophen tablets from Tanzania. This was prepared by crushing ten Equate brand 500 mg acetaminophen tablets with a mortar and pestle.
adding 100 mg of the crushed powder to a 200 mL volumetric flask, and adding 100 mL of mobile phase to the flask. After approximately 10 minutes of mixing, the solution is diluted to 200 mL with mobile phase and mixed for approximately another 5 minutes. Using a funnel and an empty flask, the solution is passed through filter paper. Finally, 1.5 mL of solution is placed in an HPLC vial.

Measurements

Each HPLC trial, for all samples, consisted of a blank run and 3 sample runs. A reference standard was prepared and tested in three separate trials. The average retention time of the reference standard was 5.04 minutes with an average peak area of 99.95%. A sample result is shown in Figure 1.

Three separate extractions of Equate brand acetaminophen tablets were prepared and tested in three separate trials each, for a total of nine trials. The average results of these extractions were used to set acceptance limits for this method as a retention time of 5.05 minutes and a peak area percent of 99.95% with a standard deviation of 0.011 and 0.053, respectively. Limits to the acceptance criteria were set based on verification that extraction test results were significantly tighter than these values. A sample result for the extractions, indicative of expected results for this method is shown in Figure 2.

One unfiltered extraction of Equate brand acetaminophen tablets was prepared and tested in three separate trials. Tablet samples were filtered for accurate results. If the sample was not filtered, it may appear similar to Figure 3, with two wide peaks between 4.5 and 5.5 minutes.

Once the universal method for acetaminophen was established, further studies were conducted on amoxicillin tablets using the HPLC.

Figure 1. Sample chromatogram for HPLC acetaminophen reference standard.

Figure 2. Sample chromatogram for HPLC acetaminophen tablet extraction is indicative of expected results for this procedure.

Figure 3. HPLC chromatogram for unfiltered acetaminophen tablet extraction.
**EDUCATIONAL MODULE 2: AMOXICILLIN**

**Purpose**

Established United States Pharmacopeia (USP) methods were referenced when shaping the proper HPLC method for amoxicillin capsules. These reference methods are governed by the USP who is responsible for the determining and publication of analysis on the quality of all medications.

Amoxicillin will be isolated and measured, and results will be compared to products of the generic tablet of the same makeup in this laboratory module.

Supplies for the module were obtained from Purdue University Industrial and Physical Pharmacy Department (amoxicillin USP-level), Sigma-Aldrich (methanol), and Milli-Q Integral System (fresh ultrapure water).

**Preparation of Standards**

First, 250 mg of amoxicillin was measured and placed in a 100 mL volumetric flask. The flask with its contents was then diluted with 100 mL of potassium phosphate buffer at pH 5 (Pump A). This mixture was then stirred for approximately 30 minutes. Utilizing a funnel and an empty flask, the contents of the volumetric flask were strained through filter paper, and 1.5 mL of this sieved solution was placed in an HPLC vial for analysis.

**Solvents**

Four solvents are essential in the universal method: water, methanol, acetonitrile, and 0.5 M potassium phosphate. For amoxicillin, only methanol and potassium phosphate were utilized.

**Preparation of Drug Tablet Assay Samples**

A single capsule of amoxicillin is fragmented, and the contents are then placed into a 100 mL volumetric flask. Amoxicillin was diluted with 100 mL of buffer from Pump A. This blend is mixed for approximately 30 minutes. Utilizing a funnel and an empty flask, the contents of the volumetric flask were strained through filter paper and 1.5 mL of this sifted solution was placed in an HPLC vial for analysis.

**Measurements**

For all samples and their HPLC trials, a blank run and three sample runs were implemented. In three separate trials, a reference standard was prepared and used as a comparison. The reference standard’s average retention time for amoxicillin was 5.76 minutes with an average peak area of 94.12%.

In total, nine trials of amoxicillin capsules were assessed. It was found that the accepted limits for the amoxicillin method were set at a retention time of 5.76 and a peak area of 94.12% with a standard deviation of 0.071 and 1.47, respectively.

**DISCUSSION**

HPLC analysis was utilized as opposed to other methods in this project for several reasons. HPLC excels at the analysis of small, organic molecules which is a feature shared by every drug analyzed or every drug intended to be analyzed. For this reason, HPLC has also been widely utilized by the pharmaceutical industry and is the main method used to analyze drugs for purity. Additionally, HPLC is highly adaptable. Many different variables can be manipulated on an HPLC, including the size and type of column, polarity of the solvents, flow rate, wavelength of excitation, injection size, and several others. This makes HPLC very versatile, able to be manipulated to analyze an endless numbers of drugs, and perfect for the development of a universal method.

Separations using high-pressure liquid chromatography (HPLC) depend upon the basic processes of adsorption, partition, ion exchange, and molecular exclusion, as previously discussed. The advantages of using HPLC as compared to classical chromatographic methods are greater speed, precision, accuracy, and ease of operation. The column materials used in HPLC are of unique structure and provide the basis for separations.

A wide variety of aqueous and nonaqueous solvents may be used as the mobile phase. Solvents must be degassed before they can be used or the detector will adsorb bubbles and erroneous readings will be obtained. The mobile phase is generally pumped through the column under pressure. Most commercial instruments are capable of providing pressures up to 7000 psi.

Ultraviolet (UV) absorbance and refractive index detectors are most frequently used in HPLC. UV detectors are available with fixed and variable wavelengths. This type of detector monitors the column effluent continuously as it passes through the microcell. Since the signal is measured in absorbance units and recorded versus time, a differential plot (peak) is obtained on the recorder.
These drugs, just like any other set of drugs, vary greatly in terms of solubility. Some are more polar and, therefore, readily soluble in our polar solvents, while others are more nonpolar. Thus, these different drugs have different solubility and different rates of dissolution. That being said, the solubility of these drugs is similar enough that we have been able to both dissolve them all in the same solvents and utilize the same column for analysis.

The thought process for choosing the original drugs has been previously discussed. This process directly influenced the process for selecting the solvents. The only solvents utilized in the acetaminophen method were readily accessible and widely used. After selecting other drugs to test and researching methods by which to analyze them, it was found that four solvents were common to HPLC methods per

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Pump A</th>
<th>Pump B</th>
<th>Injection Volume</th>
<th>Temperature</th>
<th>UV Detector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Methanol</td>
<td>Water</td>
<td>10 µL</td>
<td>25 °C</td>
<td>243 nm</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Methanol</td>
<td>1.42g/L Potassium Phosphate Adjusted with Diluted Phosphoric Acid</td>
<td>20 µL</td>
<td>45 °C</td>
<td>215 nm</td>
</tr>
<tr>
<td>Amoxicillin (Amoxicillin Capsules, Diomax 250)</td>
<td>Buffer pH=5 Potassium Phosphate (2.72g/L)</td>
<td>Methanol</td>
<td>10 µL</td>
<td>40 °C</td>
<td>210 nm</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Acetonitrile</td>
<td>Phosphoric Acid</td>
<td>10 µL</td>
<td>29-31 °C</td>
<td>278 nm</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Methanol</td>
<td>Potassium Phosphate</td>
<td>15 µL</td>
<td>25 °C</td>
<td>254 nm</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>Acetonitrile</td>
<td>0.05M Potassium Phosphate (pH=3.0)</td>
<td>20 µL</td>
<td>37 °C</td>
<td>272 nm</td>
</tr>
<tr>
<td>Loratadine</td>
<td>Acetonitrile</td>
<td>Methanol</td>
<td>15 µL</td>
<td>25-35 °C</td>
<td>254 nm</td>
</tr>
<tr>
<td>Lumefantrine/ Artemether (Lumartem)</td>
<td>0.05M Potassium Phosphate (pH=3.0)</td>
<td>Acetonitrile</td>
<td>20 µL</td>
<td>25 °C</td>
<td>303 nm</td>
</tr>
<tr>
<td>Mefloquine (Mephaquin)</td>
<td>0.05M Potassium Phosphate</td>
<td>Methanol</td>
<td>20 µL</td>
<td>30 °C</td>
<td>283 nm</td>
</tr>
<tr>
<td>Quinine (Quinine Sulphate BP)</td>
<td>0.05M Potassium Phosphate (pH=2.0)</td>
<td>Acetonitrile</td>
<td>10 µL</td>
<td>30 °C</td>
<td>347 nm</td>
</tr>
</tbody>
</table>

Table 2. Drugs with their best solvent system.
Method to Improve Drug Safety in Africa

As an example, acetaminophen tablets were tested using the method at both Purdue and KSP. Purdue students tested four tablets obtained from a local pharmacy and manufactured in the US. Eight KSP students each tested three tablets manufactured in Tanzania, China, or India.

The Purdue team found an average retention time over four experiments of 5.05 minutes ±0.01. Area and area percent of the primary peak is reported for each experiment in Table 3. It should be noted that Samples 3 and 6 were not properly filtered prior to analysis.

The results in Table 3 indicate that the developed method tested by Purdue students was repeatable and able to verify purity within 99% peak area.

### Results of Studies Performed by Students at the Kilimanjaro School of Pharmacy

In March of 2016, in our manufacturing course in Purdue’s master’s program at the Kilimanjaro School of Pharmacy, a variety of procedures were performed to help students better understand the use of HPLC. These procedures included typical validation procedures like linearity, precision, and accuracy and tablet assaying to compare the quality of different tablets.

<table>
<thead>
<tr>
<th>Sample Number (Date Tested)</th>
<th>Retention Time (min)</th>
<th>Area (mAu*s)</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (Jan. 26)</td>
<td>5.050666667</td>
<td>20052.46667</td>
<td>100</td>
</tr>
<tr>
<td>Sample 1 (Jan. 27)</td>
<td>5.067333333</td>
<td>16096.4</td>
<td>100</td>
</tr>
<tr>
<td>Sample 2 (Feb. 1)</td>
<td>5.042333333</td>
<td>16200.4</td>
<td>99.93713333</td>
</tr>
<tr>
<td>Sample 2 (Feb. 3)</td>
<td>5.06</td>
<td>16241.45</td>
<td>100</td>
</tr>
<tr>
<td>Sample 3 (Feb. 1)</td>
<td>4.929</td>
<td>104301.5</td>
<td>99.22085</td>
</tr>
<tr>
<td>Sample 4 (Feb. 3)</td>
<td>5.0505</td>
<td>16237.15</td>
<td>100</td>
</tr>
<tr>
<td>Sample 4 (Feb. 8)</td>
<td>5.096</td>
<td>16221.63333</td>
<td>99.79413333</td>
</tr>
</tbody>
</table>

**Table 3.** Retention time studies performed by students at Purdue.

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>Std. Dev.</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18604.1</td>
<td>18943.1</td>
<td>19004</td>
<td>18850.4</td>
<td>215.464545</td>
<td>1.14302373</td>
</tr>
<tr>
<td>2</td>
<td>9512.9</td>
<td>9504.3</td>
<td>9445.4</td>
<td>9487.53333</td>
<td>36.7410307</td>
<td>0.38725588</td>
</tr>
<tr>
<td>3</td>
<td>1848.7</td>
<td>1856.2</td>
<td>1853.8</td>
<td>1852.9</td>
<td>3.8301436</td>
<td>0.20671076</td>
</tr>
</tbody>
</table>

**Table 4.** Example precision data obtained from the Kilimanjaro School of Pharmacy.

As an example, acetaminophen tablets were tested using the method at both Purdue and at KSP. Purdue students tested four tablets obtained from a local pharmacy and manufactured in the US. Eight KSP students each tested three tablets manufactured in Tanzania, China, or India.

The Purdue team found an average retention time over four experiments of 5.05 minutes ±0.01. Area and area percent of the primary peak is reported for each experiment in Table 3. It should be noted that Samples 3 and 6 were not properly filtered prior to analysis.

The results in Table 3 indicate that the developed method tested by Purdue students was repeatable and able to verify purity within 99% peak area.
the preparation of multiple standard solutions. From these standard solutions, multiple AUC measurements were made and recorded. From this data, the relative standard deviation was measured and averaged across the data. An example of this data is provided in Table 4.

For the accuracy measurements, the relative closeness of the results of the analytical method to the true value is measured. This was done through the preparation of several concentrations through serial dilution. The AUC was then measured and used to estimate the concentration with the prepared calibration curve. Finally, using the estimated concentration, the percent recovery was estimated compared to the expected concentration and averaged across the data. An example of this data is provided in Table 5.

Finally, a tablet assay was used to illustrate how HPLC can be used to compare the content and quality of acetaminophen tablets produced in different countries. Tablets produced in China, Tanzania, and India were studied. The AUC was measured and the concentration was estimated using a previously prepared calibration curve. Using the known volume of the mobile phase used in the preparation of the sample solutions, the mass content of the tablets was determined from the estimated concentration. An example of this data is provided in Table 6. Tablets from all three countries were slightly below label but still within 90% of the labeled amount.

### Table 5. Example accuracy data obtained from the Kilimanjaro School of Pharmacy.

<table>
<thead>
<tr>
<th>Expected/Real Concentration (mg/mL)</th>
<th>AUC</th>
<th>Calculated Concentration (mg/mL)</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.63</td>
<td>18604.1</td>
<td>0.561023</td>
<td>89.05126984</td>
</tr>
<tr>
<td>0.315</td>
<td>9512.9</td>
<td>0.288287</td>
<td>91.51968254</td>
</tr>
<tr>
<td>0.126</td>
<td>3544</td>
<td>0.109220</td>
<td>86.68253968</td>
</tr>
<tr>
<td><strong>AVERAGE =</strong></td>
<td></td>
<td></td>
<td><strong>89.08449735</strong></td>
</tr>
</tbody>
</table>

### Table 6. Example tablet assay data obtained from the Kilimanjaro School of Pharmacy.

<table>
<thead>
<tr>
<th>Country of Origin</th>
<th>AUC</th>
<th>Calculated Concentration (mg/mL)</th>
<th>Mass Content of Acetaminophen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>15093.4</td>
<td>0.455702</td>
<td>455.702</td>
</tr>
<tr>
<td>Tanzania</td>
<td>15723.1</td>
<td>0.474593</td>
<td>474.593</td>
</tr>
<tr>
<td>India</td>
<td>15196.1</td>
<td>0.458783</td>
<td>458.783</td>
</tr>
</tbody>
</table>

### Figure 4. Example calibration curve obtained from the Kilimanjaro School of Pharmacy.
CONCLUSION

The discrepancies in the results between the two groups of students highlight a need for clarity. This module is most effective when a calibration curve is developed and drug concentrations are analyzed for percent recovery in addition to observation of the retention time and peak area percentage. Retention time verifies the correct compound is in the drug product, and the percent recovery allows for method verification as well as quality metrics. Peak area percentage can be used to understand the level of purity in the drug.

The results obtained by both the student teams at Purdue University and Kilimanjaro School of Pharmacy indicate that the concept of a universal method is a useful tool for addressing testing of SSFFC drugs. The developed method was validated and conforms to a broader attempt by students at both institutions to create HPLC methods that are simple and effective at quantifying the active pharmaceutical ingredient in an oral formulation.

NOTE

The Sustainable Medicine Program in Tanzania was established by Stephen Byrn (Purdue University College of Pharmacy), Sister Zita Ekeocha and John Chilunda (St. Luke Foundation/Kilimanjaro School of Pharmacy), and Joseph Fortunak (Howard University). The program was developed to help combat poor quality of medications due to counterfeiting and manufacturing issues. The program consists of educational programs in pharmaceutical sciences and good manufacturing practices (GMP), and a GMP pharmaceutical manufacturing facility and analytical laboratory at St. Luke Foundation/Kilimanjaro School of Pharmacy. Since its inception in 2007, this certificate program serves as a transition to a Master of Science in technology, leadership, and innovation with a concentration in biotechnology, innovation, and regulatory affairs (BIRS) through the Purdue Polytechnic Institute and its Center for Professional Studies.

ACKNOWLEDGMENTS

The HPLC equipment for this study was donated to KSP by Merck. The software for the HPLCs was donated by Agilent. We are grateful for their assistance.

REFERENCES


