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.

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Keywords

HPLC, SSFFC, universal method, acetaminophen, Tanzania, learning module

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

packaging of genuine drugs without offering the desired efficacious effect. These SSFFC drugs waste resources, put individuals at risk of continued illness, and discourage individuals from purchasing genuine and life-saving drugs.

In 2008, the World Health Organization (WHO), United Nations Educational, Scientific and Cultural Organization (UNESCO), and the International Pharmaceutical Federation (FIP) collaborated to create a Pharmacy Education Taskforce (PFT) in order to develop a Pharmacy Education Action Plan for 2008–2010. As of 2008, the WHO estimated that there was a shortage of more than four million healthcare workers worldwide; it was reported, as well, that 57 countries fell under the WHO threshold of 2.5 health care professionals per 1,000 population. These shortages can hinder other health programs and can form a barrier when trying to reach the Millennium Development Goals being set to improve the quality of life for people around the globe (Anderson et al., 2008). Pharmacists are usually not the first healthcare professionals considered when looking at countries with insufficient health care systems, however, pharmacists are a vital component of the health-care system. For many underprivileged communities, the pharmacist is the most common and, in some cases, the sole provider of healthcare for several reasons, including proximity, cost associated with visiting a doctor or physician, and lack of availability of other options (Anderson et al., 2008). In most nonurban communities, private drugstores that are licensed to sell over-the-counter drugs are the prominent source of medications for poorer citizens; the Tanzanian Ministry of Health acknowledges the importance of private drugstores throughout Tanzania, as well, in providing health care to the country's citizens (Viberg et al., 2010). In addition, in Africa over 75% of antibiotics are incorrectly prescribed to patients (Mwambete, 2014, p. 98). This is due to the incorrect drug type being prescribed and the frequency of SSFFC drugs in health-care systems in Africa (Mwambete, 2014). Having a lack of educated pharmacists in Tanzania is an issue that needs to be addressed in order to help provide better health care to the people of Tanzania. To address this issue, there needs to be a system in place to educate more pharmacists for the workforce.

This paper describes an educational module designed to educate students about SSFFC medicines and a laboratory exercise that illustrates the strategy for detecting SSFFC medicines. Indirectly, this module and laboratory exercise illustrates how drug quality can be ensured using laboratory assays and methods. Additionally, this paper describes a universal method

for distinguishing between SSFFC and non-SSFFC drugs. Additionally, we describe implementation of this method in Tanzania at the Kilimanjaro School of Pharmacy (KSP). Strategies to increase pharmaceutical education and expertise in Tanzania and ways of detecting the sale of SSFFC drugs in the Kilimanjaro region of Tanzania also are discussed. Alternatively, 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

Parameters	Values
Column	Agilent Zorbax SB-C18 4.6mmx250mm ¹
Pump A	Methanol
Pump B	Water
Mobile Phase	50 mL Methanol + 150 mL Water
Gradient	Time (min)
	A%
	B%
	0
	10
	25
	75
Injection Volume	10 µL
Temperature	25°C
UV Detector	243 nm (Reference 360 nm & 100 nm)
USP Compound	0.01 mg/ml of acetaminophen in mobile phase
Extraction Procedure and Drug Preparation	<ul style="list-style-type: none"> • 100mg of acetaminophen in a 200mL volumetric flask • Add 100mL mobile phase, stir, dilute to volume (~200 mL mobile phase) • Using a funnel and an empty flask, pass solution through filter paper • Extract 1.5mL of this solution into an HPLC test tube
Stability Information	The prepared drug buffer has been shown to remain stable for up to 2 weeks under refrigeration
Acceptance Criteria	No peaks on the blank
Acceptance Results	>99% peak area within 5 to 5.1 minutes
Average Retention Time	5.05 minutes
Average Area %	99.95%

¹ USP suggests 3.9mm x 30 cm, L1 packing

■ **Table 1.** HPLC method for acetaminophen.

phosphate. For acetaminophen, only methanol and water were utilized.

Preparation of Drug Tablet Assay Samples

Tablet 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.

Figure 1. Sample chromatogram for HPLC acetaminophen reference standard.

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

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.

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.

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 ultra-pure 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.

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

■ **Table 2.** Drugs with their best solvent system.

The USP contains a general chapter, USP<621>, on chromatography. This chapter describes approaches to separations and analysis of drugs using chromatographic methods. It also describes quantitative analysis of drugs using both external standard and internal standard. The USP sells standards for this purpose. The availability of standards makes this study much more feasible.

When first beginning the project, many important decisions, including which drugs to test and how to adapt our methods for universal drug applicability, had to be made. The initial criterion was choosing drugs that were the low-hanging fruit: cheap, readily accessible, widely utilized, and simple to test. Amoxicillin was an all but obvious first choice based on these criteria. After this selection, drugs that seemed like they were widely utilized or might be readily utilized throughout Africa were chosen. These criteria led to hydrochlorothiazide, quinine, mefloquine, lumefantrine, and artemether. For future studies, we have cross-referenced the WHO's essential medicines list with the Wal-Mart \$4 drug list. This provided a list of drugs that we know will be medically necessary and affordable in these areas, including acetaminophen, loratadine, ciprofloxacin, dexamethasone, metformin, carvedilol, amitriptyline and haloperidol.

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.

Upon starting this project, one of the major challenges was proving that the results were consistent with what was, in fact, correct. To do this, USP standards were purchased, and the results were compared to those displayed from the USP analysis in the monograph for each drug. If this method produced results comparable to the USP HPLC results, it was concluded that method was suitable for that specific drug.

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

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

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

Standard No.	AUC					
	Replicate 1	Replicate 2	Replicate 3	Average	Std. Dev.	RSD
1	18604.1	18943.1	19004	18850.4	215.464545	1.14302373
2	9512.9	9504.3	9445.4	9487.53333	36.7410307	0.38725588
3	1848.7	1856.2	1853.8	1852.9	3.8301436	0.20671076
AVERAGE =						0.57899679

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

the USP. This proved to us that they would be able to be manipulated to analyze many different drugs and, therefore, suitable for our universal method.

In an effort to make testing as simple as possible, it was decided to use four solvents. This is a challenge as the chemistry of each drug is different, and they each have HPLC procedures previously developed to optimize their elution through the instrument that may or may not involve the solvents that were chosen. All of our methods are based on the four solvents: water, methanol, acetonitrile, and 0.5M potassium phosphate at varied pH levels using phosphoric acid for all pH adjustments. These methods are being called our universal methods, and we have worked to find the right settings, which will have a single peak for each of the initial five common SSFEC drugs of interest.

RESULTS

Results of Studies Performed by Students at Purdue University

Investigations at Purdue University focused on determining which combinations of the four solvents worked best for the universal method. Table 2 shows all drugs tested and the best solvent system associated with each one.

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 $\pm$ 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.

Expected/Real Concentration (mg/mL)	AUC	Calculated Concentration (mg/mL)	% RECOVERY
0.63	18604.1	0.561023	89.05126984
0.315	9512.9	0.288287	91.51968254
0.126	3544	0.109220	86.68253968
AVERAGE =			89.08449735

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

Country of Origin	AUC	Calculated Concentration (mg/mL)	Mass Content of Acetaminophen (mg)
China	15093.4	0.455702	455.702
Tanzania	15723.1	0.474593	474.593
India	15196.1	0.458783	458.783

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

■ **Figure 4.** Example calibration curve obtained from the Kilimanjaro School of Pharmacy.

The study involved carrying out a linearity, precision, and accuracy analysis utilizing a secondary reference standard. For linearity, a proportional relationship between response and concentration is demonstrated for a desired range of concentrations. A serial dilution method was used from a stock solution of acetaminophen in mobile phase. From this serial dilution, the area under the curve (AUC) was measured and plotted against the respective concentration. These data were used to construct a calibration curve, as shown in Figure 4.

For precision, the relative repeatability of the analytical method is measured. This was done through

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.

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/Kili-

manjaro 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.

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