HPLC Analysis of Illicit Drugs Azithromycin and Amoxicillin

Hunter I. Noel
Mentor: Dr. Corina E. Brown

Abstract

Throughout the project, HPLC will be used to analyze samples of Azithromycin and Amoxicillin to determine if they are counterfeit. In order to do this, the specific Azithromycin and Amoxicillin methodology will need to be revised in order to produce an accurate calibration curve. The research goal is stated: "Through this project, we will work to detect falsified medicines efficiently in low-resource settings and track the supply chains through which these insufficient quality products move. By working with partners in the health system and with regulatory agencies, we hope to protect patients from dangerous fake drugs" (Lieberman, 2018). The samples, pulled from West African markets, will be provided by the Notre Dame DPAL (Lieberman, 2018).

Background

- The global phenomenon of drug counterfeiting is a grave threat to both patients and healthcare systems alike.
- Annually, it is estimated that 100,000 people have died from ingesting fake drugs (Blackstone et al., 2014).
- The counterfeit drug industry is a very lucrative business: It is estimated that, globally, counterfeit drugs provide around $75 billion in revenue every year to sellers (WHO, 2010).
- By replacing the expensive active drug ingredient with something inexpensive and ineffective such as cassava flour, and then charging the market price of the real drug, sellers can make several magnitudes of profit from unwitting buyers (Kelesidis & Falagas, 2015).
- A counterfeit drug vendor in Cameroon can make an average of $40 USD a day, which is much more than the average $1.25 daily wage (Jarrells, 2015).

What drugs are counterfeited the most often?

- It seems that the market is most saturated with **beta-lactam** antibiotics and anti-malarial drugs like chloroquine
- Most of these drugs merely have a reduced concentration of the active drugs, and most can be traced back to South-East Asia and Africa (Kelesidis & Falagas, 2015).
- Quantitatively, the chromatogram of a possible fake sample will vary in terms of the peak, height, retention time, and number of peaks.
- Qualitatively, the sample may have a different appearance, either physical or in its packaging.

Azithromycin

- Azithromycin is a macrolide antibacterial agent derived from the earlier antimicrobial drug, Erythromycin (Dunn & Barradell, 1996).
- It was developed in 1980 by a team of researchers at PLIVA laboratories in the former Yugoslavia, now Croatia (Jelić & Antolović, 2016).
- Azithromycin is one of the best-selling drugs in the world and marks a significant improvement from its parent drug, Erythromycin, namely due to a broader spectrum of activity, low risk of side effects, and faster treatment regimen of just three days.
- Azithromycin is effective against Gram positive and some Gram-negative bacteria that cause respiratory tract infections, such as *Haemophilus influenzae, Streptococcus pneumoniae, or Legionella pneumoniae* (Dunn & Barradell, 1996).

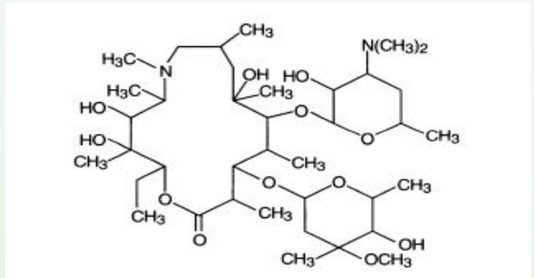

Figure 1: Structure of Azithromycin (Dunn & Barradell, 1996)

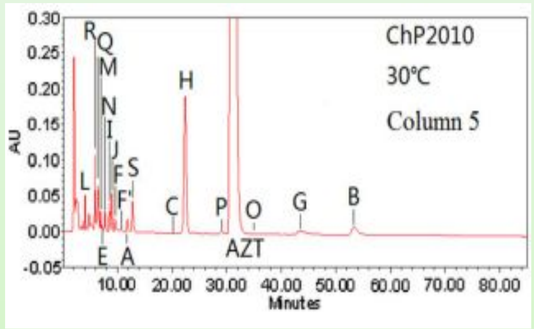

Figure 2: Chromatogram of Azithromycin (Chang et al., 2015)

Amoxicillin

- Amoxicillin is a semi-synthetic penicillin-derived drug first invented in 1972 at Beecham Research Laboratories in England (Todd & Benfield, 1990).
- It is primarily used to treat bacterial infections in the ears, sinus, skin, lungs, and urinary tract (Todd & Benfield, 1990).
- A wide variety of bacteria are susceptible, covering many gram-positive and some gram-negative strains (Akhavan et al., 2020).
- Susceptible bacteria include such species as *H. influenzae, E. Coli, and Streptococcus pneumoniae* (Carson-DeWitt, 2015).
- Amoxicillin, as a beta-lactam antibiotic, kills bacteria by binding to penicillin-binding proteins that inhibit the process of transpeptidation in bacterial cell walls (Akhavan et al., 2020).

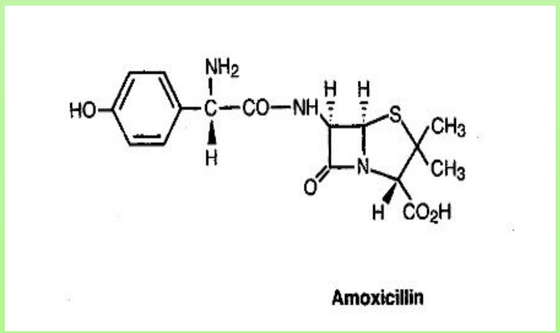

Figure 3: Structure of Amoxicillin (Todd & Benfield, 1990)

Methodology

For the analysis of Amoxicillin a gradient method was used (Lieberman, 2018).

- Column: 100x4.6mm C18
- Solvents: 20 mM Monosodium Phosphate buffer at pH 4.4 and HPLC grade methanol
- Gradient method with varying solvent ratios over a period of 12 minutes.
- Amoxicillin is injected at a sample concentration of 0.5 mg/mL, at a total injection volume of 20 µL (diluted in water).
- UV Detector set to a wavelength of 220 nm.

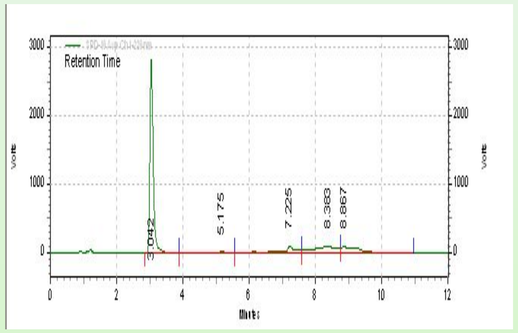

Figure 4: Amoxicillin run with 100x4.6mm column

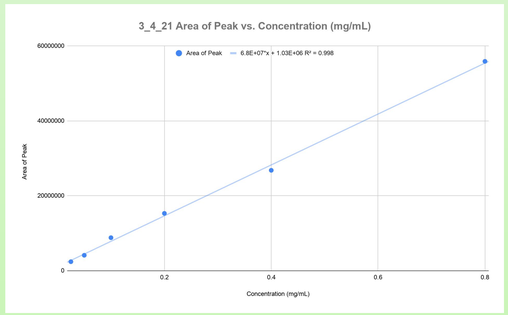

Figure 6: Standard curve of Amoxicillin

Figure 7: HPLC modules and PC. "Layla"

Accomplishments

- Building familiarity and experience with the Shimadzu HPLC Instruments and software.
- Testing multiple amoxicillin standard solutions and building several standard curves with a 100x4.6mm column.
- Testing multiple samples of drugs stated to be amoxicillin.
- Applying and earning two research grants, with total funding of $1000.
  - NHS undergraduate research fund
  - Tri-Beta Biological Honors Society

Challenges

- Azithromycin is used to treat COVID patients in order to prevent secondary bacterial infections
  - As such, it is hard to come by due to increased market demand.
- Very specific, rare, and expensive chemicals are required to make the proper buffer solutions
  - Requires time for delivery and additional funds
- Limited lab access due to school policies
  - On-campus research requires in-person presence, and the risk inherent in that during a pandemic
- HPLC instruments were left alone for months on end without proper maintenance after the initial lockdown, past spring break

Future Directions

- Analyze suspect samples of amoxicillin, report back to Notre Dame the results
- Order and test Azithromycin samples when they become available
- Order different columns for additional drug testing
  - Doxycycline?
- Researchers generally do not know the prevalence of specific counterfeit drugs by identity (Kelesidis & Falagas, 2015).
- In order to fix this, an organization such as the WHO could do a more in-depth survey of drug markets around the world, making sure to categorize and classify discovered counterfeits with details such as drug identity.

Conclusions

- Counterfeit drugs are an emerging threat to public health around the world.
- These counterfeits are much more prevalent in the developing world, such as sub-Saharan Africa, compared to a developed region such as Western Europe (WHO, 2010).
- Azithromycin, a commonly prescribed macrolide antibiotic for respiratory infections, is commonly counterfeited as well (Dunn et. al, 1996, Kelesidis & Falagas, 2015).
- We can use HPLC in order to determine whether or not a drug sample is legitimate.
- In the sample no. 19K-0002 collected 1-28-20, results indicated a concentration of 0.475 mg/mL compared to an expected 0.5 mg/mL

Acknowledgements

- Dr. Richard Hyslop, Chad Wangeline
- Tri-Beta Honors Society and the NHS office of undergraduate research for their financial support.

References

Lieberman, M. (2018). HPLC Methodology Manual

Erwin A. Blackstone, PhD, Joseph P. Fuhr, Jr, PhD, Steve Pociask, et al. (2014). The Health and Economic Effects of Counterfeit Drugs. Am. Health Drug Benefits, 7

Jarrells, H. E. (2015). History, TRIPs, and Common Sense: Curbing the Counterfeit Drug Market in Sub-Saharan Africa. *Ga*. J. Int'l & Comp. L, 43(2), 557.

Dunn, C. J., & Barradell, L. B. (1996). Azithromycin. A Review of its Pharmacological Properties and Use as 3-day Therapy in Respiratory Tract Infections. Drugs, 51(3), 483-505. doi:10.2165/00003495-199651030-00013

Jelić, D., & Antolović, R. (2016). From Erythromycin to Azithromycin and New Potential Ribosome-Binding Antimicrobials. Antibiot. (Basel, Switzerland), 5(3), 29. doi:10.3390/antibiotics5030029

Akhavan BJ, Khanna NR, Vijhani P. Amoxicillin. [Updated 2020 Aug 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-.

Waun, J., & Carson-DeWitt, R. (2015). Amoxicillin. The gale encyclopedia of prescription drugs : A comprehensive guide to the most common medications (pp. 42-45) Cengage Learning.

Todd, P. A., & Benfield, P. (1990). Amoxicillin/clavulanic acid-an update of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs (New York, N.Y.), 39*(2), 264-307. doi:10.2165/00003495-199039020-00008

Kazakevich, Y., & Lobrutto, R. (2007). HPLC for Pharmaceutical Scientists (1st ed.). Hoboken, New Jersey: Wiley.