Development of a RP-HPLC Method for the Simultaneous Quantification of Ampicillin and Cloxacillin in Ampiclox

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Introduction

This project was focused on the development of a method using Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) to simultaneously quantify the antibiotics ampicillin and cloxacillin contained in the pharmaceutical Ampiclox. The Distributed Analysis Pharmaceutical Laboratory (DPAL) is a collaborative group focused on using validated methods to identify substandard and falsified pharmaceutical products. Antibiotics are among the most commonly reported substandard and falsified drugs, thus this method will be used to analyze Ampiclox tablets obtained from DPAL.[1]

Specifically, selection of the proper column type, mobile phase, and buffer composition, gradient optimization were made. One crucial aspect of method development is the selection of a proper mobile phase and buffer composition. This drastically impacts separation efficiency and accuracy as the active pharmaceutical ingredient (API) has acid and base properties that may be controlled by the pH and other properties of the mobile phase.[2] Additionally, it is important to consider the effects of buffer concentration and additives on peak shape and symmetry.[3]

In addition to selection of the aforementioned parameters, the method was tested to ensure that analytical metrics such as tailing factors, theoretical plates, and resolution met United States Pharmacopeia (USP) guidelines.

Ampiclox

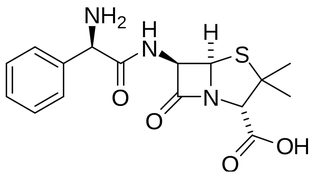

Figure 1: Ampicillin

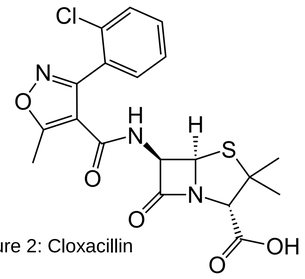

Figure 2: Cloxacillin

- Ampiclox is a combination of the two antibiotics ampicillin and cloxacillin
- Both APIs are beta-lactam antibiotics
- Ampicillin contains an amine functional group
- Cloxacillin contains a large hydrophobic phenyl group

Instrumentation and Parameters

Instrument: Agilent InfinityLab LC Series 1220 Infinity II LC System

Method Parameters

- Column: ZORBAX Eclipse Plus C18, 5µm, 4.6 x 150 mm
- Flow Rate: 1 mL/min
- Detector Type/Wavelength: Variable Wavelength Detector set to 225nm
- Mobile Phase: KH_2PO_4 buffer and acetonitrile organic solvent

Figure 3: Agilent HPLC.

Method Development

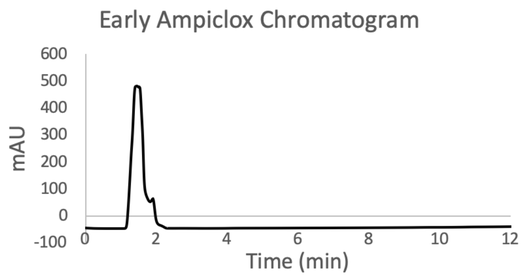

Figure 4: Early Ampiclox Chromatogram

pH	Concentration (M)	Additive	Tailing Factor
5	.5	None	N/A; instrument clogged
5	.025	1% Acetic Acid	~1.6 amp, ~2.3 clox
5	.01	None	~1.8 amp, ~2 for clox
3	.05	None	~1.8 for both
3	.05	.1% TFA	~1.6 for both
3	.025	None	~1.5 amp, ~1.6 clox
3	.025	.1% TFA	~1.5 amp, ~1.6 clox
3	.01	.1% TFA	~1.0 for both

Table 1: Summary of Buffer Manipulations

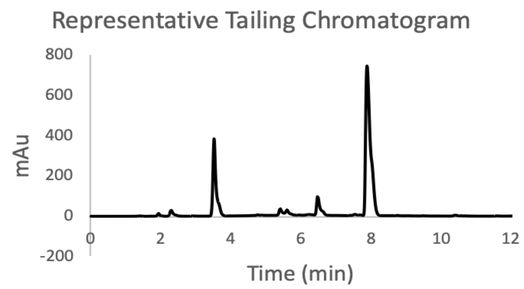

Figure 5: Example Tailing Chromatogram

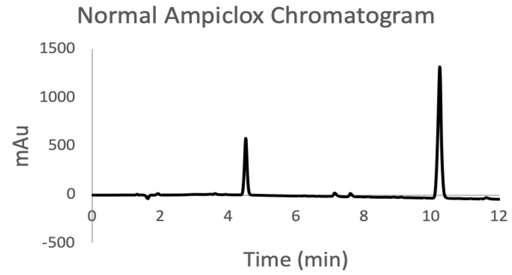

Figure 6: Normal Ampiclox Chromatogram

- Isocratic method yielded one unretained peak (Figure 4)
- Gradient method yields separation of the two peak (Figures 5 and 6)
- To meet the USP tailing factor requirements of no more than (NMT) 1.4 for ampicillin and 1.8 for cloxacillin, the following adjustments were made.
  - Manipulation of pH to reduce tailing by protonating all basic groups and reducing secondary analyte-silanol interactions in the column.
  - Manipulation of buffer concentration to limit secondary interactions by introducing additional ions to interact with the stationary phase.
  - Addition of competing acids such as trifluoroacetic acid (TFA) to limit secondary interactions in the column by protonation of ionized silanol groups.

Optimal Buffer Composition: pH3, 0.01M KH_2PO_4, 0.1%TFA

System Suitability

The following metrics were reported to Distributed Pharmaceutical Analysis Laboratory (DPAL) to prove and ensure accuracy and reproducibility of the method for the analysis of Ampiclox.

	Ampicillin	Cloxacillin
Precision (RSD of 6 replicate injections, NMT 0.2)	0.006	0.010
% Difference Overdosed (NMT±2%)	1.32%	0.86%
% Difference Normal (NMT±2%)	1.01%	0.24%
% Difference Deficient (NMT±2%)	0.67%	1.15%
% Recovery of Spiked Pill (between 90-110%)	98.71%	95.95%
% Recovery of Degraded Matrix (between 90-110%)	105.18%	94.35%
Lower Limit of Detection (ppm)	0.186	0.153
Lower Limit of Quantification (ppm)	0.620	0.510

Table 2: Summary of System Suitability

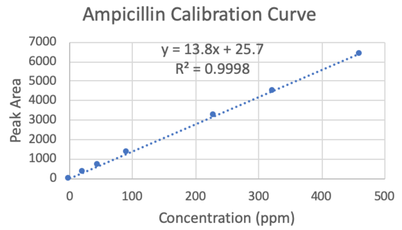

Figure 7: Ampicillin Calibration Curve

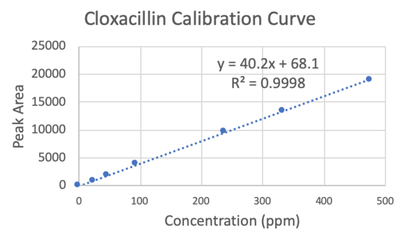

Figure 8: Cloxacillin Calibration Curve

	Average Resolution	Average Tailing Factor	Average Theoretical Plates
Ampicillin	>1.5	NMT 1.4	>1500
Cloxacillin		NMT 1.8	>1500

Table 3: USP Requirements for Selected Metrics

	Average Resolution	Average Tailing Factor	Average Theoretical Plates
Ampicillin	62.81	1.054	49,500
Cloxacillin		1.011	167,000

Table 4: Experimentally Determined Metrics

Future Directions

- Obtain Ampiclox tablets through DPAL from a pharmacy in a developing nation to analyze using the method.
- Report results to the DPAL with the ultimate goal of identifying the source of some substandard and falsified pharmaceutical products

Acknowledgements

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•University of Indianapolis Shaheen College of Arts and Sciences

References

1.Substandard and Falsified Medical Products. https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products (Accessed September 13, 2019), World Health Organization, 2018.
2. Singh, R. HPLC method development and validation- an overview. *J. Pharm. Ed. Res.* ***2013,*** *4*(1), 26-33.
3. The Secrets of Good Peak Shape in HPLC. https://www.agilent.com/cs/library/eseminars/Public/secrets%20of%20good%20peak%20shape%20in%20hplc.pdf