Identifying Substandard and Counterfeit Acetaminophen Containing Pharmaceuticals using High Performance Liquid Chromatography

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Introduction

The World Health Organization reported 42% of identified counterfeit drugs from 2013 to 2017 originated in Africa. Dr. Marya Lieberman is the director of the Distributed Pharmaceutical Analysis Laboratory (DPAL) at the University of Notre Dame. DPAL is a consortium of laboratories that analyzes suspicious pharmaceutical samples. Prior to analyzing real-world samples, DPAL laboratories are required to meet a series of system suitability requirements that include linearity, precision, accuracy and range, spiking, and specificity.

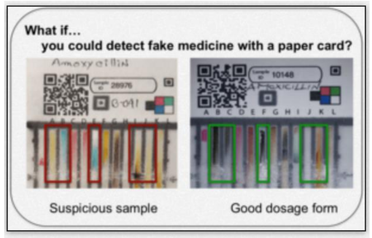
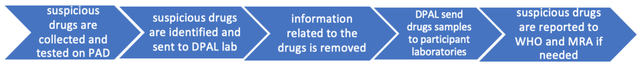


Image source: https://padproject.nd.edu/technology/

DPAL purchased acetaminophen containing tablets at pharmacies in Kenya. The drugs were initially tested on Paper Analytical Devices (PAD) before being distributed to our DPAL laboratory for HPLC analysis. When counterfeit drugs are identified, DPAL will then run additional LC-MS analysis prior to reporting to a Medical Regulatory Authority (MRA).



Chromatographic Analysis

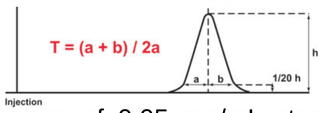
Theoretical Plates

$$N = 5.54 \times \left(\frac{t_r}{W_{0.5}}\right)^2$$

Capacity Factor

$$k = \frac{t_r - t_0}{t_0}$$

Tailing Factor



A chromatogram of 0.05 mg/mL standard and 0.04 mg/mL uracil was used to calculate the theoretical plates, column capacity, and tailing factor according to the formula shown above:

N = 4507 plates T = 1.8 k = 0.69

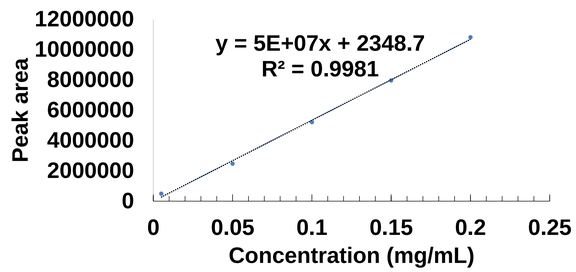
HPLC System Suitability

Precision

Six consecutive injections of 0.1 mg/mL acetaminophen standard were performed. DPAL requires that the percent relative standard deviation (%RSD) must be below 2.0%. The %RSD collected equals 0.8%.

Injections	Peak area	Retention time (minute)
1	5304100	1.977
2	5407918	2.000
3	5396411	1.997
4	5396079	1.990
5	5411955	2.001
6	5410736	1.993
RSD	0.0077	
Mean	5387867	
St. Dev	41623.6	

Linearity



We prepared a five point calibration curve with secondary standard acetaminophen with concentration from 0.005 to 0.2 mg/mL. The correlation coefficient must be at least 0.98.

Accuracy and Range

Deficient, normal and overdosed samples were injected in triplicates. A blank solvent and external standard were injected after every four runs. Percent difference of each sample must be within ± 2%.

Samples	Expected concentration (mg/mL)	Average Peak Area	Percent difference (%)
Deficient	0.035	265764	1.2
Normal	0.100	8045297	0.0
Overdosed	0.150	12121780	0.0

Specificity

Degraded acetaminophen tablet was initially heated at 60 °C for one hour. Both the control and degraded tablets were prepared at room temperature. About 30 % Active Pharmaceutical Ingredients (API) were added to each sample which yielded 0.129 mg/mL of total sample concentration.

Samples	Sample + spike concentration (mg/mL)	Sample concentration (mg/mL)	Spike recovery
Control tablet	0.129	0.099	100%
Degraded tablet	0.129	0.100	96.5%

Accuracy via Spike

0.101 mg/mL of the control sample was run against 0.130 mg/mL of the spiking sample. Triplicate injections were performed on each sample. DPAL requires the percent recovery of the spike to be within 90 – 100%. The spike sample recovery was reported to be 96.4%.

Sample	Spike + sample concentration (mg/mL)	Sample concentration (mg/mL)	Percent Recovery (%)
Control tablet	0.130	0.101	96.4%

Limit of Detection & Quantitation

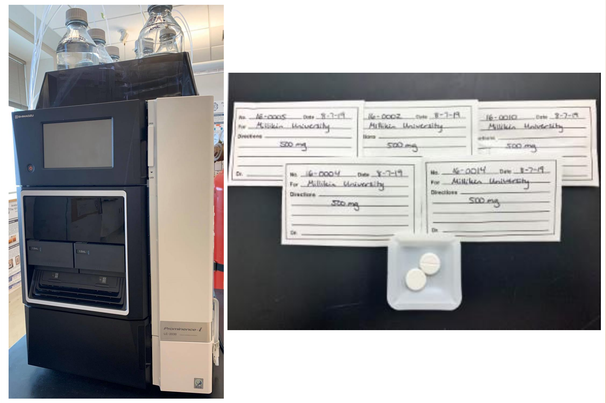
The limit of detection (LOD) and limit of quantitation (LOQ) of our HPLC acetaminophen analysis were calculated according to the equations below.

$$LOD = \frac{3 \times \text{standard error of intercept}}{\text{slope of calibration curve}}$$

$$LOQ = \frac{10 \times \text{standard error of intercept}}{\text{slope of calibration curve}}$$

LOD = 0.0102 mg/mL
LOQ = 0.0339 mg/mL

Analyzing Tablets from Kenya



Sample	Retention time (minute)	Peak area	Measured concentration (mg/mL)	Measured potency (mg acetaminophen/ tablet)
16-0004	1.964	5578792	0.0947	473.8
16-0004	1.963	5577491	0.0947	473.8
16-0004	1.963	5578340	0.0947	473.9
Mean				473.8
St. Dev				0.036
RSD				7.6E-05
Propagation of error			0.0061	

An acetaminophen sample collected from Kenya was processed with mortar and pestle before dissolving 0.201 g of tablet into 25-mL of HPLC solvent. 141 µL of this stock solution was then diluted with 10.0 mL of HPLC solvent to prepare a 0.099 mg/mL solution. The potency of 16-0004 tablet was determined to be 473.81 ± 0.04 mg acetaminophen.

Future Work

- Continuous to analyze other four acetaminophen tablets.
- New students will analyze another drug samples.

Acknowledgements

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