DEVELOPMENT AND VALIDATION OF SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY METHODS FOR THE CONCURRENT DETECTION OF SELECT ANTIBIOTICS IN NEW YORK TECH'S WASTEWATER PLANT.

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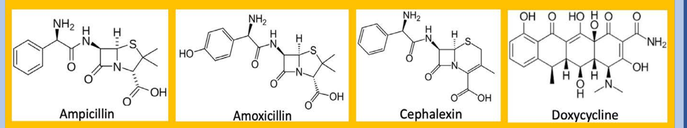
INTRODUCTION

- Proceeding their consumption or improper disposal, antibiotics tend to accumulate in wastewater, an environment characterized by the presence of microbes, such as bacteria.
- Bacterial exposure to these antibiotics poses significant consequences, such as the potential development of antibiotic resistance.
- In the medical realm, as the prominence of antibiotic resistance grows, antibiotic efficacy is reduced, contributing to bacterial infections that are more difficult to treat.

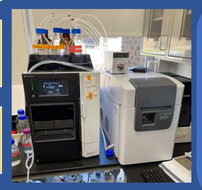
OBJECTIVE

The objective of this research is to determine, test, and validate efficient methods for detecting four antibiotics [Ampicillin, Amoxicillin, Cephalexin, Doxycycline] in wastewater samples collected from the New York Tech wastewater treatment plant in the Long Island campus.



METHODOLOGY DEVELOPMENT

1. Preparation of Antibiotic Standards at a Known Concentration
2. Mass Spectrometry (MS) Method Development, Tweaking, and Validation for each Antibiotic
3. Preparation of Standard Solutions for each Antibiotic Employing Serial Dilution Technique
4. Creation of Calibration Curves using Average Peak Areas for each Standard Solution
5. Solid Phase Extraction (SPE) Method Development, Tweaking, and Validation
6. Collection and Filtration of Wastewater from New York Tech Old Westbury Wastewater Plant.
7. Solid Phase Extraction of Wastewater Samples
8. Mass Spectrometry Analysis of Wastewater Samples using Validated Methods
9. Determine Detectability of each Antibiotic in New York Tech Wastewater Samples



Liquid Chromatography/Mass Spectrometry (LC/MS)

Used to analyze retention times and peak areas of standards and wastewater samples.

New York Tech's Wastewater Plant in the Old Westbury Campus.[3]

Solid Phase Extraction System, containing cartridges

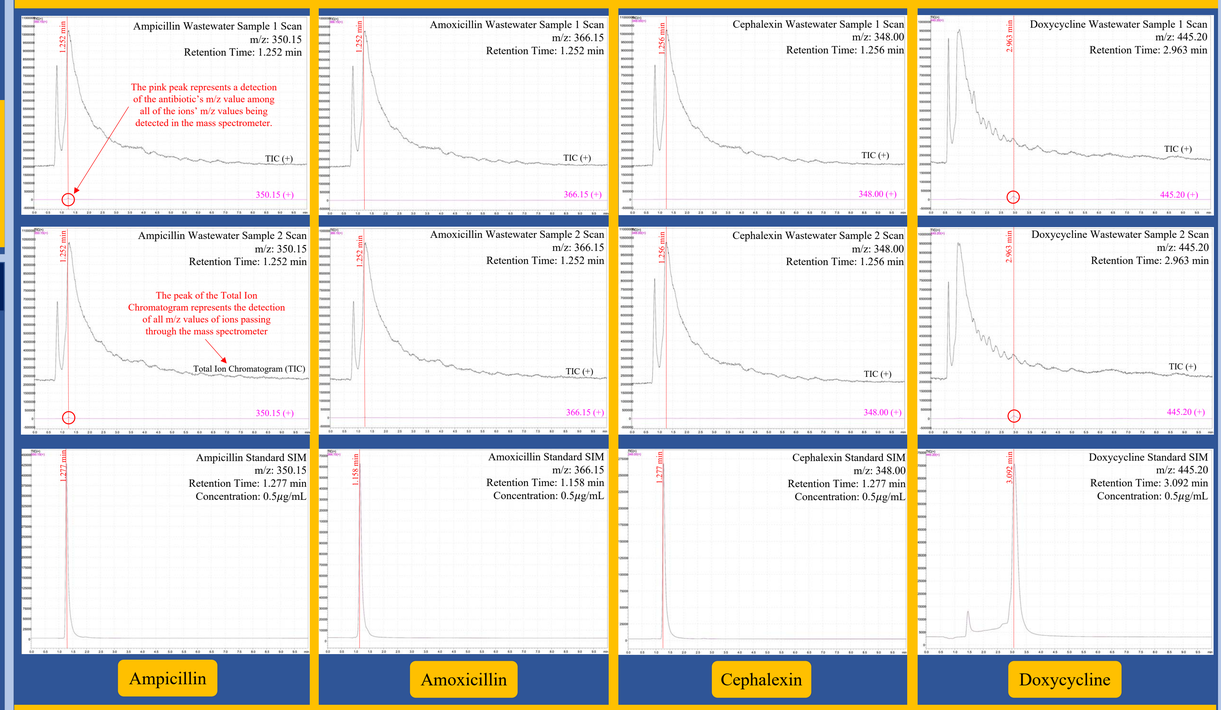
METHODOLOGY VALIDATION

LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY METHOD VALIDATION

Method Name	Antibiotic(s)	Eluent Percentages	Time	Flow Rate	Mode	Oven Temperature	Temperature Limit	Wavelength Channel 1	Wavelength Channel 2	Nebulizing Gas Flow	Interface	Desolvation Line Temperature	Heat Block Temperature
Method S_0.4FR (Isocratic)	Doxycycline	20% Acetonitrile, 10% Methanol, 70% H_2O 0.1% Formic Acid	10 min	0.400 mL/min	SIM (Selected Ion Monitoring) Low Pressure Gradient	35°C	85°C	254 nm	220 nm	1.5 L/min	DUIS (Dual Ion Source)	250°C	400°C
Method V (Isocratic)	Ampicillin, Amoxicillin, Cephalexin	24% Acetonitrile, 13% Methanol, 63% H_2O 0.1% Formic Acid	10 min	0.300 mL/min	SIM (Selected Ion Monitoring) Low Pressure Gradient	35°C	85°C	254 nm	220 nm	1.5 L/min	DUIS (Dual Ion Source)	250°C	400°C

RESULTS

MASS SPECTROMETRY CHROMATOGRAMS



CHROMATOGRAM KEY

The **BLACK** peaks refer to the detection of all ions with varying m/z (mass to charge) values passing through the mass spectrometer.
The **PINK** peaks refer to the detection of the antibiotic's, and potentially other molecules', specific m/z value.
The **Antibiotic Standard Chromatograms** represent the ability to detect the antibiotics at concentrations as low as 0.5 μg/mL.

CONCLUSIONS

The detection of a peak at the same retention time and with the same m/z value as the ampicillin and doxycycline standards indicates the presence of these antibiotics in New York Tech wastewater samples. No peak was detected for amoxicillin and cephalexin, suggesting that these two antibiotics are not present.

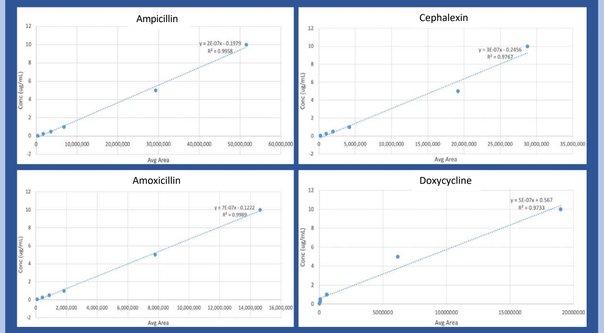
Large losses of antibiotics when performing solid phase extraction with the antibiotic standards poses a major challenge. The inability to minimize loss with the antibiotic standards means that we may be experiencing similar losses with our wastewater samples. Current losses indicate that the antibiotics are possibly detectable in wastewater, but not quantifiable.

Method optimization for solid phase extraction must continue in order to minimize the loss of the antibiotics.

SOLID PHASE EXTRACTION VALIDATION

Step	Solvent	Volume	Flow Rate
Calibration	Methanol	3 mL	3 mL/min
Equilibration	Acidified H_2O with HCl (pH ~ 3.0)	3 mL	3 mL/min
Loading	Standard/Wastewater Samples	50 mL	1 mL/min
Washing	Acidified H_2O with HCl (pH ~ 3.0)	1 mL	1 mL/min
Vacuum Drying	--	--	1 mL/min
Elution	Methanol	3 mL	3 mL/min

STANDARD CALIBRATION CURVES



- Following completion of Solid Phase Extraction, rotary evaporation is used to dry the samples. Samples are dried using a 40°C water bath.
- Dried samples are resuspended in 2 mL of H_2O 0.1% Formic Acid.
- Samples are analyzed using Liquid Chromatography/Mass Spectrometry Method S_0.4FR and Method V to determine if Doxycycline or Ampicillin, Amoxicillin, and Cephalexin are present in the wastewater samples, respectively.

ACKNOWLEDGMENTS AND REFERENCES

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[1] Do, T. C. M. V., Nguyen, D. Q., Nguyen, T. D., and Le, P. H. (2020, March 24) Development and validation of a LC-MS/MS method for determination of multi-class antibiotic residues in aquaculture and river waters, and photocatalytic degradation of antibiotics by Tio2 nanomaterials. *MDPI*. Multidisciplinary Digital Publishing Institute.
[2] Elhag DE., Abdallah BS., Hassan M., and Suliman A. (1, November 30) ESI-LC/MS method development and validation for the determination of some selected antibiotics in hospital wastewater. *Pharmaceutica Analytica Acta*. Walsh Medical Media.
[3] MacDermott, R. (2017) A wonderful visit with Horizons parent Mr. Panella at the NYIT wastewater management facility. photograph, Old Westbury.
[4] Olaitan, O. J., Anyakora, C., Bamiro, T., and Tella, A. T. (2014, June 30) Academic Journals - Journal of Environmental Chemistry and ecotoxicology - determination of pharmaceutical compounds in surface and underground water by solid phase extraction-liquid chromatography. *Journal of Environmental Chemistry and Ecotoxicology*. Academic Journals.
[5] Tran, N. H., Chen, H., Do, T. V., Reinhard, M., Ngo, H. H., He, Y., & Gin, K. Y. (2016, June 3) Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution. *Talanta*. U.S. National Library of Medicine.
[6] Unutkan, T., Bakirdere, S., and Keyf, S. (2017, August 28) Development of an analytical method for the determination of amoxicillin in commercial drugs and wastewater samples, and assessing its stability in simulated gastric digestion. *OUP Academic*. Oxford University Press.