PHARMACEUTICALS IN THE ENVIRONMENT: PREVIOUSLY UNRECOGNIZED SOURCES IN WATER RESOURCES

by

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ABSTRACT

NICOLE L. KENNEDY NETH. Pharmaceuticals in the environment: previously unrecognized sources in water resources. (Under the direction of DR. OLYA KEEN).

It is known that wastewater treatment plants are one of the major routes through which pharmaceuticals enter the environment. The following work investigated the effect of previously unrecognized sources of pharmaceuticals that enter water resources via wastewater treatment plants.

Specifically, this work focuses on determining the role of healthcare facilities in the load of pharmaceuticals to solid waste and wastewater, examining whether landfill leachate may contribute to the pharmaceutical concentration in wastewater treatment plants more significantly than previously known, and investigating pharmaceutically active transformation products as pharmaceuticals go through wastewater disinfection (with antibiotics as a subset of pharmaceuticals investigated).

The research revealed that most healthcare facilities dispose of unregulated pharmaceuticals in a responsible manner. However, a single facility can make a significant impact due to the large amounts of pharmaceuticals discarded. The study found improved recovery and reproducibility when size exclusion is used in solid phase extraction for emerging contaminants from leachate. Lastly, active transformation products were discovered in four of the six antibiotics studied in wastewater disinfection. This work found three previously unrecognized sources of pharmaceuticals in water resources that will be of great importance to the scientific community and to public health awareness and education.

DEDICATION

I would like to dedicate this to my husband, Bryan, whose support planted the seed and whose encouragement never allowed me to say “I can’t.” Your confidence in me gives me strength to set goals I once thought were unobtainable. I would also like to dedicate this to Lucia, Jameson, Dallas, and Ralphie. Your unconditional love and support helped me through the toughest of times.

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LIST OF ABBREVIATIONS

ARG	antibiotic resistant genes
BPA	bisphenol-A
CBZ	carbamazepine
CEC	contaminants of emerging concern
CIP	ciprofloxacin
CTC	chlortetracycline
DNA	deoxyribonucleic acid
DOM	dissolved organic matter
DOX	doxycycline
DW	drinking water
EPA	environmental protection agency
FAC	free available chlorine
HLB	Hydrophilic-Lipophilic Balance
HPLC/MS	high performance liquid chromatography/mass spectrometry
IBU	ibuprofen
LVF	levofloxacin
MS	mass spectrometry
MS/MS	tandem mass spectrometry
OFL	ofloxacin
OSHA	occupational safety and health administration
OWC	organic wastewater contaminants
PE	Polyethylene

RMW	regulated medical waste
RCRA	Resource Conservation and Recovery Act
RCS	reactive chlorine species
RT	retention time
SMX	sulfamethoxazole
SPE	solid phase extraction
TMP	trimethoprim
TP	transformation product
UP	ultrapure water
USGS	U.S. Geological Survey
WW	wastewater
WWTP	wastewater treatment plant

CHAPTER 1: INTRODUCTION

Pharmaceuticals were first introduced in the early 1800s[1-2], since their conception they have revolutionized the world. Different classes of pharmaceuticals have evolved to form hundreds of medicinal drugs readily available for prescription use.[3] Common types of pharmaceuticals include antibiotics, statins, steroids, anesthetics, hypertension, anticonvulsants, antidepressants, anti-inflammatory, anticoagulants, and vaccines.[3]

When pharmaceuticals were first introduced the quality of life improved and mortality rates sharply declined, thanks in part to the introduction of antibiotics and vaccines.[2] Pharmaceuticals continue to improve public health efforts around the world. In 1980, the World Health Organization announced the global eradication of smallpox in humans.[4] In 2010, thanks in large part to the Food and Agriculture Organization of the United Nations, rinderpest (a viral disease largely infecting cattle) was announced to have been completely eradicated.[5] This is currently the only disease of livestock to be eradicated by human efforts.[5]

Before the 1800s, pharmaceuticals were plant-based and could be found at the local apothecary shop. Nowadays, some pharmaceuticals may be derived from plant-based substances but all retain some form of chemical activity.[2] The pharmaceutical industry has continued to grow and evolve to be on the forefront of the latest technologies, clinical trials, and groundbreaking discoveries in pharmacological sciences. With the vast improvements that have been made to human and animal health it can be easy to ignore the decline in environmental efforts that has resulted.

While the effects of most environmental pharmaceuticals on human health are unclear, some classes such as antibiotics have been linked to human health concerns.

With the insurgence of pharmacological compounds into our environment through improper disposal or excretion, it is not a far stretch to see how antimicrobial resistance is of paramount global concern.[6-7] One significant concern is the role healthcare systems play in the load of pharmaceuticals to solid waste and wastewater. Though their disposal methods may be widely known by those in the field, their compliance is of more concern. To date, there have not been many studies that were able to grasp the amount of disposal accumulated and compliance of disposal by a variety of healthcare providers.[8-12] Responsible disposal options for major hospital systems especially is urban areas are more readily available. Areas of interest as well are smaller and private healthcare institutions especially in rural areas.

Another source of pharmaceuticals in the environment that has received little attention is through leachate from municipal solid waste landfills. Leachate is the liquid that collects at the bottom of landfills and is commonly pumped to nearby wastewater treatment plants (WWTPs). The concern with leachate is the improper disposal of pharmaceuticals, i.e. throwing them into solid waste, which is sent to landfills. A concern is the unknown amount of pharmaceuticals entering WWTPs via leachate and whether this entry is a significant contributor to the overall amount of pharmaceuticals in WWTPS.

Recent studies have detected contaminants of emerging concern (CECs), which includes pharmaceuticals, industrial chemicals, household chemicals, nonprescription pharmaceuticals, steroid hormones and animals/plant sterols in varying concentrations within leachate.[13-15] Bisphenol A, a component of plastics and thermal paper, was seen in the highest concentration of 17,200,000 ng/L whereas pharmaceuticals ranged from 10 –

10,000 ng/L.[13] The CEC concentrations were seen to be greater in active landfills compared to closed landfills.[13] Furthermore, CEC concentrations were greater in untreated leachate compared to treated leachate. The CEC concentrations were also significantly greater in leachate discharged to WWTPs from lined landfills compared to leachate released to groundwater from closed unlined landfills.[13]

An additional source of these pharmacological compounds into the environment has been found to be WWTPs.[16-20] Through no fault of their own, as WWTPs are not designed to remove pharmaceuticals from their final effluent, they have become ground zero for investigating these rising concerns. Although the emergence of pharmaceuticals in wastewater is becoming widely known, their transformation products (TPs) open another door for possible entry of pharmacologically active substances into the environment. TPs refer to the altering of a parent compound, e.g. a common antibiotic, through a chemical treatment process (typically, disinfection) at the WWTP. Altering the parent compound can refer to the breaking down of the compound into smaller fragments or attachment of structural parts, such as chlorine atom or a hydroxyl group, onto the compound. Concentrations of pharmaceuticals at WWTPs vary widely[21-25] but little is known of their transformation products. These TPs deserve a closer inspection as some may retain their pharmacological potency[26] and greatly add to the concentrations of active pharmaceuticals found in the environment.

There is a particular concern for antimicrobial compounds in the environment and the effects these compounds have on human health. Civil and environmental engineers now find themselves protecting waterways not only from harmful pathogens and harsh chemicals but also from what may be the biggest global health undertaking of the 21st

century, antimicrobial resistance. Understanding the importance of different sources of pharmaceuticals being released into waterways will greatly aid in this present and future undertaking.

1.1 Disposal of pharmaceuticals in healthcare settings

The elusive world of pharmaceuticals in healthcare settings has not been studied in depth[8-9, 11-12] partly because collecting the data is a monumental task. Healthcare facility procedures for waste disposal can vary widely. Larger hospital systems may have stricter internal protocols simply because of the volume and type of waste produced. While managing large amounts of medical waste can be overwhelming, it is important for staff to be informed on the procedures for proper waste disposal. Studies have found a recurrent theme that medical workers are under-trained and uninformed.[27-28]

Healthcare medical waste can be extremely confusing adding to the frustration of properly disposing of waste. There are at least seven different forms of healthcare waste, each with its own container color.[29-35] Separating each category of medical waste is extremely important as incorrectly disposing can lead to costly fines. Additionally, when waste is not properly categorized, healthcare systems can find themselves paying for excess waste. An example of this would be when intravenous medication delivery systems (IVs) are used. The IVs have a specific disposal color associated with them depending on a substance being administered through the IV; however, their packaging can be discarded into municipal waste. This amount of waste is calculated into the total cost of disposal. This is an important key element that healthcare systems can overlook.

However, if waste is improperly disposed into more costly receptacles, the overall cost of disposal and incorrect accumulations prove to be a major financial burden.

The main goal of studying pharmaceutical waste in healthcare settings was to understand how they handle these disposal issues. Particular interest was placed on disposal of unregulated pharmaceutical substances, as there is a large degree of freedom within the facilities of how these pharmaceuticals are managed. Some disposal options medical facilities may choose would have greater effect on the environment. It is important to determine whether they contribute a significant load through sewage disposal or by landfill leachate. Significant interest was also placed on comparing urban settings to rural settings. There may be less awareness in rural areas and potentially fewer programs available for segregating non-regulated pharmaceutical waste compared to urban areas.

1.2 Leachate as a source of pharmaceuticals

Landfill leachate is the liquid that collects at the bottom of the landfill. This liquid leaches or drains from the waste and is heavily affected by heavy rainfalls and seasonal variations.[36] Leachate can vary quite significantly in composition depending on the age of the landfill and type of waste.[36] Leachate can exhibit extremes in pH, biochemical oxygen demand, chemical oxygen demand, heavy metals and will usually contain both dissolved and suspended solids.[37]

One of the main environmental concerns associated with landfills is the discharge of leachate into water resources via WWTPs. Leachate is produced from the beginnings of the landfill to many decades after the landfill has been closed.[37] In modern lined

landfills, the leachate is typically sent to the local municipal WWTP. Leachate has been shown to be a source of potential long-term persistence and transport of organic wastewater contaminants (OWCs) in ground water.[38] On the other hand, the role of leachate in contributing OWCs to surface water via WWTPs is not well understood.

Reducing the amount of waste to be landfilled and utilizing recycling centers, incinerations and composting can help in reducing the production of leachate.[37] Separating hazardous materials of municipal waste such as expired medicines, mercury lamps and pesticides can help in reducing leachate concentrations of heavy metals, halogenated hydrocarbons and other toxic compounds.[37] Pharmaceuticals can find their way into landfills through household trash, medical waste, or industry (pharmaceutical) waste. However, the concentration of these pharmaceuticals can vary quite significantly.

The technique necessary for analyzing leachate for pharmaceuticals is solid phase extraction (SPE). SPE is necessary to concentrate pharmaceuticals, which are present at low concentrations and to isolate them from the sample background. Leachate can be difficult to work with in regards to the large variety of anthropogenic compounds and possible TPs. These compounds can have wide ranges of polarities thus the extraction methods need to be adapted to the specific chemical characteristics of the samples.[39] Additionally, leachates contain very high concentration of other organics, such as humic substances, that can interfere with the extraction by saturating the resin media.

Commonly, SPE is used to extract the compound of interest; the compound can then be analyzed through either HPLC/MS or GC/MS depending on which analytical method works best for the specific compound. The U.S Geological Survey (USGS) when investigating pharmaceuticals and other organic wastewater contaminants of a leachate

plume used both HPLC/MS and GC/MS methods for their purposes.[38] While the analytical methods are straightforward, what is challenging is the best SPE method for extracting a variety of compounds from such a complex matrix.

SPE utilizes cartridges filled with adsorption resin, through which the sample solution, i.e. leachate, will pass. The leachate is able to pass through these porous cartridges with the aid of a vacuum pressure manifold, or an automated instrument working on the same principle.[40] Leachate has large-size organics that can saturate the resin and prevent good recovery of small organics such as pharmaceuticals. There are a variety of cartridges available commercially for different applications. These cartridges can vary widely in size and adsorption media. Some work better with polar compounds while others work best with non-polar compounds and some are even able to work efficiently with both polar and non-polar compounds. Some cartridges offer size exclusion in addition to adsorption where only small molecules are allowed to diffuse into the resin pores where adsorption in happening. But no research is available to understand which cartridges will work best for extremely complex water matrices such as leachate.

Studies have reported pharmaceuticals in leachate previously but most use HLB (Hydrophilic-Lipophilic Balance) cartridges for extraction with pharmaceuticals,[41-46] although others have also used PPL cartridges.[47] HLB cartridges are widely used in wastewater, drinking water, surface water, and groundwater and have been efficient for recovery of known pharmaceuticals in these matrices.[48-53] Because leachate has higher non-target organics than most other environmental samples, it can quickly coat the media and prevent pharmaceuticals from being properly extracted. For this reason three

cartridges have been evaluated and compared to HLB. These cartridges have different size exclusion capabilities to see if an improved pharmaceutical detection could be obtained in comparison to traditional methods. The four cartridges evaluated were HLB, ENV, PPL and PLEXA.

1.3 Fate of Antibiotics in WWTPs

Pharmaceuticals in wastewater effluent have been of interest to researchers for decades due to their effects on the aquatic life and implications for human health as well. For example, the feminization of fish[54] and the rise of antibiotic resistant bacteria[55] are some of the consequences of pharmaceuticals in the environment linked to treated wastewater discharges.[56]

Antibiotics especially have clear implications for human health. Streams that receive treated effluent show higher levels and diversity of antibacterial resistance genes, and it has been postulated that trace levels of antibiotics in wastewater cause the rise of antibacterial resistance in the environment.[57,58,59,60] TPs are known to some extent, however their properties (e.g. toxicity, endocrine disrupting potential, etc.) are not well studied. Transformation typically occurs during chemical disinfection processes, although some pharmaceuticals can be biodegraded. For this study, the focus has been on the antibiotic class of pharmaceuticals for their public health significance.

Apart from the antibiotics that are known to be present in wastewater, other antibacterially active compounds could be forming when antibiotics go through chemical reactions in wastewater treatment (biodegradation is typically not a major pathway for antibiotics).[61] Most of the chemical transformations in wastewater treatment occur when

antibiotics are exposed to disinfectants. Chlorine is one of the most commonly used wastewater disinfection chemicals. Studies show that many pharmaceuticals readily react with chlorine forming a number of products.[62, 63, 64, 65] Products of antibiotics could potentially retain the antibacterial activity and contribute to the development of antibiotic resistance in the environment[66].

Prior research on the fate of antibiotics in disinfection processes (particularly, the properties of the products that form) has focused on UV[67,68] and ozone[69]. Chlorine has also received some attention regarding the products that form in the reaction between the disinfectant and the antibiotics found in wastewater. For example, researchers have examined the reaction rates between various pharmaceuticals and chlorine[70,71] and some identified the reaction pathways and postulated product structures.[72,73] Some of the studies specifically stressed that chlorine incorporation is a major pathway in the reactions between pharmaceuticals and aqueous chlorine, yet the toxicological or other relevant properties of chlorinated products of pharmaceuticals need to be explored more.[74] For example, recently, a study showed testosterone inhibition in fish by chlorinated products of a cholesterol-lowering drug gemfibrozil.[75] When it comes to antibiotics, studies have examined the toxicity of some antibiotics and possible TPs, but limited studies have evaluated the changes of antibacterial activity of products resulting from reactions with chlorine.[16, 76]

A recent study examined the genotoxicity of quinolone antibiotics that produced chlorinated disinfection byproducts.[77] Another study on levofloxacin saw an increase in toxicity during chlorination that suggests the first TPs that were formed were more toxic than the parent compound.[78] Some studies have measured the toxicity of the

transformation byproducts and proposed transformation pathways.[79, 80] One study inferred that a chlorination product of levofloxacin may retain the antibacterial activity of the parent compound because the core molecular structure is preserved in the reaction.[78, 81] Additionally, one study arrived at the same conclusion for trimethoprim stating that the core structure had not changed enough in the reaction, and the products likely retain the antibiotic properties of the parent compound.[82]

Previous studies have examined degradation of antibiotics in disinfection processes. Trimethoprim is among one of the most commonly detected antibiotics in the environment appearing at several hundred ng/L in wastewater effluents.[83-84] Trimethoprim has been shown to react quicker with reactive chlorine species (RCS) ($ClO^{\cdot}$) over $HOCl/OCl^{-}$ to form TPs when advanced oxidation with UV/chlorine was applied.[85] Another study has shown that the trimethoprim structure was not substantially degraded when reacted with free available chlorine (FAC) even though transformation of the parent compound was substantial with conditions typical of wastewater and drinking water chlorination.[82]

Another antibiotic that is commonly prescribed and combined with trimethoprim is sulfamethoxazole.[86] Sulfamethoxazole has been detected at concentrations on the order of 200- 2000 ng/L in wastewater effluents.[55, 87-88] It has been shown that sulfamethoxazole is reactive with free chlorine but no transformation products were identified.[89-90] Other experiments with sulfamethoxazole and FAC have seen chlorinated products, but have not examined the properties of the products.[91]

Fluoroquinolones are a class of antibiotics with particular interest because they are not completely metabolized, for this reason a substantial amount can be discharged

into wastewater treatment facilities. Studies have reported fluoroquinolones present in wastewater effluents at concentrations in the range of 70 – 500 ng/L.[49, 92-93] A recent study found ciprofloxacin chlorinated products in drinking water distribution systems.[94] The results indicated that the measured increase of antibiotic resistant genes was from the growth of bacteria in the presence of the chlorinated TPs.[94]

Another recent study examined the reaction between FAC and ofloxacin, another fluoroquinolone class of antibiotics. Several transformation products were seen including some chlorinated transformation products.[95] Ofloxacin is a racemic mixture with 50% levofloxacin (the biologically active component) and 50% of its enantiomer dextrofloxacin.[96] Dextrofloxacin is 8-128 times less potent than levofloxacin, maintaining some antibiotic properties.[97] Levofloxacin, was investigated under chlorination process to investigate four transformation products that arose.[78] The first transformation products that formed showed more toxicity than the parent compound. *V. fisheri* was used as a test organism to test the toxicity of the products compared to the parent compound.

To date there has not been a comprehensive study of doxycycline, trimethoprim, sulfamethoxazole, ciprofloxacin, levofloxacin, and ofloxacin (the most commonly prescribed and detected antibiotics) with exposure to chlorine disinfection in wastewater using microbial assays for transformation product potency. This study investigated possible transformation product properties and whether these products retained their antibacterial potency. If so, these TPs will be of interest to the scientific community and their presence in the environment is a public health concern. Furthermore, this study observed the outcome of sulfamethoxazole and trimethoprim together in their prescribed dosage of 5:1 ratio. This ratio was investigated to observe if the synergetic relationship

between the two antibiotics produced varying results for TPs compared to their individual results.

CHAPTER 2: MATERIALS AND METHODS

2.1 Surveys of non-regulated pharmaceutical waste disposal in healthcare settings

The free online survey software, Survey Monkey ®, was used to create the survey to gage the waste disposal procedures in healthcare settings. It was important to gather information on disposal of unused expired and partially used medication as healthcare systems may significantly contribute to pharmaceuticals in waterways if these substances are improperly discarded. Also of interest were the volumes of medications being disposed of and the specific classes of pharmaceuticals. It was important to have the respondents give an estimated amount of disposal volume (either in liquid or solid measurements) to know where disposal efforts would be of most benefit. Another interest of concern was rural versus urban healthcare settings and their overall understanding of disposal and amount generated. Due to the lack of regulation on certain types of pharmaceutical wastes, it was hypothesized that rural healthcare facilities are less likely to participate in voluntary pharmaceutical collection programs and more likely to dispose of medication via sewer or municipal solid waste.

A link was generated and distributed with the help of the organization Practice Greenhealth. This organization is a non-profit membership organization and helps to create better, safer, greener workplaces and communities for healthcare facilities. The surveys were also distributed with the help of Eastern Kentucky University's Environmental Health and Safety Alumni program. Ten questions were asked on the survey and are included in Table 1.

The main types of medical waste include hazardous, non-hazardous, chemotherapy, and regulated medical waste. The Environmental Protection Agency (EPA) regulates hazardous waste and non-hazardous waste under the Resource Conservation and Recovery Act (RCRA). Nine chemotherapy pharmaceuticals are considered hazardous waste and are regulated under RCRA. The chemotherapy pharmaceuticals that are not considered hazardous waste and have no more that 3% by weight in the paraphernalia (i.e. IVs, tubing, gloves, syringes, and vials) can be disposed of in 'chemotherapy only' (yellow receptacles) and will be incinerated. Regulated medical waste (RMW) also known commonly as biohazardous waste includes blood, body fluids, or other materials that may pose potential risk of transmitting infection. This waste can be autoclaved, incinerated, microwaved, or chemically disinfected and then disposed into the municipal waste. Controlled substances, including opioids, are regulated by the Drug Enforcement Administration (DEA). For the purposes of this study the wastes of concern are not regulated by the DEA as controlled substances and not classified as biohazardous or hazardous waste.

Table 1. Survey questions

	Survey questions/statements asked
1	What kind of medical facility are you? • Private general practice, outpatient only • Private specialized practice, outpatient only • Multi-practitioner general practice, outpatient only • Multi-practitioner specialized practice, outpatient only • General hospital, includes inpatient and surgery • Specialized hospital, included inpatient and surgery • Assisted living/long-term care facility • Hospice care • Veterinary clinic • Pharmacy • Dentistry • Other (please specify)
2	Do you serve mainly rural population, mainly urban, or both? • Rural • Urban • both
3	Does your facility have an unused expired medication disposal protocol for the whole facility? • Yes • No • If yes, does this disposal protocol vary by department
4	Does your facility have organized unused medication disposal days/events, or is medication disposal done on as-needed continuous basis? • Specific days/events • As needed
5	How frequently does your facility inventory medication and dispose of unused expired medication? • Weekly • Monthly • Every few months • Once a year or less frequently • This is not applicable to our facility • Please add any comments_____

6	What amount of expired medication typically accumulates between disposal times? Please provide your best mass or volume approximation: e.g. 5 lb. or 10 gal.
7	How do you dispose of partially used non-expired medication? • Down the sink drain • Into municipal solid waste • With DEA regulated medical waste • Special pharmaceutical waste collection program • If special pharmaceutical waste collection program is used please specify
8	How do you dispose of expired medication? • Down the sink drain • Into municipal solid waste • With DEA regulated medical waste • Special pharmaceutical waste collection program • If special pharmaceutical waste collection program is used please specify
9	What are some of the highest volume medications that your facility routinely disposes of? • Antibiotics • Anticonvulsants • Hormones • Statins • Blood pressure • Anti-inflammatory • Antidepressants • Steroids • Vaccines • Anesthetics • Other (please specify)
10	Please provide any details or comments that you would like to share with the study.

2.2 Solid phase extraction (SPE) for investigation of pharmaceuticals in leachate

The glassware used for all experiments were baked in the furnace at 550°C for 3 hours prior to use. Any glassware that was not able to be heated to 550°C was soaked in an acid bath overnight, then rinsed at least 5 times with ultrapure water and one time with HPLC grade methanol. A glass block vacuum manifold (Figure 1) was used along with

four solid phase extraction (SPE) cartridges: HLB, ENV, PPL and Plexa. The ENV, PPL, and Plexa cartridges were all bond-elut and were obtained from Agilent Technologies (Santa Clara, CA). The Supel-Select HLB cartridges were purchase from Supelco [part of Sigma Aldrich] (Bellefonte, PA).

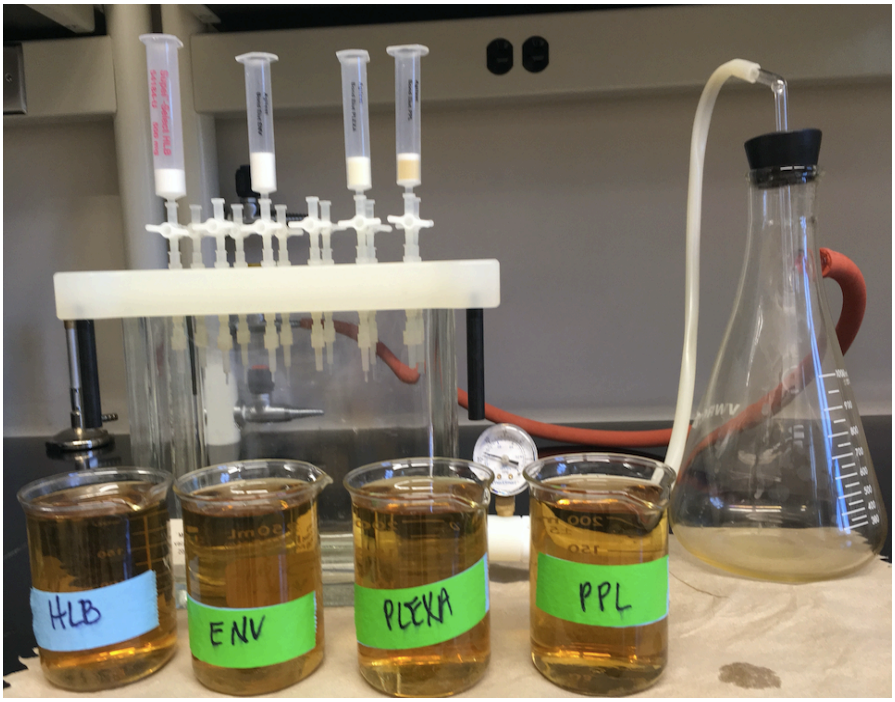


Figure 1. The glass block vacuum manifold that was used for all SPE experimentation, with chosen cartridges, and respective leachate.

A description of the four SPE cartridges chosen can be seen in Table 2, all the cartridges with the exception of HLB exhibit size exclusion capabilities. A schematic of size exclusion principle can be seen in Figure 2. All the cartridges were rinsed with 5 mL of HPLC grade methanol (Sigma Aldrich, St. Louis, MO) and then 5 mL of ultrapure water.

Table 2. Comparison of the four SPE cartridges chosen.

SPE cartridge	Features
HLB	The material consists of Polyethylene (PE) (20 µm) with a hydrophilic modified styrene polymer. Can be used with broad range of compounds from aqueous samples. Retention is predominately based on reversed-phase but can be adaptable to polar compounds as well.
ENV	A polymer, designed for the extraction of polar organic residues. It contains 125 µm spherical particles with cross linking; advantageous for high volume and fast flowthrough applications.
PPL	A styrene-divinylbenzene (SDVB) polymer that has been modified with a nonpolar surface. Will retain even the most polar classes of analytes, including phenols. Large particle size allows for particular-rich water samples with strong hydrophobicity.
PLEXA	Has a nonretentive, hydroxylated, amide-free surface, and a nonpolar core for retaining small molecules. Binding of proteins and lipids on the polymer surface is minimized, resulting in cleaner samples and reduced ion suppression.
Source: Agilent technologies product information and Sigma Aldrich product information	

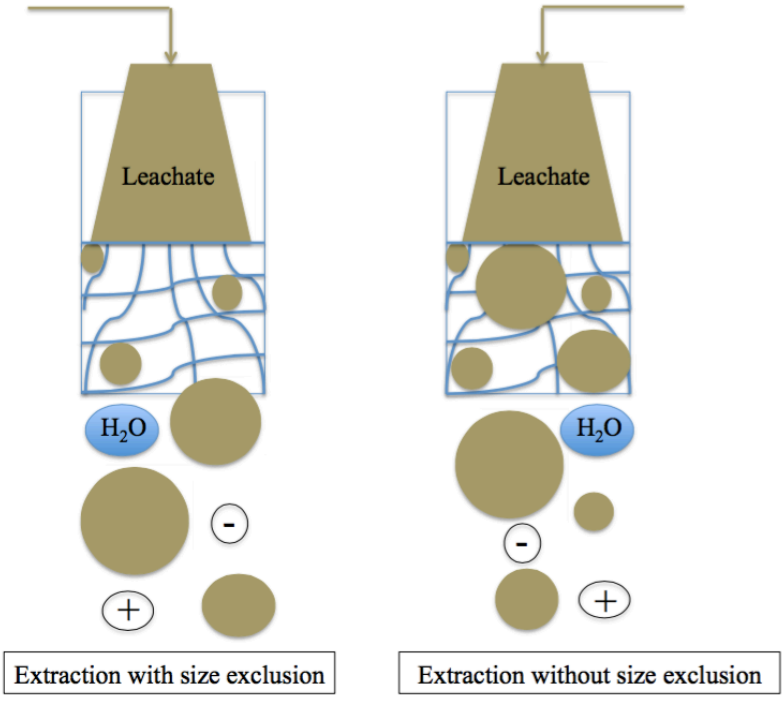


Figure 2. A schematic of SPE cartridges extraction with size exclusion (retains only small organics) and without size exclusion (retains organics of various sizes).

The leachate used was collected from a local landfill and was first acidified to a pH of 3.5 per typical extraction methods for pharmaceuticals from environmental samples to ensure all the target pharmaceuticals were in their adsorbable form.[98] The pK_{a1} of carbamazepine is 13.9[99], the pK_{a1} of bisphenol-A is 9.6[100], and the pK_{a1} and pK_{a2} of ciprofloxacin is 6.09 and 8.74, respectively.[101] These compounds were chosen in anticipation of finding them in leachates. BPA is a plasticizer and present in many consumer plastic products that get discarded. CIP is a commonly prescribed antibiotic and CBZ is an anticonvulsant that is one of the least biodegradable pharmaceuticals.[102] The leachate was then filtered first through 0.8 µm glass fiber filters (Merck Millipore Ltd., Billerica, MA) and then through 0.45 µm membrane filters (Whatman, Piscataway, NJ), Figure 3.

Figure 3. Leachate before filtration (left) and leachate after being filtered through glass fiber filters (right).

Additionally, after final elution the procedure for which is described below, the 1 mL sample was filtered through 0.2 µm membrane syringe filters (Advanctec, Matthews,

NC) (Figure 4). This was to both allow better performance of the cartridges and also to minimize interferences with the HPLC/MS instrumentation.

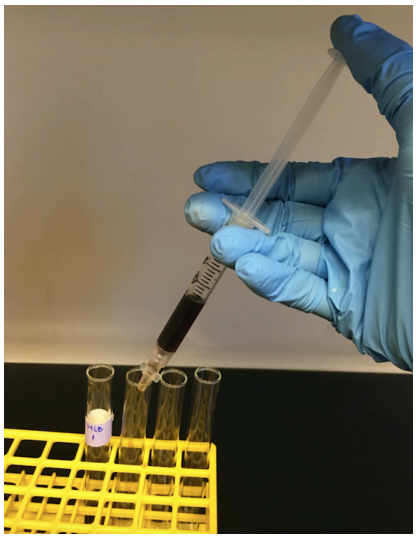


Figure 4. Sample being filtered through 0.2 µm membrane syringe filter.

Once 1 L of acidified leachate had been filtered it was then spiked with 1 µg/L of carbamazepine-d10, bisphenol A-d16 and ciprofloxacin-d8 hydrochloride (all obtained from Sigma-Aldrich, St. Louis, MO). The deuterated form of each compound was chosen to differentiate the spiked compounds from the same compounds that may already exist in the leachate sample. The 1 L solution was then divided into four 250 mL sterile beakers each being designated to one of the four cartridges. The leachate from the respective beakers passed through the cartridges at 1-2 drops per second. A blank was also run immediately after the experiments with the cartridges. This was to capture any potential contamination in the protocol. The entire experiment was run three times.

After the samples were loaded onto the cartridges, the wastebasket was removed and glass tubes placed in the racks to the corresponding cartridge. The adsorbed substances were then eluted with 2.5 mL of HPLC grade acetonitrile (EMD Millipore

Corporation, Billerica, MA) and 2.5 mL of HPLC grade methanol, allowing 5 mL of volume to be captured into the glass tubes. Figure 5 shows the samples after they were eluted from the cartridges in the 5 mL glass tubes.

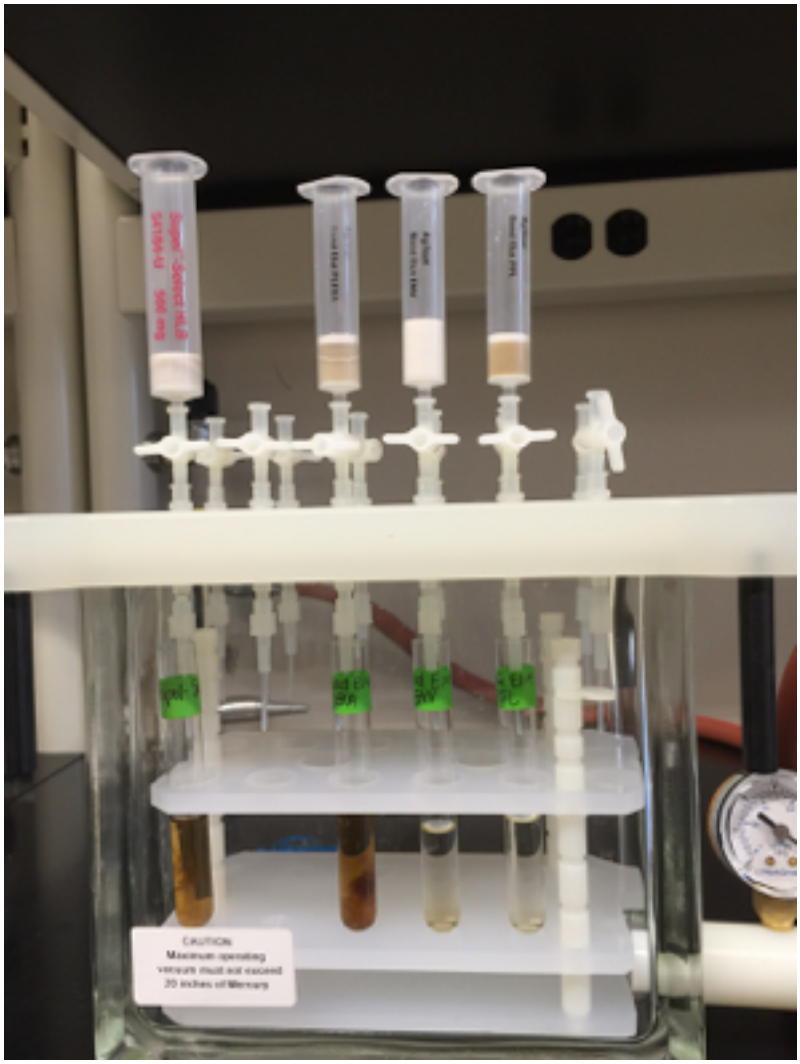


Figure 5. Sample after final elution in 5 mL glass tubes.

The glass tubes were then placed in a nitrogen hot water bath evaporator (N-evap 111 nitrogen evaporator OA-HEAT model 5085, Organomation, Berlin, MA) and allowed to evaporate down to 1 mL. The glass tubes were weighed before the volume was added and after the volume was evaporated down to capture the final volume and

add the appropriate amount of HPLC grade water so that the final sample volume was 1 mL. The resulting extracted sample was transferred to HPLC vials for HPLC/MS analysis.

2.3 HPLC/MS methods for SPE

Analysis was done using high performance liquid chromatography with mass spectrometry (HLPC/MS) Vanquish flex quaternary HPLC system and a Velos pro dual-pressure linear ion trap mass spectrometer with electrospray ionization (ESI) source (Thermo Fisher Scientific, Waltham, MA), seen in Figure 6.

Figure 6. High performance liquid chromatography with mass spectrometry (HLPC/MS).

The method was run in full scan mode (m/z range 200-1000) under positive ionization. The chromatography mobile phase consisted of solvent A (HPLC grade water with 0.1% formic acid) and solvent B (HPLC grade acetonitrile with 0.1% formic acid). The method gradient began with 10% Solvent B and involved a minute delay before ramping. The flow during the first minute was diverted to waste to ensure inorganic constituents and first column flush would be diverted from the mass spectrometer. This was then followed by gradual ramp up from 10% solvent B to 100% solvent B over 54 min. In the last 6 min of the method, solvent B was dropped to 10% and allowed to equilibrate. The flow was at 0.25 mL/min, the column temperature was 35°C with the injection volume of 10 µL. The column was obtained from Agilent Technologies (Santa Clara, CA) and was a 2.1x100 mm Zorbax Eclipse Plus C-18 rapid resolution high throughput with 1.8 micron particle size with a 1000 Bar maximum pressure.

2.4 Samples and Reagents

Different classes of antibiotics were chosen to enhance the understanding of formations of TPs. These classes include tetracycline (doxycycline), pyrimidine inhibitors/sulfonamides (trimethoprim/sulfamethoxazole), and fluoroquinolones (ciprofloxacin, levofloxacin, and ofloxacin). Doxycycline hyclate, ciprofloxacin, sulfamethoxazole, and trimethoprim were obtained from Sigma-Aldrich (St. Louis, MO). 10-15% solution of sodium hypochlorite and 30% solution of hydrogen peroxide were reagent grade and were obtained from Sigma-Aldrich (St. Louis, MO). The deuterated compounds carbamazepine-d10, bisphenol A-d16, and ciprofloxacin-d8 hydrochloride

were all obtained from Sigma-Aldrich (St. Louis, MO) as well. Bisphenol A is a common industrial chemical in consumer products and was applied in leachate analysis along with pharmaceuticals to assess other commonly present contaminants of emerging concern.

Ciprofloxacin, trimethoprim and sulfamethoxazole were initially tested for antibiotic potency as some of the most commonly prescribed and detected in water resources antibiotics. After initial experiments showed antibacterially active products of ciprofloxacin, more of the common fluoroquinolone class antibiotics (levofloxacin and ofloxacin) were tested. Levofloxacin (98% powder) and ofloxacin (98.5% powder) were obtained from Alfa Aesar (Ward Hill, MA). Ofloxacin is a racemic mixture of 50% levofloxacin (active component) and 50% dextrofloxacin (8-128 times lower activity than levofloxacin).

Wastewater effluent was collected from a local wastewater treatment plant that uses UV disinfection. The samples were collected right after UV disinfection and filtered within hours of collection. Samples were first filtered through a nylon 0.8 μm membrane filter (Whatman, Piscataway, NJ) and then a nylon 0.2 μm membrane filter (Merck Millipore Ltd., Billerica, MA) to remove any microorganisms that would compete with the test organism in the antibacterial activity assay. The samples were disinfected again right before experimentation with UV disinfection at dose of 250 mJ/cm^2 using a benchtop low pressure mercury lamp collimated beam. This was done to ensure that the wastewater was fully sterile and indigenous microorganisms not susceptible to the chosen antibiotics would not grow in the assay. The wastewater sample was collected once and used for the entirety of the experiments. The water quality parameters remained stable throughout the duration of the experiments.

Water quality parameters of the effluent were measured immediately after wastewater effluent was filtered, and the water was then placed in storage at 4 °C. Water quality parameters for the wastewater sample can be seen in Table 3.

Table 3. Effluent water quality parameters (values rounded to significant digits replicated in repeated measurements)

Test	Measurement	Method/Instrument used
Nitrite	< 0.012 mg-N/L	nitrite HACH test TNT 839
Nitrate	21.2 mg-N/L	nitrate HACH test TNT 835
Ammonia	< 0.024 mg/L NH_3-N	ammonia HACH test TNT 831
Total Organic Carbon	6.8 mg/L	Shimadzu TOC-LCPN
Absorbance at 254 nm	$0.11cm^{-1}$	HACH DR 6000 UV Vis Spectrophotometer
pH	7.0	HACH pH meter H280G
Alkalinity	42.4 mg/L as $CaCO_3$	HACH alkalinity digital titrator, AL-DT

2.5 Chlorination Procedure

Doxycycline solution with concentration of 10 mg/L, ciprofloxacin solution with concentration of 2.33 mg/L, trimethoprim solution with concentration of 20 mg/L, sulfamethoxazole solution with concentration of 2 mg/L, sulfamethoxazole in tandem with trimethoprim at a 5:1 ratio solution with concentration of 2 mg/L and 0.4 mg/L respectively, levofloxacin solution with concentration of 5 mg/L, and ofloxacin solution with concentration of 2 mg/L were prepared in ultrapure water or in effluent. These concentrations were used in order for the antibiotic to be effective in the assay and to improve the detection of the products (Table 4). Any background antibiotics that may be present in the effluent would have concentration orders of magnitude lower and would not interfere with the assay.

Table 4. LD_{50} values for the selected antibiotics

Antibiotic	LD50 range (mg/L)
Ciprofloxacin	0.25 - 1.48
Levofloxacin	0.34 - 1.34
Ofloxacin	0.24 - 1.38
Trimethoprim	0.46 - 2.8
Sulfamethoxazole	0.39 -1.65
Sulfamethoxazole/Trimethoprim	0.36 - 1.44
Doxycycline	0.32 - 2.75

The purpose of conducting experiments in ultrapure water was for better detection of the products in a clean matrix without interferences. The solution was sampled before chlorination to be able to measure the change in concentration of antibiotic with chlorine exposure over time. The remaining antibiotic solution was chlorinated and samples were taken at the following time intervals: 0.5, 1, 5, 10, 30, 60 and 120 minutes. At each time interval a sample was withdrawn and placed into an HPLC vial (for product structure analysis) and a 1.5 mL sterile centrifuge vial (for antibacterial activity assay). Initial chlorine dose was determined with a goal of 0.5 ± 0.2 mg/L of Cl_2 residual at the end of the experiment to assure that chlorine was present for the full 2 hours of the exposure. At the end of the experiment, the chlorine concentration was measured using a HACH DR 2800 spectrophotometer with N,N-diethyl-p-phenylenediamine colorimetric method (HACH DPD powder pillows).

The reaction with chlorine was stopped after a desired time interval by transferring a volume of the sample into a vial containing hydrogen peroxide (H_2O_2) which immediately reacted with chlorine.[68] H_2O_2 was chosen over other commonly used reagents such as thiosulfate because H_2O_2 does not add background levels of inorganics in the sample, which can interfere with mass spectrometry instrumentation. Preliminary

experiments were also performed with the antibiotics and H_2O_2 to ensure no reaction takes place and no intermediates form. The amount of H_2O_2 used was determined by stoichiometric ratio for chlorine quenching (1 mg/L of H_2O_2 to 2.1 mg/L of Cl_2) based on the initial concentration of chlorine. Residual H_2O_2 was then quenched by bovine catalase (Sigma-Aldrich, St. Louis, MO) at a dose of 1 mg/L with the reaction time of at least 30 min. This was done to make sure the H_2O_2 would have no effect on the assays. Furthermore, H_2O_2 control assays were performed to make sure that H_2O_2 at possible residual concentrations did not inhibit bacterial growth. Treated wastewater effluent control assays were also performed to confirm the effluent matrix did not inhibit bacterial growth in the assay.

2.6 Antibacterial Activity Assay

The chosen antibiotics are effective against gram-negative cells, so a non-pathogenic, non-resistant strain of *Escherichia coli* (ATCC 11303) was used as a test organism in the antibacterial activity assays. The bacterial culture was grown in sterile broth in a shaking incubator set at 37 °C for 24 hours or until cloudy. The broth recipe is as follows: 500 mL of ultrapure water, 5 g of tryptone (Fisher Scientific, Fair Lawn, NJ), 2.5 g of yeast extract (Fisher Scientific, Fair Lawn, NJ) and 2.5 g of sodium chloride (Fisher Scientific, Fair Lawn, NJ). Then 1 mL of overnight culture was transferred to 50 mL of fresh broth where it was grown for an hour to the optical density (absorbance) at 600 nm (OD_{600}) of 0.20 +/- 0.03 cm^{-1}. The 1 hr culture was then diluted 1:10 to achieve the desired cell concentration of approximately 1 million cells per mL. Optical density measurements were used as a measure of bacterial growth in the broth. The correlation

between OD_{600} and cell count for this strain of *E. coli* was determined previously and incorporated into the assay protocol that was adopted for this study.[68] The OD_{600} was measured using the HACH DR6000 spectrophotometer (Hach Corporation, Loveland, CO) and can be seen in Figure 7.

Figure 7. HACH DR6000 spectrophotometer that was used to read assays.

A serial factor-of-2 dilution of the samples before chlorination and after different intervals of chlorine exposure was prepared in a sterile, flat bottom, non-treated Cellstar 96-well plate (Greiner Bio-one, Monroe, NC). Sterile phosphate buffered saline (PBS) was used for dilution to ensure an environment acceptable for microbial cells. PBS recipe includes 400 mL of ultrapure water, 3.20 g of sodium chloride (Fisher Scientific, Fair Lawn, NJ), 0.08 g of potassium chloride (Fisher Scientific, Fair Lawn, NJ), 0.72 g of dibasic sodium phosphate dihydrate (Fisher Scientific, Fair Lawn, NJ) and 0.10 g of potassium phosphate monobasic (Sigma Aldrich, St. Louis, MO). An *E. coli* culture was then added to each sample well. Each plate had positive and negative controls, and growth in each well was calculated as the percentage of the growth in the positive controls. The positive control contained 100 µL of *E. coli* culture in broth and 100 µL of PBS, and served as a measure of bacterial growth not restricted by antibiotics. The

negative control contained sterile broth and sterile PBS (100 µL each) and was expected to contain no growth and was used to monitor if contamination of the sterile solutions or of the assay had occurred.

Sterility control was performed with UV-predisinfected wastewater effluent by using the effluent matrix in a control assay that replicated the conditions of the actual assay. The change in OD_{600} in the sterility control after incubation was 0.002 ± 0.003 (average and standard deviation of 60 wells), which is representative of the instrumental noise and is consistent with negative controls. For comparison, OD_{600} of positive controls was on the order of 0.24. The perimeter well readings were affected by evaporation during the incubation period. Therefore, a buffer zone was created around the perimeter of each plate that contained only 100 µL of sterile PBS solution. The absorbance of the wells was measured using a Bio-Tek Instruments µQuant microplate reader model # MQX200 (Winooski, VT) at 600 nm immediately after the plate preparation and following a period of four hours when the plates were incubated at 37 °C in a shaking incubator. The period of incubation was selected to ensure the bacteria were still within their exponential growth phase and that OD_{600} readings above 0.1 could be obtained in positive controls. Change in OD_{600} of the wells was associated with bacterial growth.

A probit table analysis[103] was used to transform the data from a dose response curve to a straight line that can be analyzed by a linear regression. An example of the transformation from dose-response to linear regression can be seen in Figure 8.

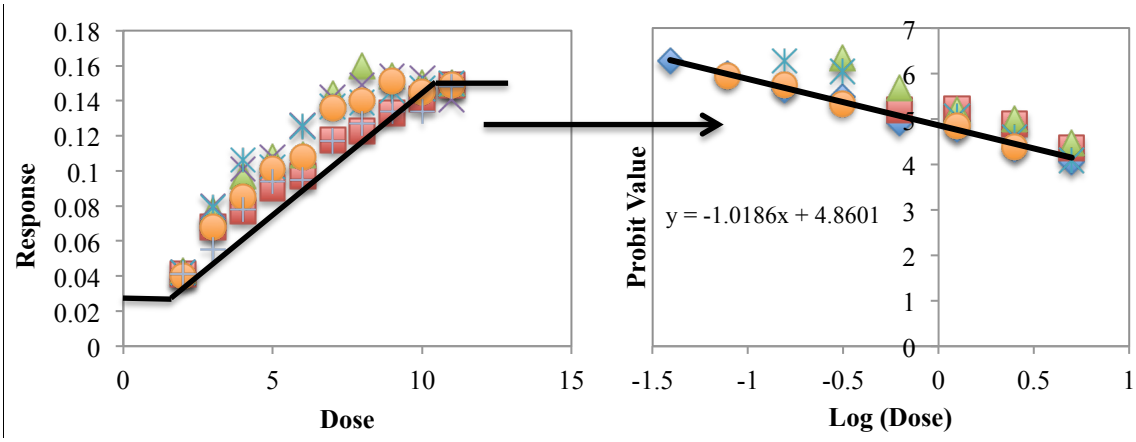


Figure 8. Example of transforming data from dose-response curve to linear regression.

The data is linearized, using the y-intercept and the slope to determine the LD_{50}. After the data has been linearized, the x-axis is the log of the concentration and the probit value of 5 on the y-axis corresponds to the LD_{50}. The LD_{50} was the concentration of the antibiotic at which the OD_{600} increase would be 50% of the OD_{600} increase in the positive control. The potency equivalent (PEQ)[82] was calculated for each sample and was equal to the LD_{50} of the untreated sample divided by the LD_{50} of the treated sample. Decreased growth in antibacterial assays is associated with increased antibiotic potency of the parent/product mixture. PEQ of each sample was compared to the concentration of the parent antibiotic remaining (as measured by HPLC/MS). PEQ values that are higher than the fraction of the parent antibiotic remaining demonstrate that new antibiotics have formed. Full replication of the chlorination experiment was done three times. For each of the replicates, duplicate assays were run.

2.7 HPLC/MS methods for transformation products

The fraction of antibiotic remaining and the structure of the products were analyzed using high performance liquid chromatography with mass spectrometry (HPLC/MS) using a Vanquish flex quaternary HPLC system and a Velos pro dual-pressure linear ion trap mass spectrometer with electrospray ionization source. UV detection was also used at 235 and 275 nm wavelengths to aid with the location of products in the chromatogram. The method was run in full scan mode (m/z range 150-1000) under positive ionization. The chromatography mobile phase consisted of Solvent A (HPLC grade water with 0.1% formic acid) and Solvent B (HPLC grade acetonitrile with 0.1% formic acid). Method gradient started at 10% Solvent B and involved a 1 minute delay before ramp during which the flow was diverted to waste to assure that inorganic wastewater effluent constituents could be diverted from the mass spectrometer. This was followed by a 15 minute ramp to 100% Solvent B, one minute at 100% Solvent B, and ramp down to 10% Solvent B followed by a 2 minute relaxation before next injection. The flow was at 0.4 mL/min, the column temperature was 35°C and the injection volume was 10 µL. The column was a Thermo Scientific Hypersil GOLD C8, 100 x 2.1 mm, particle size of 3 µm.

Tetracyclines are known to cause experimental issues due to hydrolysis reactions and complexation with dissolved metals, Ca^{2+} or other cations. Complexation was possible in wastewater matrix; however, the LD_{50} of doxycycline in the wastewater would be higher than in clean water, if a portion of the antibiotic was inactivated by complexation, which was not observed. The concentrations of the antibiotics were likely sufficiently high so that the effects were negligible. No decrease in antibiotic

concentration was observed over the span of the experiment, therefore the hydrolysis reaction can also be considered insignificant for this study.

Some of the samples from the antibiotics were further analyzed using MS/MS to identify the TPs. Samples were collected prior to chlorination and after two specific time intervals of chlorine exposure. The timing was selected to capture the early transformation products (10 min of exposure) and those that may take longer to form and could be secondary products (120 min of exposure). Collision induced dissociation (CID) with the following parameters was used: isolation width of 1.0 m/z, normalized collision energy of 35 V, activation Q of 0.25 and an activation time of 10 ms. Identification of chlorine isotopes (difference of two m/z between fragments that are 1/3 the ratio of each other) were crucial in identifying chlorinated TPs.

CHAPTER 3: RESULTS

3.1 Pharmaceutical Disposal

The number of responses received was 37 and the results from the survey can be seen in Table 6. With the sample size being small, it becomes important to look at the percentage of the respondents' answers to the questions. For example, when asked, "Does your facility have an unused expired medication disposal protocol for the whole facility?" 92% answered "yes." This high percentage reduces the probability of this answer to chance if most all the 37 responses reported "yes". There may exist some biases toward responsible disposal of pharmaceuticals amongst the responses gained from the participating members of the Practice Greenhealth organization whose goal is to promote sustainability and environmental health in healthcare facilities. However, some bias was mitigated by involving Eastern Kentucky University's Environmental Health and Safety Alumni program. The average time spent on the survey was 5 minutes. Table 5 displays which questions were skipped compared to which questions were answered by the respondents. The most skipped question was number 5, involving the frequency of disposal and collecting inventory on unused expired medication. The respondents who skipped this question reported not knowing the answer. The second most skipped question was number 6, involving how much medication accumulates for disposal. Respondents reported difficulty in knowing the exact number as a cumulative amount was not recorded.

The results were further divided into rural and urban classifications (Table 7 and Table 8, respectively). Out of the 37 responses received, 6 designated themselves as

serving only rural populations. There were 7 responses out of the 37 that designated themselves as serving only urban populations.

Further comments pertaining to question 10 expressed interest in knowing more about the study. Others wanted to relay information regarding standards and requirements pertaining to medical waste disposal for their specific healthcare facility. Some respondents expressed interest through email about the outcome of the study including professionals in the healthcare setting, environmental consultants and environmental policy attorneys.

Table 5. Answered versus skipped responses for the 9 questions.

Question	Answered	Skipped
1	36	0
2	36	0
3	37	0
4	37	0
5	34	3
6	34	2
7	36	1
8	36	1
9	35	1

Most of the respondents reported serving both rural and urban populations (63%), Table 6. 92% of the respondents reported that their facility has an unused expired medication disposal protocol for the whole facility. When asked if their facility has an organized unused medication disposal days/events or on an as-needed continuous basis, 89% answered disposal was on an as-needed basis. 41% of the respondents reported that their facility inventoried medication and disposed of unused expired medication on a weekly basis. The amount of expired medication varied greatly, the lowest being 1-5 gallons per year from a university student health center and the highest being 12,000

pounds per week from a pharmaceutical warehouse. 66% of the respondents dispose of partially used non-expired medication by special pharmaceutical waste collection programs, 25% dispose with DEA regulated medical waste, 3% into municipal solid waste, and 6% into sewer. When asked how they dispose of expired medication 72% answered using a special pharmaceutical waste collection program, 19% with DEA regulated medical waste, 6% into municipal solid waste, and 3% into sewer. The highest volumes of reported medications of routine disposal were antibiotics (40%) and other (46%). "Other" referred to several different medications including: blood thinners, anesthetics, antipsychotics, inhalers, saline, nicotine patches, IVs, testosterone, and diabetes management medication.

Interestingly, 6% of respondents reported disposing of partially used non-expired medication by sink drain and 3% used municipal solid waste. When asked about disposing of expired medication 3% reported by sink drain and 6% disposed into municipal solid waste. Therefore, from the responses received, around 10% of non-expired and expired medication is being disposed into municipal utilities (either landfill or WWTP). Facilities that reported disposing of pharmaceuticals into municipal solid waste or down the sink drain included a general hospital (including inpatient and surgery), a laboratory, and a specialized hospital (including inpatient and surgery). Their amount of waste generated ranged from 2 pounds per week to 0.63 gallons per week. None of the rural facility responders reported disposing of pharmaceuticals into municipal solid waste or sewer. Very few facilities reported using such practices in general, and all of those respondents serve strictly urban population. Facilities that serve mixed urban and rural populations are presumably larger medical centers that cover

broader geographical areas. It is possible that larger facilities are more likely to participate in pharmaceutical waste collection programs.

Table 6. Overall results from pharmaceutical disposal survey.

Total responses	37
What kind of medical facility are you?	
private general practice outpatient only (2)	
multi-practicioner general practice, outpatient only (2)	
multi-practicioner specialized practice, outpatient only (1)	
general hospital, includes inpatient and surgery (17)	
specialized hospital, includes inpatient and surgery (3)	
veterinary clinic (1)	
pharmacy (3)	
laboratory (1)	
VA medical center with outpatient, same day surgery and long term care (1)	
medical school, dental school, nursing school, and allied health (1)	
university student health center (1)	
integrated health care system (1)	
primary care/specialty care systems, outpatient only (1)	
outpatient general and specialized practice (1)	
pharmaceutical warehouse (1)	
Do you serve mainly rural population, mainly urban, or both?	
rural	17%
urban	20%
both	63%
Does your facility have an unused expired medication disposal protocol for the whole facility?	
yes	92%
no	8%
Does your facility have organized unused medication disposal days/events, or is medication disposal done on as-needed continuous basis?	
specific days/events	11%
as needed	89%
How frequently does your facility inventory medication and dispose of unused expired medication?	
weekly	41%
monthly	23%
every few months	21%
once a year or less frequently	6%
this is not applicable to our facility	9%
What amount of expired medication typically accumulates between disposal times? Please provide your best mass or volume approximation: e.g. 5 lb. or 10 gal.	
1-5 gal/yr	
2.5 gal/twice a yr	
0.375 lbs/wk	
0.5 lbs/wk (2)	
0.56 lbs/wk (2)	

0.63 gal/wk	
1.2 lbs/wk	
1.25 lbs/wk (2)	
1.25 gal/wk	
1.5 lbs/wk	
1.7 gal./wk	
1.75 lbs/wk	
< 5lbs/wk	
5 lbs/wk	
7 gal./wk	
10 gal/wk	
10 lbs/wk	
13.8 gal/wk	
20 gal/wk	
25 lbs/wk	
70-90 lbs/wk	
158 lbs/wk	
375 lbs/wk	
500 lbs/wk	
12,000 lbs/wk	
How do you dispose of partially used non-expired medication?	
down the sink drain	6%
into municipal solid waste	3%
with DEA regulated medical waste	25%
special pharmaceutical waste collection program	66%
How do you dispose of expired medication?	
down the sink drain	3%
into municipal solid waste	6%
with DEA regulated medical waste	19%
special pharmaceutical waste collection program	72%
What are some of the highest volume medications that your facility routinely disposes of?	
other*	46%
antibiotics	40%
vaccines	34%
anesthetics	34%
anti-inflammatory	29%
blood pressure	17%
antidepressants	14%
anticonvulsants	12%
hormones	9%
statins	6%
steroids	6%
*Includes: blood thinners, anesthetics, antipsychotics, inhalers, saline, nicotine patches, IVs, testosterone, and diabetes management medications	

The respondents for rural population facilities consisted of hospitals, pharmacy, multi-practitioner general practice outpatient practice, and private general outpatient practice. 83% reported having an unused expired medication disposal protocol for the whole facility. 100% answered having an organized unused medication disposal day/event on an as-needed continuous basis. Most (50%) inventory medication and dispose of unused expired medication on a monthly basis. The rest dispose of medication weekly (33%) or every few months (17%). The amount of accumulated expired medication ranged from 0.56 pounds per week to 158 pounds per week. When referring to disposing of either partially used non-expired medication or expired medication most (67%) use a special pharmaceutical waste collection program and 33% dispose of unregulated pharmaceuticals with DEA regulated medical waste. The most common medications routinely disposed of were anti-inflammatory (29%) and antibiotics (22%).

Table 7. Results from rural responses of the pharmaceutical disposal surveys.

Total responses	6
What kind of medical facility are you?	
specialized hospital, includes inpatient and surgery (1)	
general hospital, includes inpatient and surgery (2)	
pharmacy (1)	
multi-practitioner general practice, outpatient only (1)	
private general practice, outpatient only (1)	
Do you serve mainly rural population, mainly urban, or both?	
rural	100%
urban	0%
Does your facility have an unused expired medication disposal protocol for the whole facility?	
yes	83%
no	17%
Does your facility have organized unused medication disposal days/events, or is medication disposal done on as-needed continuous basis?	
specific days/events	0%
as needed	100%
How frequently does your facility inventory medication and dispose of unused expired medication?	
weekly	33%
monthly	50%
every few months	17%
once a year or less frequently	0%
this is not applicable to our facility	0%
What amount of expired medication typically accumulates between disposal times? Please provide your best mass or volume approximation: e.g. 5 lb. or 10 gal.	
0.56 lbs/ wk	
1.25 lbs/wk (2)	
1.7 gal./wk	
10 lbs/wk	
158 lbs/wk	
How do you dispose of partially used non-expired medication?	
down the sink drain	0%
into municipal solid waste	0%
with DEA regulated medical waste	33%
special pharmaceutical waste collection program	67%
How do you dispose of expired medication?	
down the sink drain	0%
into municipal solid waste	0%
with DEA regulated medical waste	33%

special pharmaceutical waste collection program	67%
What are some of the highest volume medications that your facility routinely disposes of?	
anti-inflammatory	29%
antibiotics	22%
vaccines	14%
anesthetics	14%
diabetic	7%
hormones	7%
statins	7%

The facilities that responded to serve mainly urban populations represented laboratory, university student health center, outpatient general and specialized practice, multi-practitioner general outpatient practice, and general hospital. 100% of the respondents reported having an unused expired medication disposal protocol for the whole facility. 71% of the respondents answered that their facility disposed of unused medication on an as-needed continuous basis. Most (43%) of the respondent facilities inventory medication and dispose of unused expired medication weekly, the rest dispose of medication every few months (29%), once a year or less frequently (14%), and few reported this question was not applicable to their facility (14%). Typically between 1-5 gallons per year to 500 pounds per week of expired medication accumulates between disposal times for these facilities. Overall, a higher volume of pharmaceuticals for disposal accumulated in urban populations (0.56 - 500 lb/wk) compared to rural population (0.56 - 158 lb/wk) facilities. Partially used non-expired medication (57%) and expired medication (72%) is disposed with the aid of special pharmaceutical waste collection programs. However, 14% dispose of partially used non-expired medication down the sink drain and 14% dispose of expired medication into municipal solid waste.

The highest volume of medication that these facilities routinely dispose of are antibiotics (27%) and anesthetics (27%).

Table 8. Results from urban responses of the pharmaceutical disposal surveys.

Total responses	7
What kind of medical facility are you?	
multi-practicioner general practice, outpatient only (1)	
general hospital, includes inpatient and surgery (3)	
laboratory (1)	
university student health center (1)	
outpatient general and specialized practice (1)	
Do you serve mainly rural population, mainly urban, or both?	
rural	0%
urban	100%
Does your facility have an unused expired medication disposal protocol for the whole facility?	
yes	100%
no	0%
Does your facility have organized unused medication disposal days/events, or is medication disposal done on as-needed continuous basis?	
specific days/events	29%
as needed	71%
How frequently does your facility inventory medication and dispose of unused expired medication?	
weekly	43%
monthly	0%
every few months	29%
once a year or less frequently	14%
this is not applicable to our facility	14%
What amount of expired medication typically accumulates between disposal times? Please provide your best mass or volume approximation: e.g. 5 lb. or 10 gal.	
1-5 gal/yr	
0.56 lbs/ wk	
0.63 gal/wk	
10 gal/wk	
20 gal/wk	
500 lbs/wk	
How do you dispose of partially used non-expired medication?	
down the sink drain	14%
into municipal solid waste	0%
with DEA regulated medical waste	29%

special pharmaceutical waste collection program	57%
How do you dispose of expired medication?	
down the sink drain	0%
into municipal solid waste	14%
with DEA regulated medical waste	14%
special pharmaceutical waste collection program	72%
What are some of the highest volume medications that your facility routinely disposes of?	
antibiotics	27%
anesthetics	27%
vaccines	18%
blood pressure	9%
antidepressants	9%
anti-inflammatory	9%

When comparing the remaining facilities that serve both rural and urban areas 91% have an unused expired medication disposal protocol and 92% dispose their unused medication on an as-needed basis (Table 9). Most facilities (41%) dispose of unused expired medication weekly, the rest dispose monthly (23%), every few months (18%), once a year or less frequently (5%), and a few responded that this question was not applicable to their facility (14%). Most of the facilities use a special pharmaceutical waste collection program for disposal of partially used non-expired medication (71%), the others use DEA regulated medical waste bins (21%), or dispose into municipal solid waste (4%), or down the sink drain (4%). The disposal responses were the same for both non-expired medication and expired medication. The highest volume medications that are routinely disposed of include antibiotics, vaccines, and anesthetics.

Table 9. Results from both rural and urban responses of the pharmaceutical disposal surveys.

Total responses	24
What kind of medical facility are you?	
private general practice outpatient only (1)	
multi-practicioner general practice, outpatient only (1)	
general hospital, includes inpatient and surgery (12)	
specialized hospital, includes inpatient and surgery (2)	
veterinary clinic (1)	
pharmacy (2)	
VA medical center with outpatient, same day surgery and long term care (1)	
medical school, dental school, nursing school, and allied health (1)	
integrated health care system (1)	
primary care/specialty care systems, outpatient only (1)	
pharmaceutical warehouse (1)	
Do you serve mainly rural population, mainly urban, or both?	
rural	0%
urban	0%
both	100%
Does your facility have an unused expired medication disposal protocol for the whole facility?	
yes	91%
no	9%
Does your facility have organized unused medication disposal days/events, or is medication disposal done on as-needed continuous basis?	
specific days/events	8%
as needed	92%
How frequently does your facility inventory medication and dispose of unused expired medication?	
weekly	41%
monthly	23%
every few months	18%
once a year or less frequently	5%
this is not applicable to our facility	14%
What amount of expired medication typically accumulates between disposal times? Please provide your best mass or volume approximation: e.g. 5 lb. or 10 gal.	
2.5 gal/twice a yr	
0.375 lbs/wk	
0.5 lbs/wk(2)	
1.25 gal/wk	
1.25 lbs/wk	
1.5 lbs/wk	
1.75 lbs/wk	
5 lbs/wk	

7 gal./wk	
7 lbs/wk	
25 lbs/wk	
70-90 lbs/wk	
375 lbs/wk	
12,000 lbs/wk	
How do you dispose of partially used non-expired medication?	
down the sink drain	4%
into municipal solid waste	4%
with DEA regulated medical waste	21%
special pharmaceutical waste collection program	71%
How do you dispose of expired medication?	
down the sink drain	4%
into municipal solid waste	4%
with DEA regulated medical waste	21%
special pharmaceutical waste collection program	71%
What are some of the highest volume medications that your facility routinely disposes of?	
anesthetics	17%
vaccines	14%
antibiotics	13%
blood pressure	9%
anti-inflammatory	9%
antidepressants	8%
anticonvulsants	8%
hormones	4%
blood thinners	4%
statins	2%
steroids	2%
testosterone	2%
inhalers	2%
saline	2%
antipsychotics	2%
nicotine patches	2%

After obtaining the results from these survey responses, questions 7 and 8 became of interest in learning which special pharmaceutical waste collection programs are being utilized by these facilities. For those respondents that answered yes to using special pharmaceutical waste collection programs, further information was obtained in regards to what type of facilities they are and which specific program they are using (Table 10).

Interestingly, if the medication has expired, most healthcare facilities will try to send back to the vendor. This is advantageous, as vendors will try to give a financial incentive for returning expired medication to them. For partially used non-expired medication these facilities use a variety of disposal programs that best fit the types of medications they have. For example, pharmacies will try to send their medication back to the vendor/wholesaler, whereas a hospital will use hazardous waste disposal, RCRA, DEA, and non-RCRA disposal. An example of special pharmaceutical waste collection company that specialize in collecting and disposing of regulated medical waste is Stericycle, which was used by some of the respondents. An example of a company that specializes in pharmaceutical returns is Pharmalogistics, this company was reported being used mainly by pharmacies. One concerning response was reported by a general hospital facility (serving both rural and urban areas) that disposed of pharmaceuticals down the sink or with other waste depending on the pharmaceutical. Discarding of pharmaceuticals down the sink should never be thought of as an acceptable disposal method. This stresses the need for further clarity within healthcare facilities regarding their pharmaceutical waste program. Some facilities may be complaint with their regulated pharmaceutical waste management; however, they may not be using the best practices for disposal of non-regulated substances.

Table 10. Responses to question 7 and 8 regarding disposal of partially used non-expired and expired medication

How do you dispose of partially used non-expired medication?	How do you dispose of expired medication?	Type of Facility
hazardous waste disposal, Rx disposal, DEA, and non-RCRA separately, Stericycle, send to vendor, RCRA incinerator, per EPA regulations, depends on the medications (in the sink or with other waste), hazardous and non-hazardous pharmaceutical waste is collected separately to be incinerated		general hospital, includes inpatient and surgery
guaranteed returns to wholesaler		pharmacy
n/a	any expired drug samples will be returned to manufacturer	multi-practitioner specialized practice, outpatient only
hazardous waste disposal	reverse distributor or hazardous waste	integrated health care system
facility-wide pharmaceutical waste program that encompasses hazardous, non-hazardous, and DEA regulations		specialized hospital, included inpatient and surgery
most are designated as hazardous waste and are managed by thermal destruction incineration technology at an EPA permitted RCRA facility		university student health center
determine if it qualifies as RCRA waste and dispose into appropriate waste receptacle	medication will be disposed into proper receptacle depending on type	medical school, dental school, nursing school, allied health
all departments return pharmaceuticals to the pharmacy were they will sort them for returns, hazardous waste, or non hazardous waste		VA Medical Center with outpatient, same day surgery, and long term care
Stericycle		primary care/specialty care systems, outpatient only
medications are dissolved in a solution and sent to the proper disposal vendor		private general practice, outpatient only

Waste disposal in healthcare settings can be divided into several color-coded categories that are standard (Table 11). This includes hazardous waste, with the designated receptacle color of black, and the waste collected being incinerated. Several respondents reported using special pharmaceutical waste collection programs. These can include receptacles that are designated with the color of white or blue. The category of waste is typically considered non-hazardous (with some exceptions, including pharmaceuticals listed as hazardous by OHSA and drugs categorized as carcinogenic by the U.S. Department of Health and Human Services National Toxicology Program). The waste that is accumulated here will eventually be incinerated.

The color yellow is designated for chemotherapy waste, which can include empty IV bags and tubing. Ultimately, the waste collected here will go to the incinerator. Regulated medical waste (or bio-hazardous waste) is designated with the color red and will be collected for incineration. Some examples of bio-hazardous waste are anything used in surgery that will need to be disposed. This includes items that have pathogens (i.e. blood) contained within or on them.

Some healthcare facilities also have sequestration devices. These devices are specially designated for the disposal of controlled substances. The sequestration device was a new development, within the last decade, and has been gaining further attention since the opioid crisis in healthcare settings. This device works by allowing controlled substances, such as oxycontin, morphine, opium, codeine, and methamphetamines to be inserted into the top and not being able to be released by any other mechanism. These substances will remain trapped in the device, not even obtainable by the nurses, until the container is full and ready to be collected for disposal. The contents of these devices will eventually go to the incineration as well.

Some major healthcare facilities will outsource their disposal to consulting companies working with waste management companies. These companies can be responsible for supplying the proper disposal receptacles and appropriate training protocols for employees who will handle medical waste disposal. Though most of the healthcare systems' waste will be incinerated, not all will be incinerated "in house". Some hospital systems do not have an incinerator and ones that do may not be able to incinerate hazardous waste. Smaller facilities such as dental offices, veterinary clinics,

private practice entities and pharmacies will not have incinerators on site and will have to outsource this task to other companies.

There are some types of waste that are designated as being allowed to be put down the sink eventually leading to the local WWTP. Some examples of these approved wastes are IV salts and sugars. The items that have been approved to be disposed into the trash for municipal solid waste are empty vials (that did not initially contain hazardous or regulated substances) and packaging. This accumulated disposal will go to the local landfills.

Table 11. Summary of healthcare waste by receptacle color, category and disposal method.

Color associated	category of waste	examples of waste	waste disposal
	hazardous	mercury, arsenic trioxide, epinephrine, cleaning chemicals, laboratory chemicals, pesticides	EPA location - chemically treated, incineration
	non-hazardous	lactic acid, endocrine-disrupting hormones, pharmaceuticals listed as carcinogenic by OSHA and categorized as carcinogenic by US Department of Health and Human Services Toxicology Program	incineration
	chemotherapy	items contaminated with chemotherapy pharmaceuticals (empty IV bags, tubings, vials, gowns, gloves)	incineration
	regulated medical waste (biohazardous)	Anything that posing a risk for transmitting infections (blood, body fluids or other infectious materials)	autoclave, incineration, microwave, chemically disinfected
	sequestration device (i.e. cactus smart sink)	controlled substances wastage (including opioids)	incineration
	down the sink drain	IV salts and sugars	wastewater treatment
	trash; municipal solid waste	empty vials, packaging (not RCRA regulated)	landfill
Source: Kathy Skibinski, PharmEcology Services, WM sustainability Services and Barb Bickford, Medical Waste Coordinator and Hydrogeologist (Waste and Materials Mgt/Environmental Management Division of Wisconsin Department of Natural Resources)			

3.2 SPE for Pharmaceuticals in Landfill Leachate

All the deuterated compounds (carbamazepine-d10, bisphenol A-d16 and ciprofloxacin-d8 hydrochloride) were detectable down to 8 ng/L. The calibration curves for the compounds can be seen in the following Figures 9, 10, and 11. When the samples are extracted, they are concentrated 250 times; therefore the concentration of 1 μg/L in the extracted sample corresponds to concentration 4 ng/L in the non-extracted sample. Figure 9A, shows the calibration curve for CBZ, as well as Figure 9B shows the curve for the lower concentrations. Figure 10A, shows the calibration curve for BPA, as well as Figure 10B shows the curve for the lower concentrations. Figure 11A, shows the calibration curve for CIP, as well as Figure 11B shows the curve for the lower concentrations.

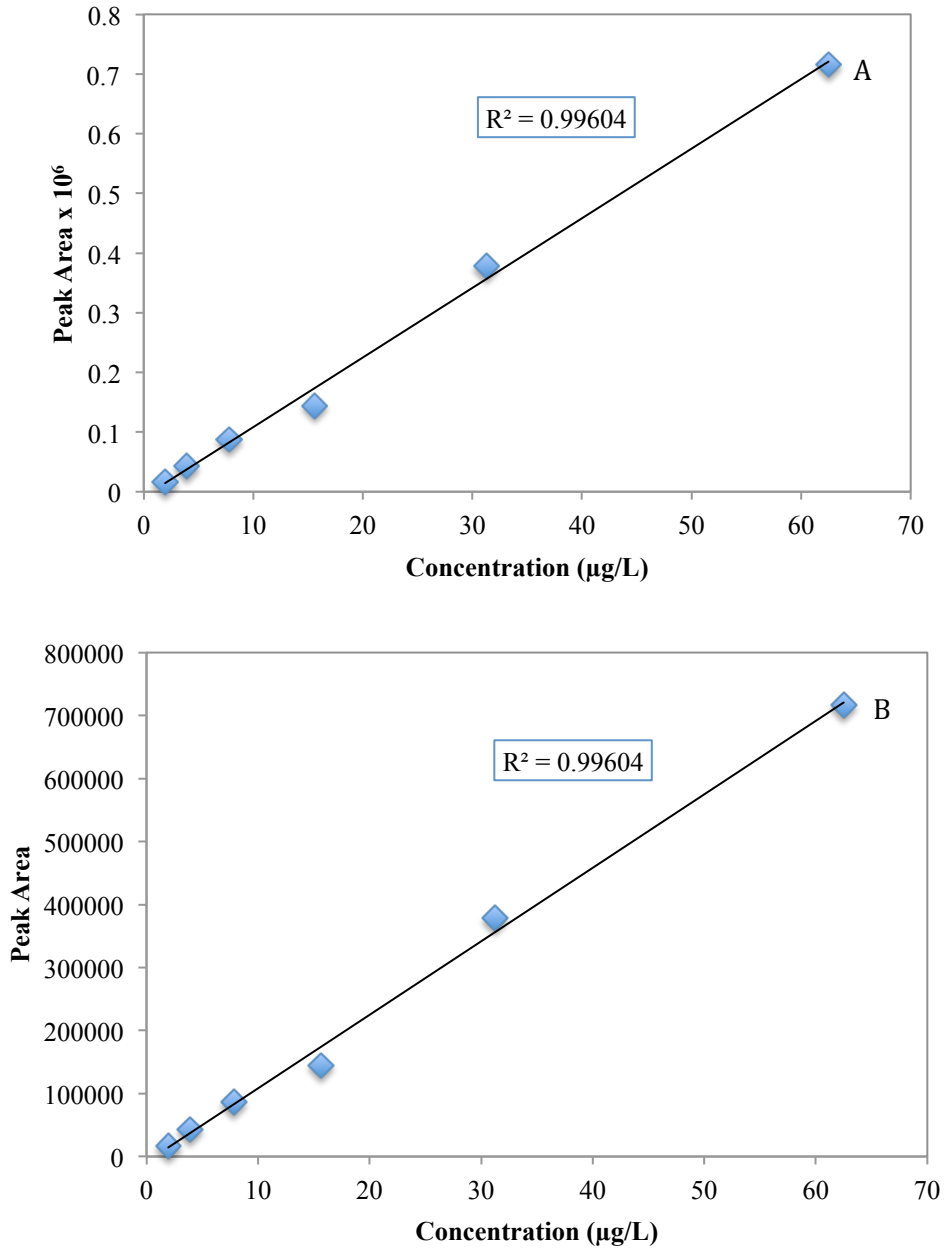


Figure 9. CBZ calibration curve, 1000 – 1.95 µg/L based on two repeated experiments (A) and 62.5 – 1.95 µg/L (B) based on two repeated experiments.

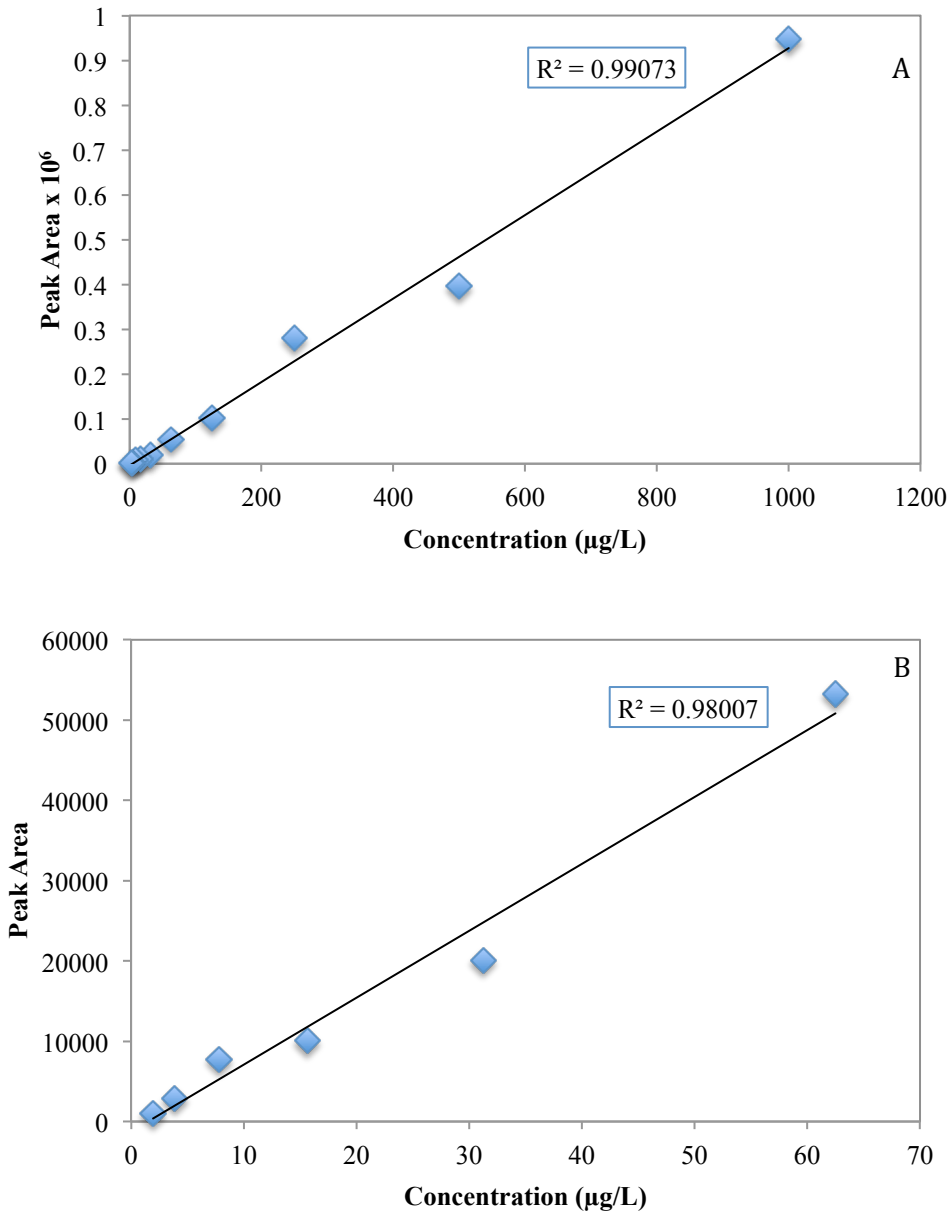


Figure 10. BPA calibration curve, 1000 – 1.95 µg/L based on two repeated experiments (A) and 62.5 – 1.95 µg/L (B) based on two repeated experiments.

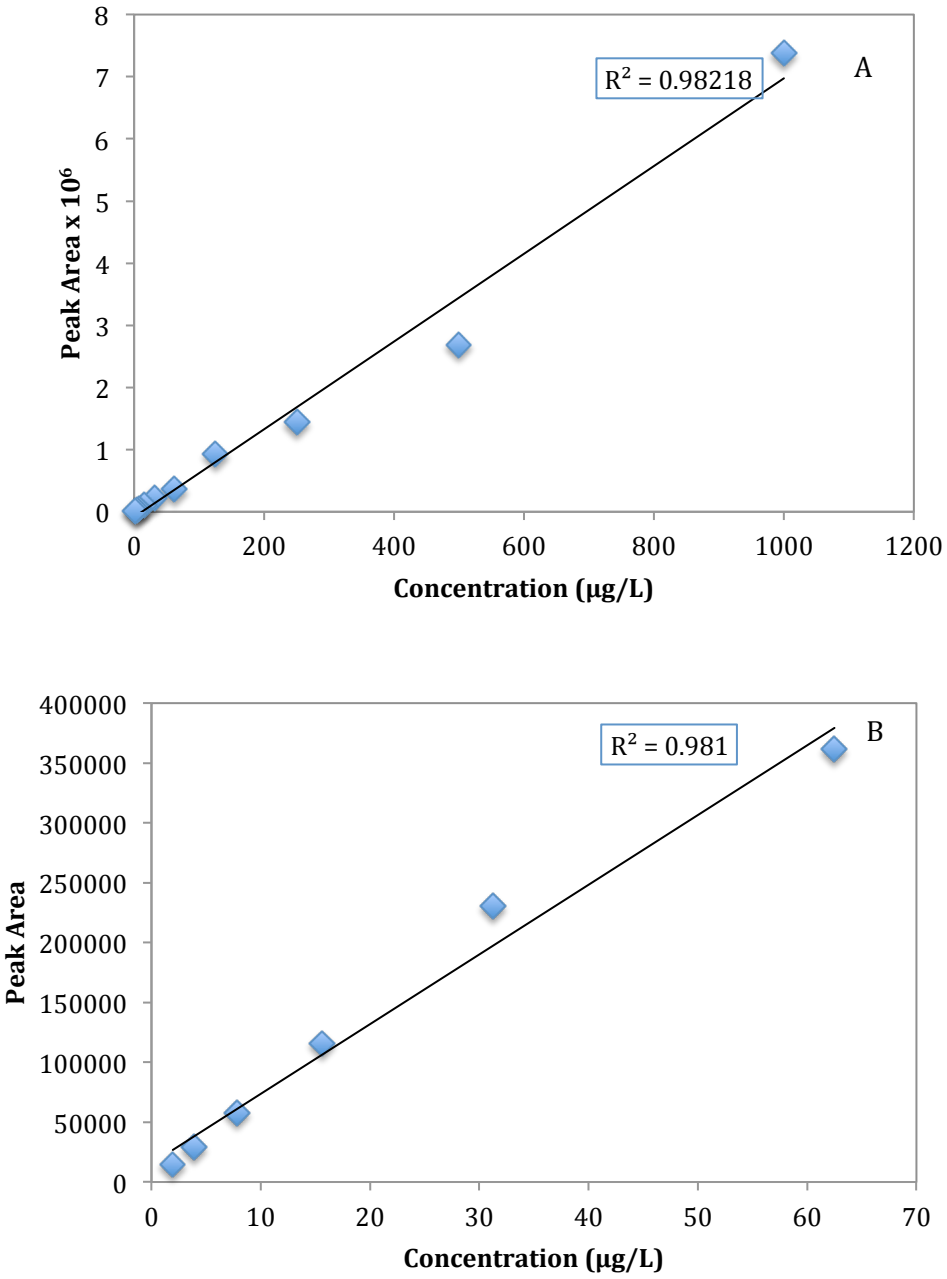


Figure 11. CIP calibration curve, 1000 – 1.95 µg/L based on two repeated experiments (A) and 62.5 – 1.95 µg/L (B) based on two repeated experiments.

Percent recovery was calculated from the peak area of the compound within the sample spiked at 1 µg/L and concentrated to 250 µg/L in relation to the standard at 250 µg/L. The percent recovery for the three compounds can be seen in Figure 12. Statistical analysis (t-tests) was performed for the four cartridges comparing the recovery of the three compounds. There was no statistically significant difference between recovery of the compounds and the cartridges (Table 12).

Table 12. P-values for selected compounds and cartridges

Compound	ENV vs. HLB	ENV vs. PLEXA	ENV vs. PPL	HLB vs. PLEXA	HLB vs. PPL	PLEXA vs. PPL
CBZ	0.32	0.55	0.74	0.27	0.35	0.77
BPA	0.21	0.45	0.87	0.11	0.25	0.52
CIP	0.38	0.35	0.33	0.46	0.57	0.68

However, the percent recovery for ENV and PPL cartridges displayed a high recovery for BPA (bisphenol-A) and CIP (ciprofloxacin). While they are both comparable, PPL did perform slightly better than ENV. Variability was observed between the individual experiments with CBZ and each of the SPE cartridges. For CBZ the four cartridges had a percent recovery between 50-70%. However, CBZ performed the best with the spiked blank, which suggests that CBZ was significantly affected by leachate and was unable to be extracted with great efficiency. PLEXA performed the third best and HLB was last out of the four cartridges for compound recovery. Although, HLB has been known to be used frequently for SPE analysis with water samples, it may not be the optimal cartridge of choice for leachate samples given the complexities of the sample matrix.

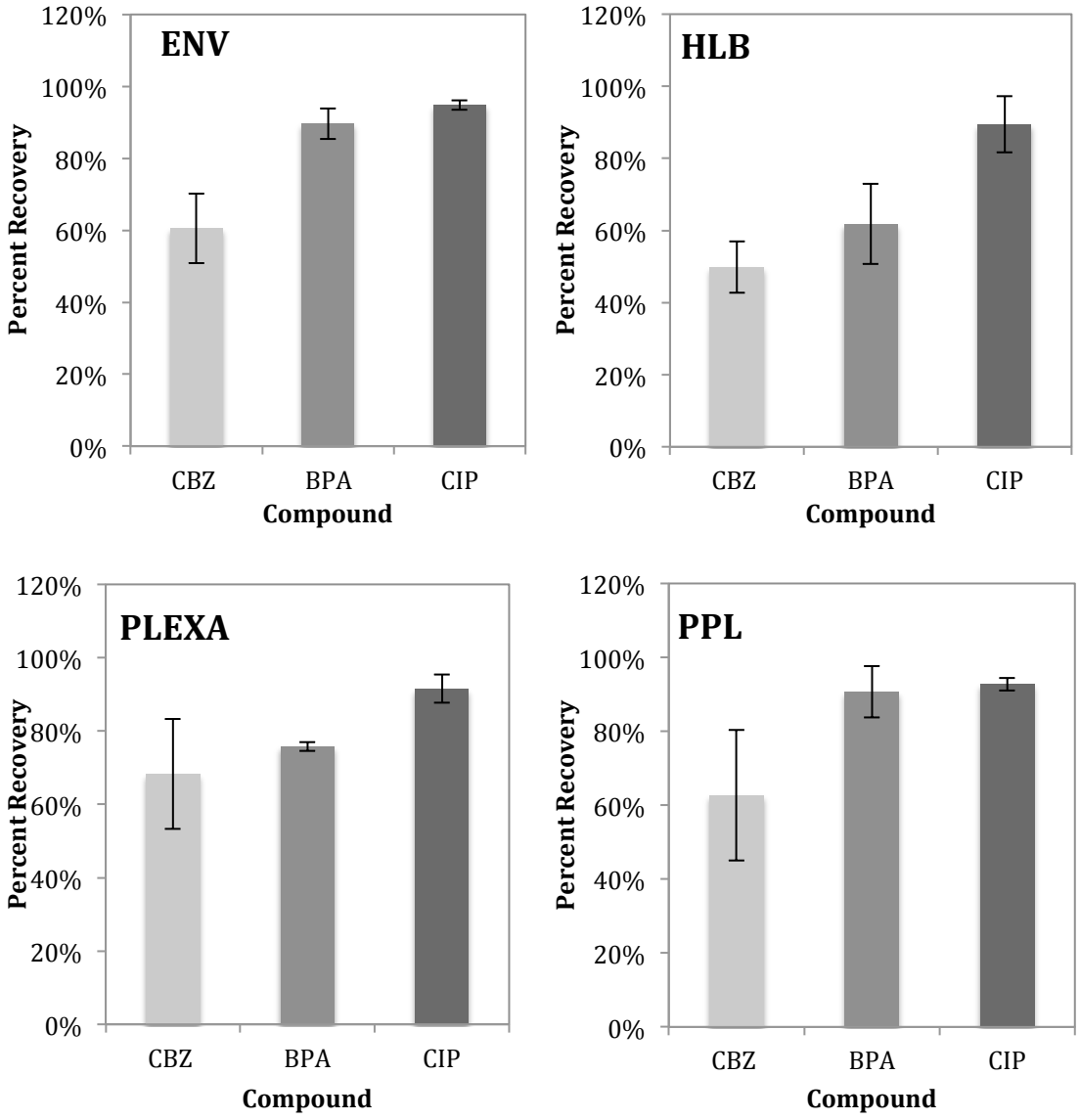


Figure 12. Percent recovery of the three compounds chosen within the four SPE cartridges. Error bars represent standard deviation from three repeated experiments.

A spiked blank was performed as well to test how the compounds extract without having leachate as a matrix; this consisted of ultrapure water and the three compounds. The three compounds performed with 97% (CBZ), 94% (BPA), and 96% (CIP) recovery. Percent recovery experiments were performed with filtered leachate as the goal was to compare the performance of the cartridges and not the overall leachate extraction process. Any loss in percent recovery from filtering the leachate would be the same for all cartridges.

CIP had the best recovery amongst all the compounds chosen; the reproducibility was best for CIP with all the cartridges (the most variability occurring in the HLB cartridge). The percent recovery remained within the 90% +/- 4% amongst all the cartridges. BPA had the second best recovery amongst all the SPE cartridges with limited variability. BPA had the best recovery with the ENV cartridge and the lowest recovery with the HLB cartridge. CBZ displayed the least recovery amongst all the compounds, performing best in PLEXA (68%) and having the lowest recovery (50%) in HLB. However, CBZ performed the best with the spiked blank (97%), which suggests that CBZ was significantly affected by leachate and was unable to be extracted with great efficiency.

Through these experiments it can be concluded that ENV and PPL may offer slightly better extraction for leachate matrices. However, studies performed previously using HLB cartridges likely captured pharmaceuticals in leachate samples reasonably well.

Analysis was also performed to detect the non-deuterated form of these compounds if present in the leachate background. The compounds appeared at negligible

concentrations (within the background noise and below the detection limit of (< 8 ng/L) with RT's of 17.08 min (CBZ), 20.99 min (BPA), and 17.27 min (CIP).

3.3 Overview of antibiotics

When comparing ultrapure water and wastewater experiments, wastewater constituents appeared to have competed with the antibiotics for the chlorine, as was expected. Therefore, higher initial chlorine concentrations were used for wastewater experiments. The antibiotics reacted at comparable chlorine residual concentrations and reaction times compared to ultrapure water. Most of the initial chlorine dose was consumed by rapid reactions within seconds, and the residual of > 0.2 mg/L was maintained in all experiments.

The CT values for the antibiotics can be seen in Table 13 for both ultrapure water and wastewater at 10 min. and 120 min. The CT values are comparable to the values used in wastewater treatment, which usually range from 0.6 to 192[104]. CT values in wastewater treatment vary greatly depending on wastewater characteristics, the concentration of chlorine, and the contact time the chlorine had with the wastewater matrix.

Table 13. CT values for selected antibiotics

Antibiotic	CT Value			
	Ultrapure water (mg·min/L)		Wastewater (mg·min/L)	
	10 min.	120 min.	10 min.	120 min.
Ciprofloxacin	6.76	59.80	6.86	14.00
Levofloxacin	1.70	13.20	6.35	15.00
Ofloxacin	4.25	27.40	5.36	19.60
Trimethoprim	3.03	20.00	11.16	12.40
Sulfamethoxazole	8.21	93.20	4.85	20.60
Sulfamethoxazole/Trimethoprim	8.01	86.60	5.05	14.00
Doxycycline	7.00	51.00	5.00	38.00

Residual chlorine was measured at each time point during preliminary experiments and can be seen in Table 14 as well as the initial chlorine and antibiotic concentrations for all antibiotics in both ultrapure and wastewater.

Table 14. Summary of antibiotics used, including initial concentration, initial chlorine concentration and residual chlorine for both ultrapure water and wastewater Error represents standard deviation from three repeated experiments.

Antibiotic	Antibiotic Initial concentration (mg/L)	Initial chlorine concentration (mg/L)		Residual Chlorine (mg/L as Cl_2)			
		Ultrapure water	Waste water	Ultrapure water	error	Waste water	error
CIP	2.33	2	6.5	0.99	0.023	0.23	0.02
LVF	2	3	7	0.22	0.046	0.25	0.01
OFL	2	3	7	0.46	0.025	0.33	0.006
TMP	20	5	10	0.33	0.015	0.21	0.006
SMX	2	2	5	1.55	0.03	0.34	0.09
SMX/TMP	2	2	5	1.44	0.03	0.23	0.02
DOX	10	9.26	18.52	0.34	0.08	0.22	0.03
*Ciprofloxacin (CIP), Levofloxacin (LVF), Ofloxacin (OFL), Trimethoprim (TMP), Sulfamethoxazole (SMX), Sulfamethoxazole/Trimethoprim (SMX/TMP), Doxycycline (DOX)							

All the antibiotics were dissolved in ultrapure water and adjusted to a pH of approximately 6.95 – 7.25. The pH remained stable with all the antibiotics within this range for wastewater and no future pH adjustment was needed. The pK_a values of the antibiotics chosen are listed in Table 15, and the hypochlorous acid pK_a is 7.6[105] This indicates that predominantly the same antibiotic species and chlorine species were present in both ultrapure water and wastewater matrix throughout the duration of the experiments. All of the antibiotics have pK_a values sufficiently close to pH 7, thus the experiments captured mainly the species that would be present within the range of pH typical for wastewater, drinking water, and natural waters (pH 6-8).

Table 15. Antibiotics and their respective pK_a values

Antibiotic	**pK_{a1}**	**pK_{a2}**	**Reference**
ciprofloxacin	6.09 (caboxylic group)	8.74 (nitrogen on piperazinyl ring)	101
sulfamethoxazole	1.6	5.7	106
trimethoprim	7.12	-	107
levofloxacin	6.24	7.94 (piperazinyl ring)	108 109
ofloxacin	5.97 (carboxylic acid)	9.28 (piperazinyl ring)	110
doxycycline	3.5	7.7	111

The results from the chlorinated experiments with the chlorine concentration over time can be seen in Figure 13 for ultrapure water and Figure 14 for wastewater. Here it can be easily seen which antibiotics react more readily with the available chlorine.

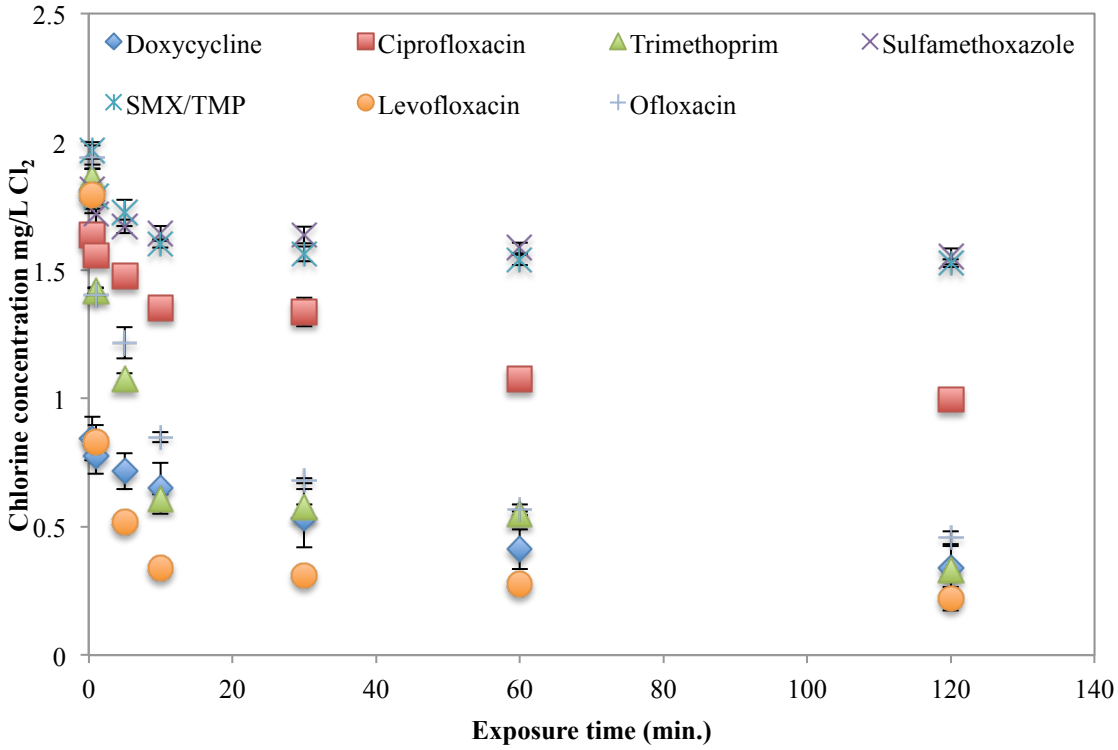


Figure 13. Chlorine concentration over time in ultrapure water for all tested antibiotics. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

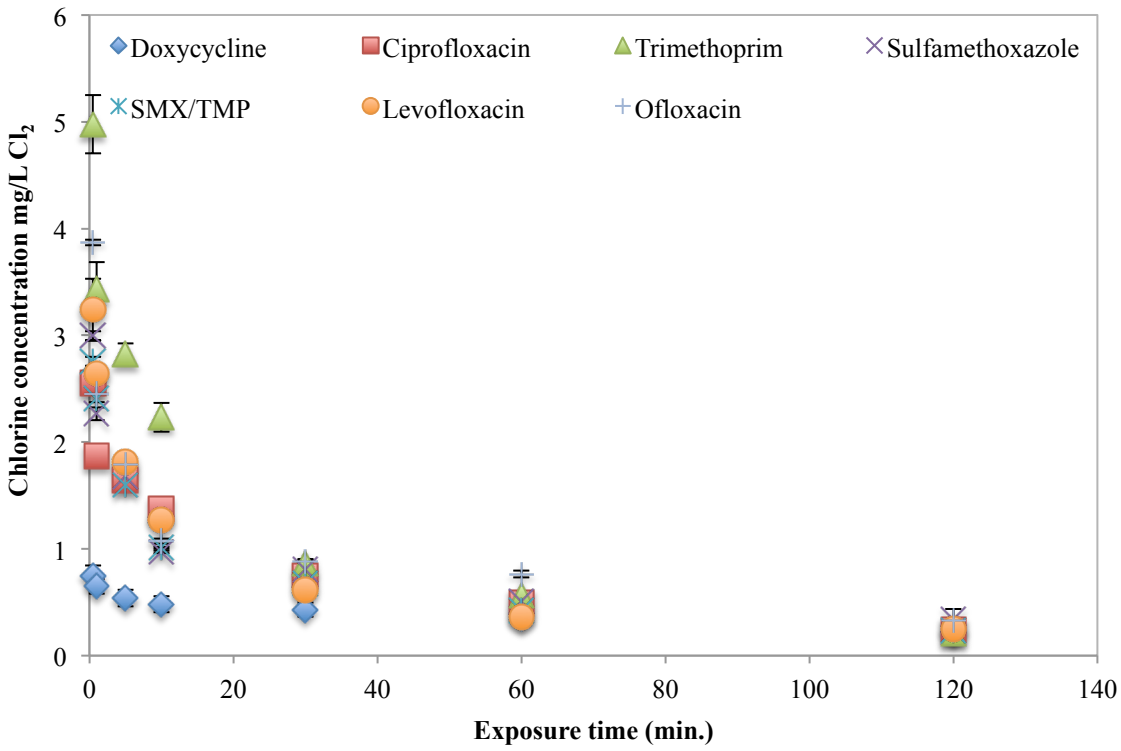


Figure 14. Chlorine concentration over time in wastewater for all tested antibiotics. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

3.4 Trimethoprim

Trimethoprim is a pyrimidine inhibitor of dihydrofolate reductase, it is an antibacterial agent related to pyrimethamine, and it can sometimes be used alone as an antimalarial pharmaceutical.[112] It is a commonly used antibiotic when paired with sulfonamides.[112]

No antibacterially active TPs were detected for trimethoprim. Therefore, detailed MS analysis of the products was not performed. The results of these experiments can be seen in Figure 15. As shown in Figure 15 the PEQ values were not consistently above the trimethoprim concentration over time. Therefore, new antibiotics or antibacterially active TPs were not formed. These figures also show a progressive decline of the trimethoprim over time as it reacts with chlorine. Trimethoprim reacted quicker in the wastewater experiments than the ultrapure water. This can be attributed to the higher initial concentration of chlorine used to overcome chlorine demand of wastewater and maintain a residual within the selected range. A recent study[113] with wastewater showed that TMP was demethylated by chlorine, then quickly hydroxylated, oxidized, and cleaved via intermediate TPs.

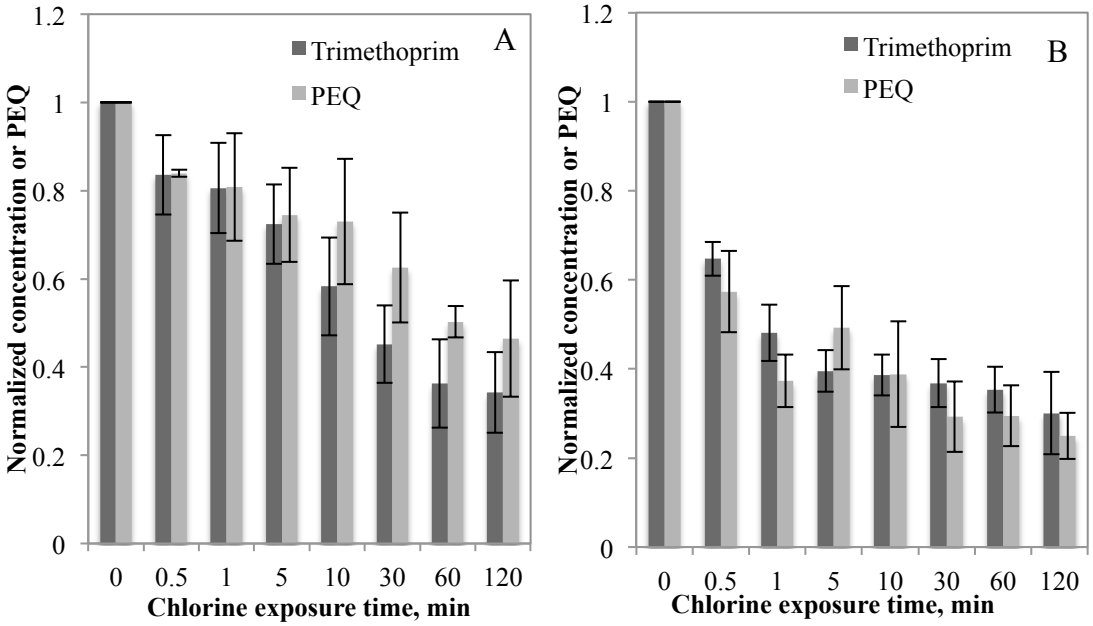


Figure 15. PEQ vs. normalized concentration of trimethoprim in ultrapure matrix (A) and wastewater effluent matrix (B) after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.5 Sulfamethoxazole

Sulfamethoxazole is an anti-bacterial sulfonamide and is a commonly prescribed antibiotic when paired with trimethoprim.[114] It is not common to see sulfamethoxazole prescribed by itself as it is not effective at all without the aid of trimethoprim. This was the slowest antibiotic degradation seen amongst the six antibiotics indicating that the compound is not as reactive with chlorine. However, the wastewater chlorine concentration degraded significantly over time with a steady decrease due to chlorine being reactive with other constituents in wastewater.

There were no antibacterially active TPs detected in sulfamethoxazole experiments, and for this reason no detailed MS analysis of the products was performed. The results for these experiments can be seen in Figure 16. The sulfamethoxazole concentration slowly decreased over time in ultrapure water and wastewater. The PEQ however never consistently surpassed the antibiotic concentration to indicate formation of active products.

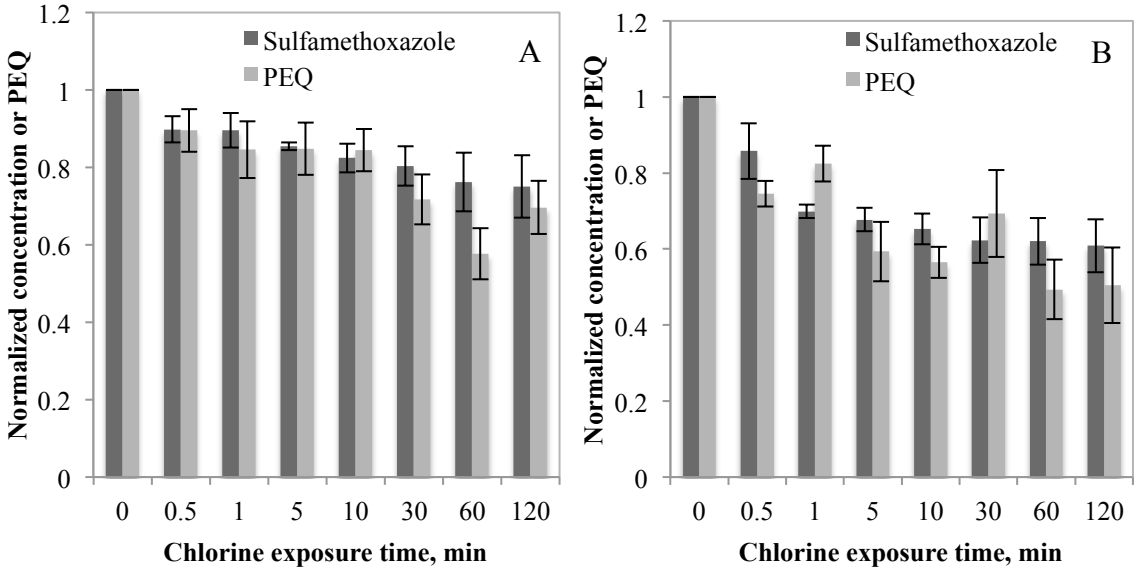


Figure 16. PEQ vs. normalized concentration of sulfamethoxazole in ultrapure matrix (A) and wastewater effluent matrix (B) after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.6 Sulfamethoxazole and Trimethoprim in tandem

The trimethoprim and sulfamethoxazole drug combination is the form most commonly used as sulfonamides potentiate pyrimidine inhibitors.[112] Together both pharmaceuticals reduce the ability of certain bacteria to utilize folic acid for increasing growth.[114] Bactrim is a combination of these two pharmaceuticals and is used to treat urinary tract infections, shigellosis, pneumonia, traveler's diarrhea and methicillin-resistant *Staphylococcus aureus* (MRSA).[114]

The antibiotic concentration and PEQ values for both ultrapure water and wastewater were fairly similar (Figure 17). There was slightly more degradation of the compounds when the wastewater matrix was used, again due to the higher initial chlorine necessary to overcome chlorine demand of the wastewater matrix. The PEQ for the antibiotics was not consistently higher than the antibiotic concentration and therefore there were no antibacterially active TPs detected. From the results of these experiments, it was concluded that no detailed MS analysis of the products was needed. While sulfamethoxazole and trimethoprim work in tandem as antibiotics, there appears to be no synergistic effects among the TPs.

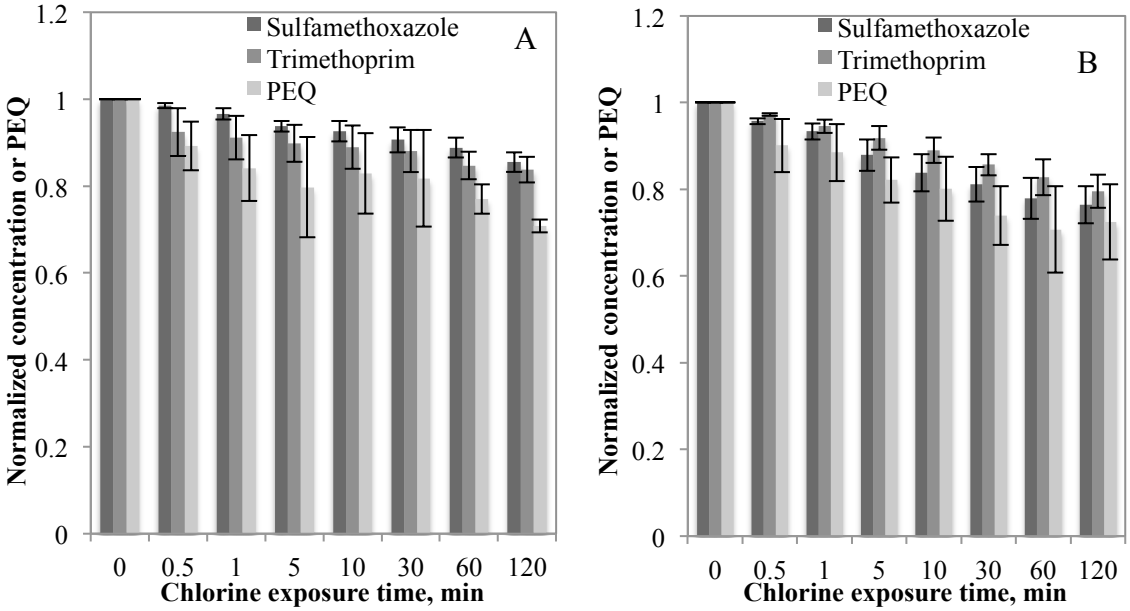


Figure 17. PEQ vs. normalized concentration of sulfamethoxazole and trimethoprim in tandem in ultrapure matrix (A) and wastewater effluent matrix (B) after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.7 Doxycycline

Doxycycline is a commonly used antibiotic to treat several infections caused by bacteria and protozoa. Some examples include bacterial pneumonia, early Lyme disease, cholera, and when paired with quinine it can be used for the treatment of malaria.[115] This pharmaceutical belongs to the tetracycline class of antibiotics. Antibacterial activity assays (Figure 18) demonstrated that some of the products that formed in ultrapure water still maintained their antibacterial properties. The graphs are plotted against the time intervals chosen for this study. It should be noted that, the reaction between doxycycline and chlorine happens within the first thirty seconds and most of the products formed within the first ten minutes. No residual antibacterial activity was observed for the

products that formed in the wastewater effluent matrix. It is possible that the activity was contributed by another product that was not detected by the analytical method and only formed in ultrapure water. One of the water quality parameters that may affect the products that form in chlorination of antibiotics is dissolved organic matter (DOM). DOM is highly reactive with chlorine, and can provide a major competing reaction, possibly preventing formation of some transformation products.

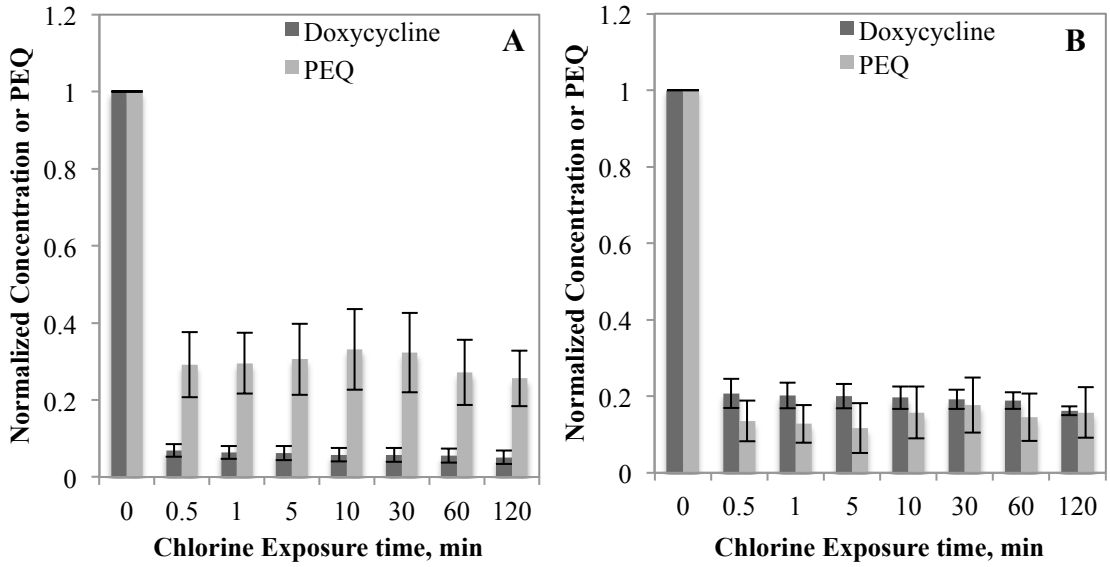


Figure 18. PEQ vs. normalized concentration of doxycycline after specific time intervals of chlorine exposure in ultrapure matrix (A) and wastewater effluent matrix (B). Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.8 Transformation products – Doxycycline

Several chlorinated and non-chlorinated products formed in the samples with ultrapure water and wastewater. Five major transformation products were found (Figure 21). The major and minor fragments of the products from MS/MS, likely reaction pathways, as well as chlorine isotope identification were used for proposing the structures of the transformation products. All five products formed in the ultrapure water matrix and in the wastewater matrix. The most prominent peaks that formed had m/z values (protonated masses) of 417 (non-chlorinated), 451 (mono-chlorinated), 479 (mono-chlorinated), 513 (di-chlorinated) and 547 (tri-chlorinated). Figure 19 shows the transformation products as well as the corresponding m/z value. All the products except m/z 417 displayed chlorine incorporation.

For m/z 417 the proposed reaction involves a loss of two methyl groups [$(CH_3)_2$] from the amine [R-N$(CH_3)_2$] group, this reaction is similar to what has been reported by other researchers[116]. The proposed m/z 451 adds a chlorine to the structure of m/z 417. The m/z 479 product has an addition of a chlorine in the para position to the phenol group on the aromatic ring of doxycycline (ortho/para directing)[117-119,120]. This position was chosen over the ortho position because it is slightly more stable due to fewer steric interactions[121]. This is a common reaction as it is seen with the formation of chlortetracycline[122]. Chlortetracycline is a known antibiotic with m/z of 479 and was compared to the m/z 479 product found[122, 123, 124, 125]. The m/z 479 product that formed in the experiments is different from chlortetracycline based on the different retention time compared to chlortetracycline standard (10.49 min and 9.41 min for standard and product respectively). The starting structure for chlortetracycline is tetracycline, and for product

with m/z 479 observed here the starting structure is that of doxycycline, and the product likely preserves the functional group locations of the doxycycline molecule.

Addition of a chlorine to *m/z* 479, likely in the ortho position to the phenol group on the aromatic ring, gives a product with *m/z* of 513. The ortho position on the aromatic ring next to the hydroxyl group was chosen as a likely location for chlorine addition because the hydroxyl group is ortho/para directing. The preferential para position is already taken by the addition of chlorine from the previous *m/*z 479 proposed pathway. Lastly, addition of another chlorine to *m/z* 513 yields tri-chlorinated proposed product of *m/z* 547. Amides have been shown to be reactive with chlorine, for this reason the chlorine was added to the amide functional group.[126,127] From the reactions listed above, it is clear that chlorination reactions, used in water treatment, have a profound effect on the transformation product formations as at least one chlorine atom appeared in all but one of the products.

The results also indicate that most products form in the initial rapid reaction between doxycycline and chlorine, and little change in products is observed over the 2 h of chlorine exposure. The non-chlorinated product (m/z 417) decreases slightly over time, while product with m/z 479 increases. The rate of decrease in m/z 417 product and the rate of increase of m/z 479 does not appear to be related, thus m/z 417 is probably not an intermediate for formation of m/z 479. Additionally, proposed structures also suggest that m/z 417 cannot lead to formation of m/z 479. Figure 19 shows the formation of each product over time in each matrix.

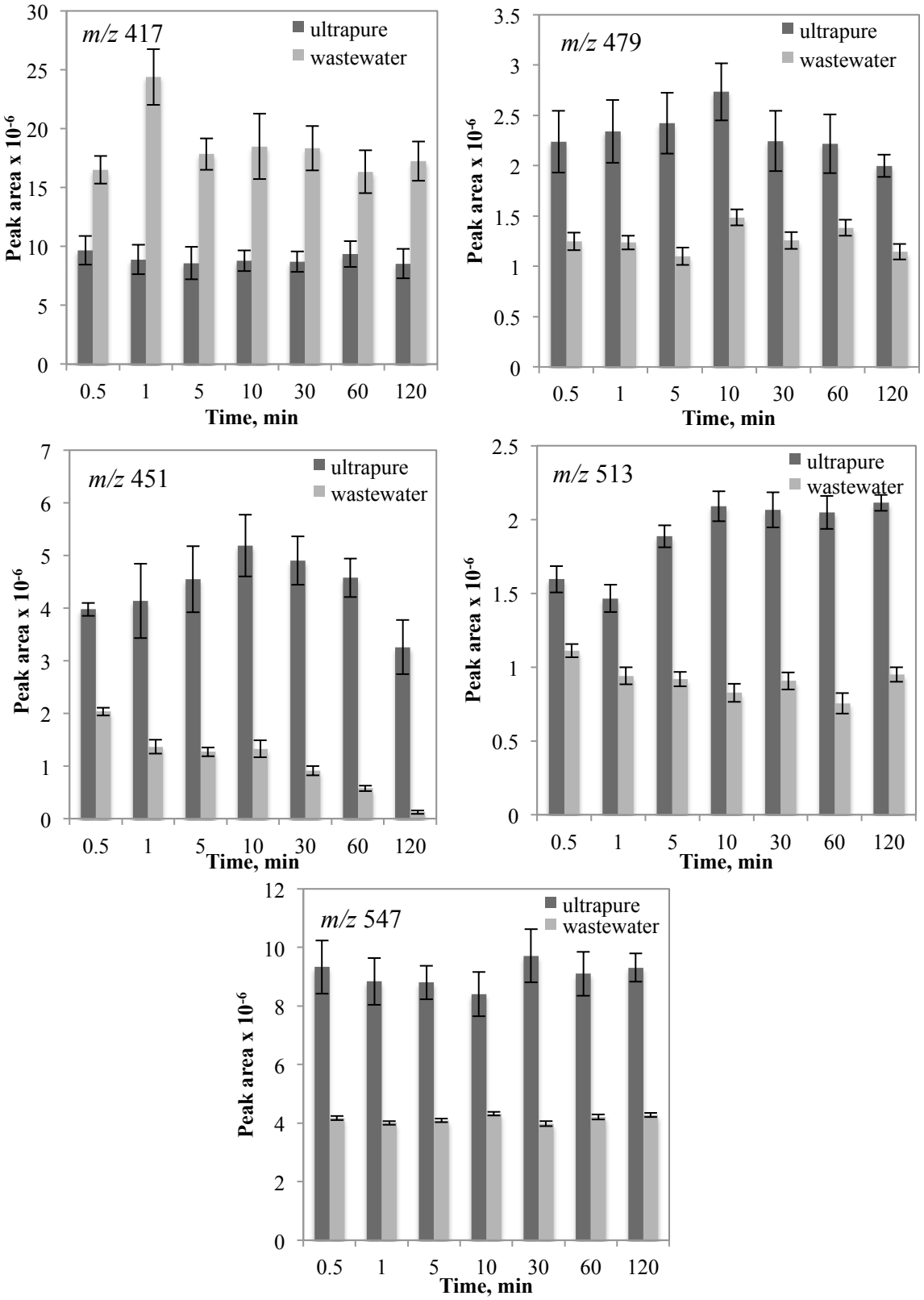


Figure 19. Transformation products of doxycycline as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

The minor and major fragments obtained in MS/MS analysis (Figure 20) helped determine the structure of the transformation products (Figure 21). The retention time for the transformation products helped determine the relative hydrophobicity of each product compared to doxycycline and make sure that it is consistent with proposed structure (chlorination generally making the product more hydrophobic and leading to longer retention time). In fragmentation analysis, the main fragment losses were hydroxyl group (-17 u, m/z 417 to m/z 400), HCl (-36 u, m/z 547 to m/z 511) and $N(CH_3)_2$ (-45 u, m/z 479 to m/z 434 and m/z 513 to m/z 468). The loss of HCl in fragmentation was observed in only one product (m/z 547), which confirms that the location of the third chlorine addition is different and less stable than the location of the first two chlorination reactions, although location other than the proposed one is also possible.

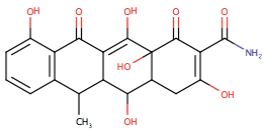
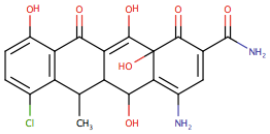
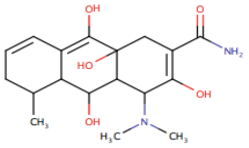
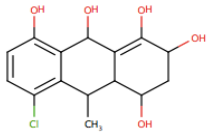
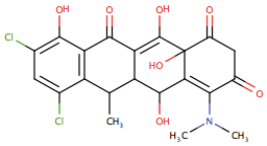
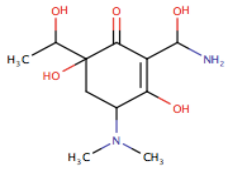
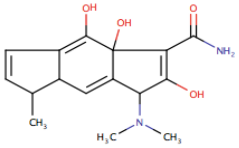
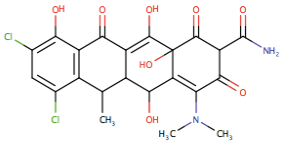
Transformation Product	Major Fragment	Proposed Structure of Fragment	Minor Fragment	Proposed Structure of Fragment
417[116]	400		-	-
451	433		-	-
479[117-121]	349		312	
513	468		260	
547[126-127]	303		511	

Figure 20. Proposed transformation products with corresponding MS/MS fragments and proposed major/minor structures.

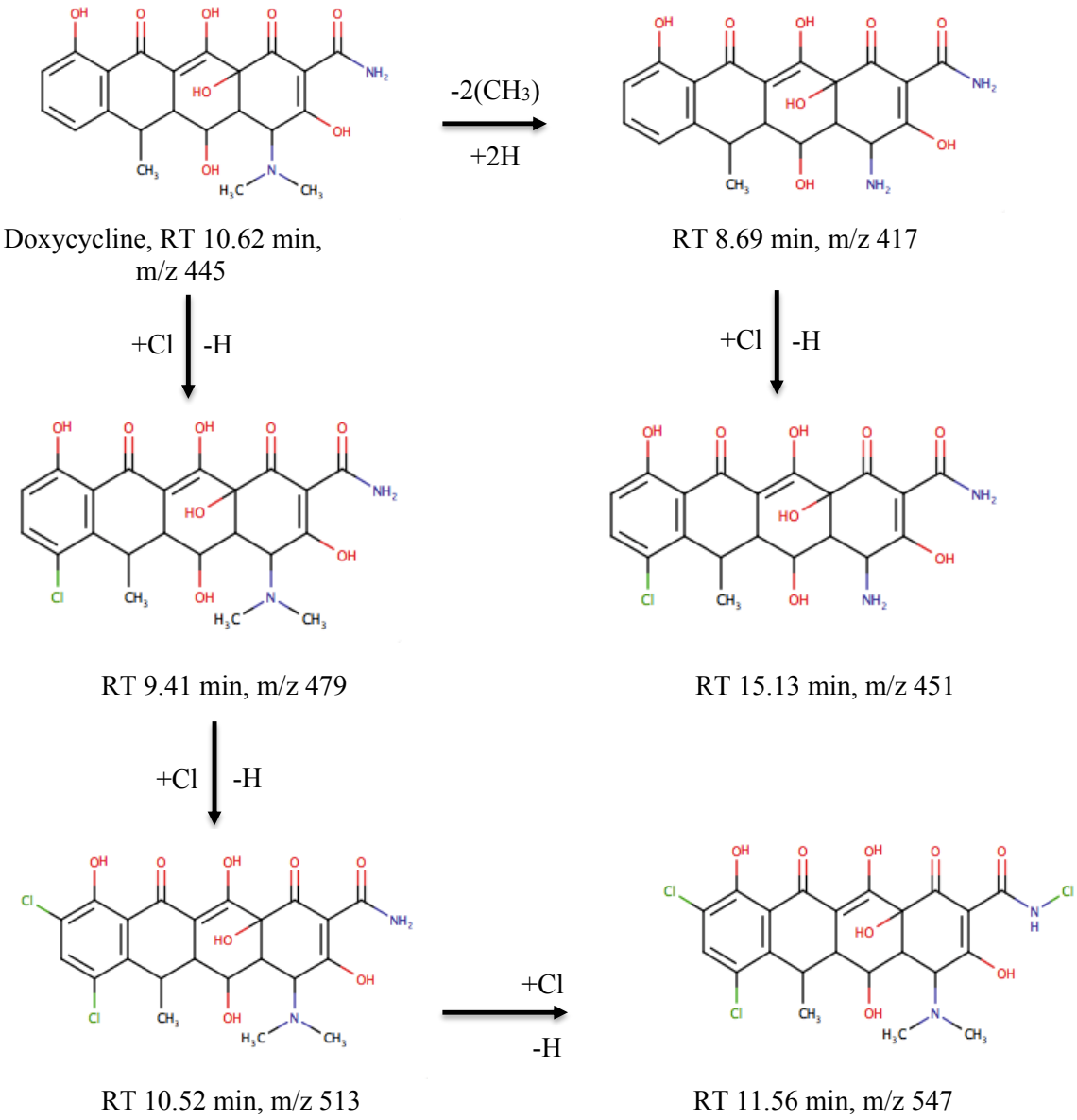


Figure 21. Chemical structures of transformation products and of parent compound, doxycycline, with corresponding m/z values and retention time (RT).

Figure 19 shows that the non-chlorinated product formed in greater abundance in wastewater, while chlorinated products were favored in ultrapure water. This suggests that one or more of the chlorinated products may be exhibiting antibacterial activity. Tetracycline antibiotics are protein synthesis inhibitors, and the functional groups responsible for their ability to bind to proteins are hydroxyl and keto groups located on the upper side of the molecule as drawn in Figure 21.[128] Another important group is the dimethylamine group. Since epimerization of this group leads to the loss of biological activity by the molecule,[128] it is reasonable to expect that more dramatic changes, such as demethylation proposed in the products with m/z 417 and 451, would lead to a formation of an inactive product. Modifications to the other amine group, such as that proposed for the product with m/z 547, have also been shown to remove antibacterial activity.[128] Therefore, the products with m/z 479 and 513 are most likely the ones exhibiting antibacterial activity, as the important functional groups are unaffected in those products. The remaining products will likely exhibit no or significantly diminished antibacterial activity.

3.9 Ciprofloxacin

Ciprofloxacin belongs to the fluoroquinolone class of antibiotics and is used to treat bacterial infections. It stops bacteria from multiplying by inhibiting the reproduction and repair of their DNA.[129-130]

The assay experiments clearly show retention of antibiotic potency by the transformation products (Figure 22). Ciprofloxacin shows stable formation of active

products over time in both ultrapure water and wastewater (Figure 22). The concentration of active products is similar in ultrapure water compared to wastewater.

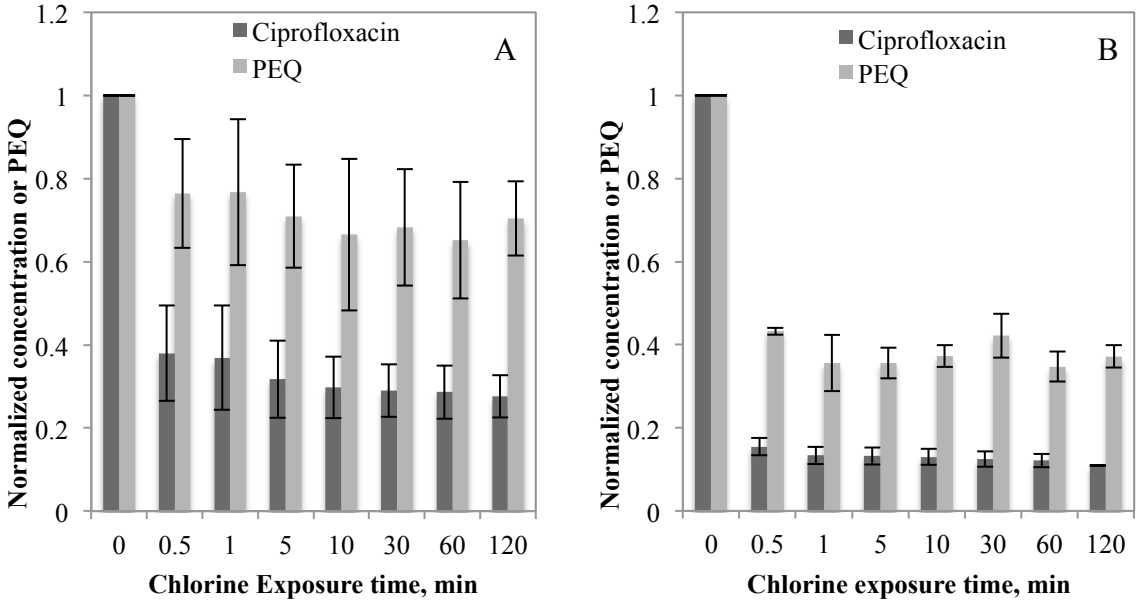


Figure 22. PEQ vs. normalized concentration of ciprofloxacin in ultrapure matrix (A) and wastewater effluent matrix (B) after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.10 Transformation Products - Ciprofloxacin

Several chlorinated and non-chlorinated products formed in the samples with ultrapure and wastewater where ciprofloxacin was present. Five major transformation products were formed with ciprofloxacin as the parent compound; two of them chlorinated. Their probable reaction pathways and chlorine isotope identification were used to propose the structures of transformation products. All five products formed in the ultrapure water matrix and in the wastewater matrix. The products had *m/z* values

(protonated masses) of 306 (non-chlorinated), 263 (non-chlorinated), 389 (di-chlorinated), 300 (non-chlorinated), and 367 (mono-chlorinated). Figure 23 displays the relative abundance for product formation for ciprofloxacin TPs. The TPs with m/z 306 and 389 formed better in wastewater. The products with m/z 263, 300 and 367 showed no difference in formation in two matrices. For the m/z 300, the results show slight continual increase in ultrapure water, while it stays stable in wastewater after initial formation.

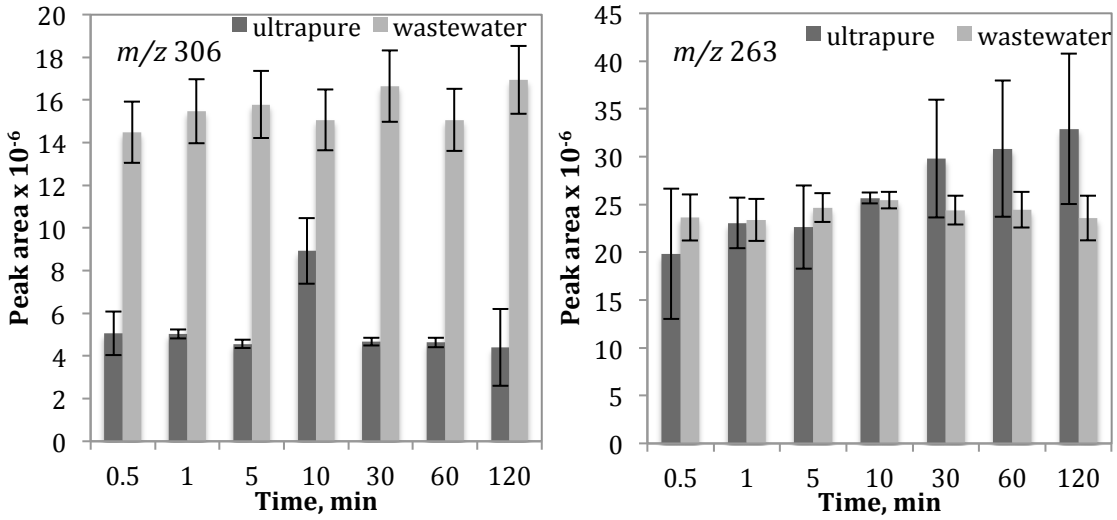


Figure 23. Transformation products of ciprofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

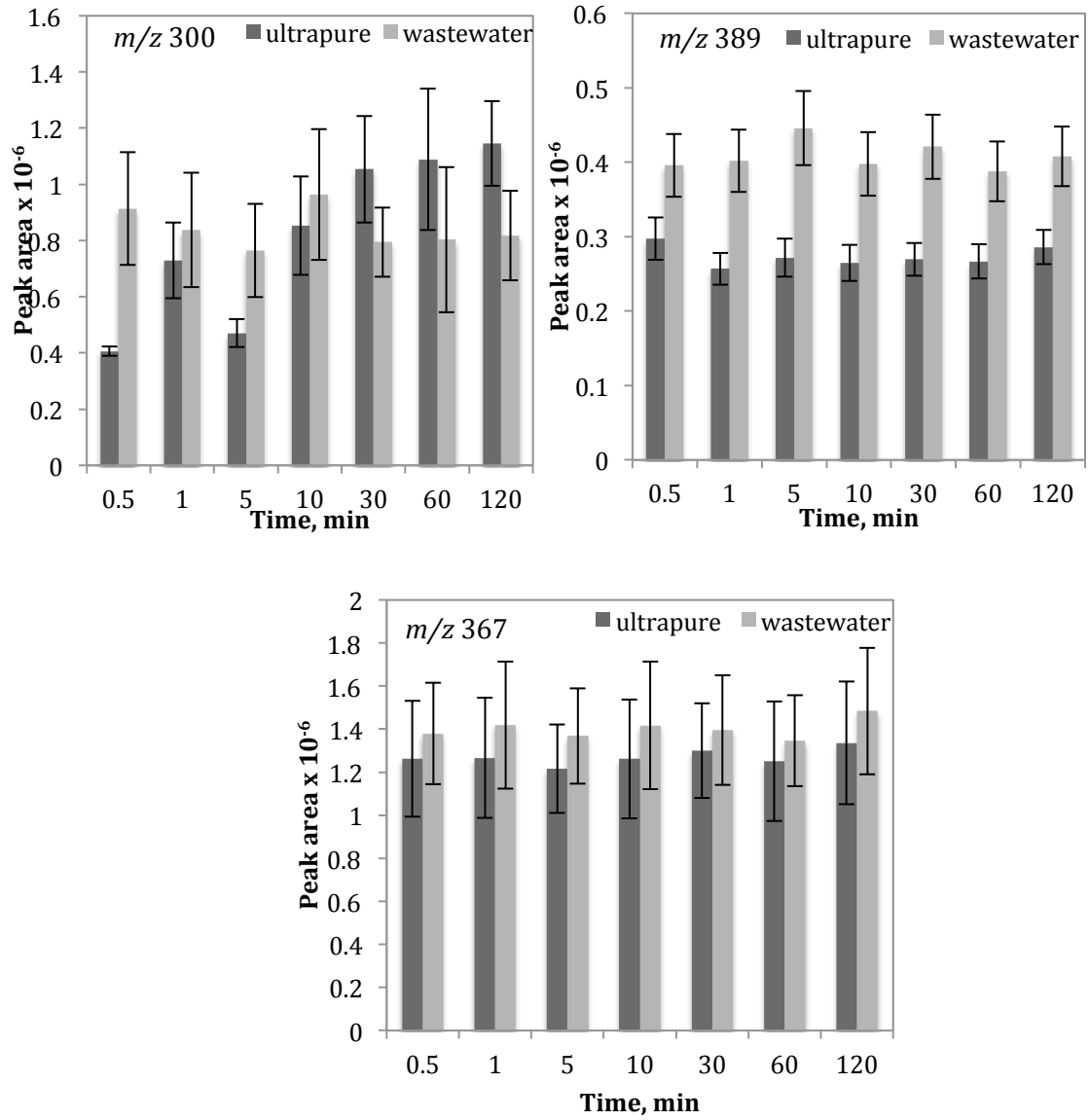


Figure 23. Transformation products of ciprofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

The essential structure of the fluoroquinolone class antibiotics can be seen in Figure 24. H can be replaced by other elements to form antibiotics. Those groups would be more accessible and likely to participate in reactions than the two rings. This essential structure was maintained for four of the five proposed TPs of CIP.

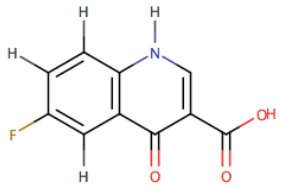


Figure 24. Essential structure of fluoroquinolone class antibiotics

Figure 25 shows the transformation products as well as their corresponding *m/z* values. The product *m/z* 306 loses two carbons and two hydrogens from its piperazinyl ring. From the *m/z* 306 structure, the m/z 263 loses two carbons, five hydrogens, and a nitrogen. The *m/z* 263 has been seen in a previous study,[131] where most of the piperazinyl ring is released, leaving an amine group. The *m/z* 300 can be seen in other work as well[132], where the piperazinyl group initially obtains a doubled-bonded oxygen but then loses a C_2H_4 and a flourine.[132]

The product *m/z* 389 adds two chlorines (evidenced from isotope identification and the proposed locations can be seen in Figure 25). These locations were chosen as favorable from previous studies placing the initial chlorine on the essential fluoroquinolone structure.[133-134] The second chlorine is placed on the piperazinyl ring, while there is a loss of a carbon and hydrogen on the cyclopropane structure.

Product *m/z* 367 has a simple addition of +35 (the mass of one chlorine), and upon LC/MS analysis, one chlorine isotope was discovered. As previously discussed, the

chlorine atom was placed in the most favorable location. This structure was reported in previous work[134] however a proposed alternative structure can be seen in Figure 25.

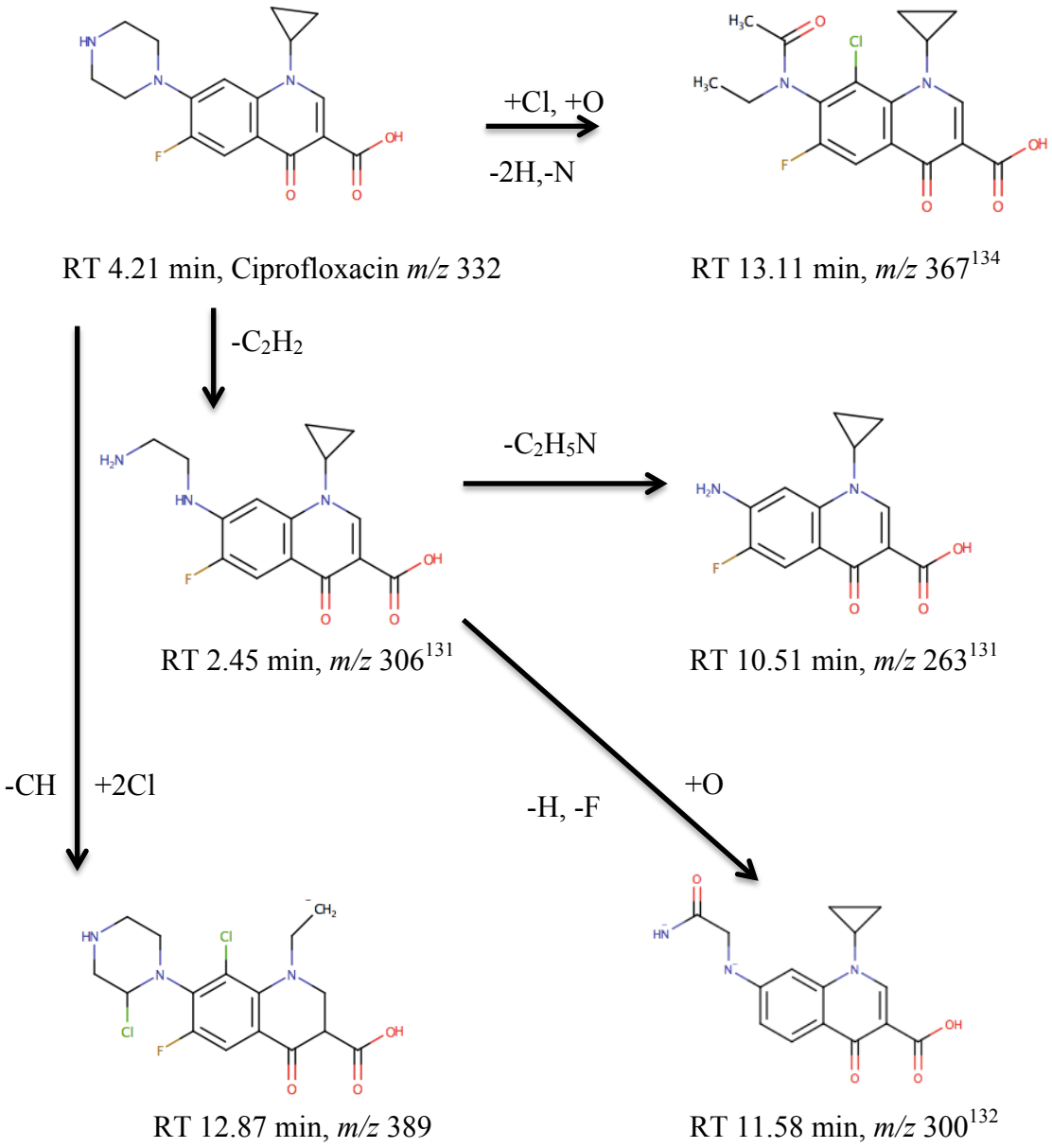


Figure 25. Chemical structures of the transformation products and parent compound, ciprofloxacin, with their corresponding m/z values and retention time (RT).

The results show the reaction between ciprofloxacin and chlorine happens over the 2 h in ultrapure water and wastewater. The products appear within the first 15 min of the reaction with little change over the remaining time of chlorine exposure. A higher concentration of chlorine was used for wastewater than in ultrapure water. This was because the wastewater constituents competed with ciprofloxacin for the chlorine.

3.11 Levofloxacin

Levofloxacin belongs to the fluoroquinolone class of antibiotics and can be used to treat sinus, skin, lung, and urinary tract infections.[135] The normalized concentration of levofloxacin decreased quicker in ultrapure water than in the wastewater matrix. Active TPs were seen in both ultrapure water and wastewater for the experiments with levofloxacin. The formation of active products from levofloxacin was observed to increase with longer exposure times in ultrapure water. The formation of active products of levofloxacin seem to be hindered by the wastewater matrix (Figure 26).

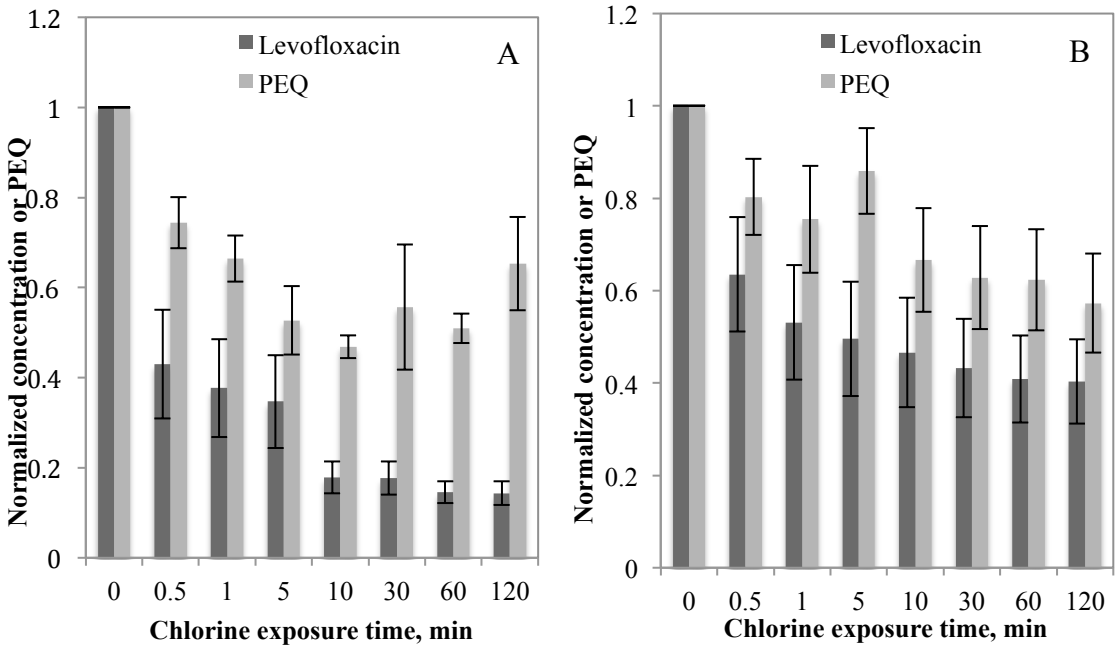


Figure 26. PEQ vs. normalized concentration of levofloxacin in ultrapure matrix (A) and wastewater effluent matrix (B) after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.12 Ofloxacin

Ofloxacin is a part of the fluoroquinolone class of antibiotics and is used for prostatitis, infectious diarrhea, and along with other pharmaceuticals can be used for multidrug resistant tuberculosis.[135-136] Ofloxacin is a racemic mixture of the enantiomers of levofloxacin and dextrofloxacin. Levofloxacin is the more biologically active enantiomer. Dextrofloxacin, has significantly less biological activity. With dextrofloxacin being an enantiomer of levofloxacin, the corresponding TPs can be enantiomers and can potentially have different antibacterial activity. Levofloxacin and dextrofloxacin differ only by the chirality of the carbon to which the methyl is attached. Although the products of levofloxacin and ofloxacin may have different activities, any enantiomers will not be

distinguishable from each other using mass spectrometry analysis without the use of a chiral crown.

After the initial drop from exposure in the first 0.5 min, the normalized concentration of ofloxacin saw a steady decrease for both ultrapure water and wastewater. Active TPs were seen in both ultrapure water and wastewater for the experiments with ofloxacin. The active products of ofloxacin appear to form with longer exposure times in both matrices (Figure 27). Different trends in wastewater matrix for levofloxacin and ofloxacin suggest that dextrofloxacin is capable of forming transformation products that are more active than parent compound, levofloxacin or dextrofloxacin.

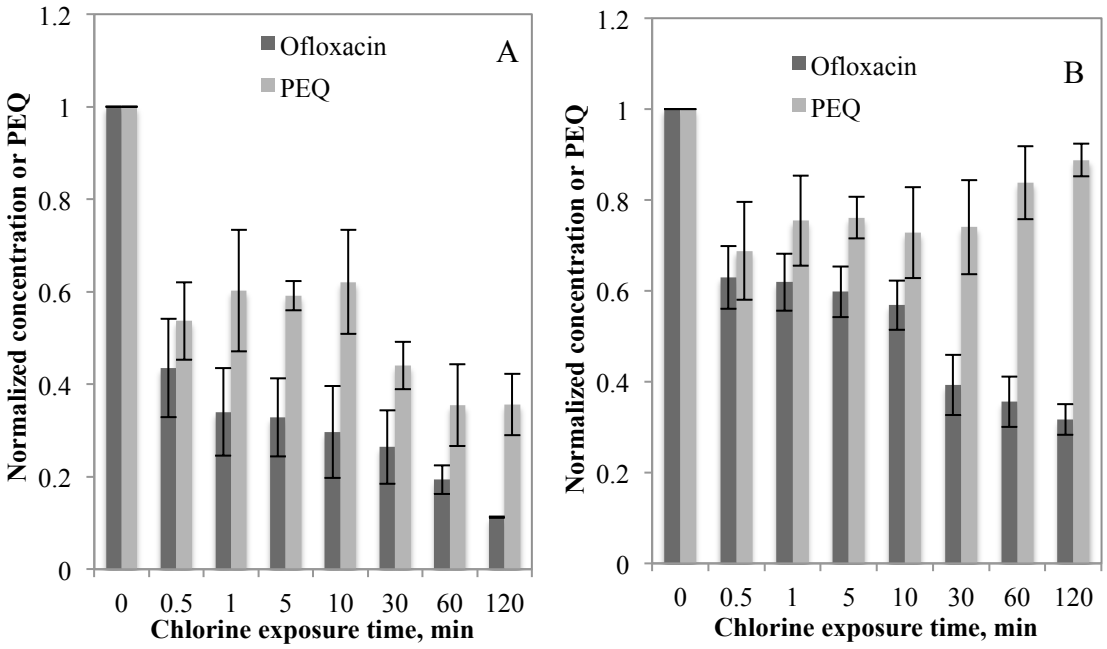


Figure 27. PEQ vs. normalized concentration of ofloxacin in ultrapure matrix (A) and wastewater effluent matrix (B), after specific time intervals of chlorine exposure. Results are based on three sets of replicated data. Error bars represent standard deviation from three repeated experiments.

3.13 Transformation products – Levofloxacin and Ofloxacin

Several chlorinated and non-chlorinated products formed in the samples with ultrapure and wastewater matrices where levofloxacin and ofloxacin were present. Both antibiotics had similar products with the same *m/z* value, but there were differences in which products were more pronounced for these antibiotics. Numerous TPs formed from these antibiotics with the most prominent seven shown in Table 17 and Table 18 for the corresponding antibiotic. Some TPs were equally prominent for both compounds. The parent stereoisomer compounds for levofloxacin and ofloxacin can be seen in Table 16. The different orientation between the two compounds is depicted. The relative abundance of these products in each matrix can be seen in Figure 28 and Figure 29. Of these eight transformation products, six are chlorinated.

One, *m/z* 269, was present in all experiments involving levofloxacin (in ultrapure water and wastewater) and ofloxacin (in ultrapure water and wastewater). This product formed during the initial rapid reaction between the antibiotic and chlorine and had a RT of 8.0 min for LVF and OFL. Though this product has not been previously seen in other studies, the proposed degradation pathway is similar to products seen in previous work where the piperazinyl and carboxyl groups are affected.[137] The proposed degradation process involves the loss of the piperazinyl ring and a chlorine replacing the carboxyl group.

The *m/z* 279 has been seen in previous work[137-138] involving photocatalytic degradation with the elimination of the piperazinyl ring.[137]

The *m/z* 304 has a substitution of the carboxyl group with a hydroxyl, opening of the piperazinyl ring with an addition of a hydroxyl, and an elimination of the morpholine ring leaving behind a hydroxyl group and adding a chlorine.[95]

The proposed product with *m/z* 326 has been detected previously[95], where the piperazinyl rings opens and loses C_2H_6 and the carboxyl group is replaced with a chlorine. The *m/z* 352 was also reported in the same study.[95] In this product chlorine replaces the carboxyl group.

Previous work has shown the possibility of the pyridone ring opening[139-140] plus the addition of the Cl give the proposed *m/z* 360. The *m/z* 378 has an addition of a hydroxyl group on the morpholine ring as shown in a previous study.[137]

Table 16. The parent compounds of levofloxacin or ofloxacin (dextrofloxacin), both antibiotics having an m/z 362.

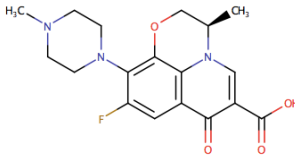
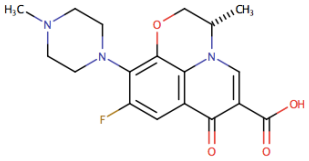
m/z	RT	Chlorine	Levofloxacin	Ofloxacin (Dextrofloxacin)
362	3.27	0		

Table 17. Chemical structures of the transformation products and their corresponding *m/z* values and retention time (RT). "X" indicates presence of the transformation product.

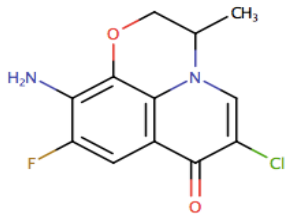
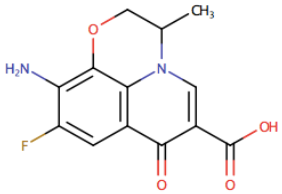
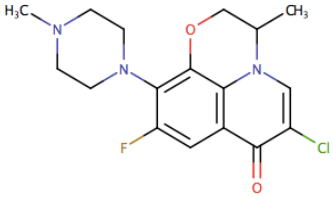
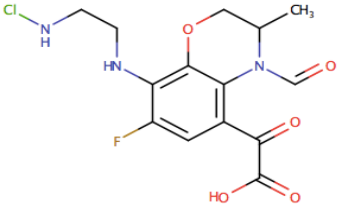
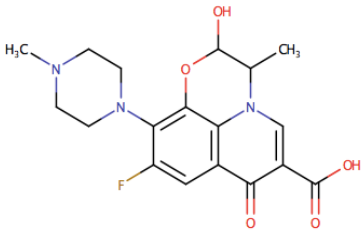
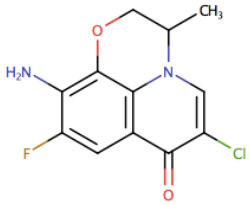
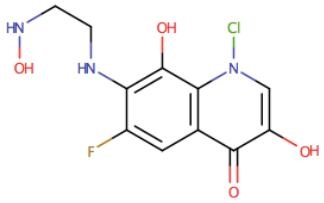
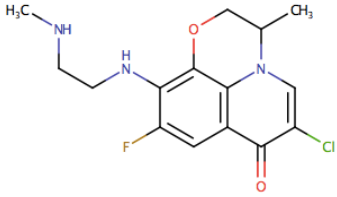
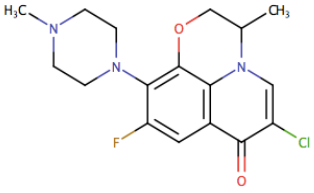
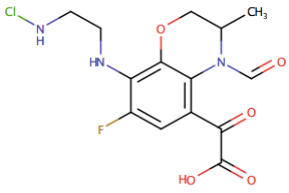
m/z	RT	Chlorine	Structure	Levofloxacin	
				Ultrapure water	Wastewater
269	8.02	1		X	X
279[138]	9.98	0		X	-
352[95]	9.95	1		-	X
360[78]	13.77	1		X	-
378[137]	9.04	0		-	X

Table 18. Chemical structures of the transformation products and their corresponding *m/z* values and retention time (RT). "X" indicates presence of the transformation product.

m/z	RT	Chlorine	Structure	Ofloxacin	
				Ultrapure water	Wastewater
269	8.02	1		X	X
304[95]	12.05	1		-	X
326[78]	10.94	1		-	X
352[95]	9.95	1		X	-
360[78]	13.77	1		-	X

The TP abundance for levofloxacin from experiments in ultrapure water and wastewater can be seen in Figure 28. The m/z 269 (mono-chlorinated) had higher abundance in ultrapure water than wastewater. The m/z 279 (non-chlorinated) appeared only in ultrapure water with slow decline in formation over time. The m/z 360 (mono-chlorinated) appeared in ultrapure water and increased in formation over time. M/z 352 had steady formation throughout time and m/z 378 increased formation over time eventually decreasing after 1 h of reaction time, both appearing in wastewater only. For these experiments, antibacterial activity increased with time suggesting that some or all of the products showing an increase in abundance over time are likely the ones with antibacterial activity.

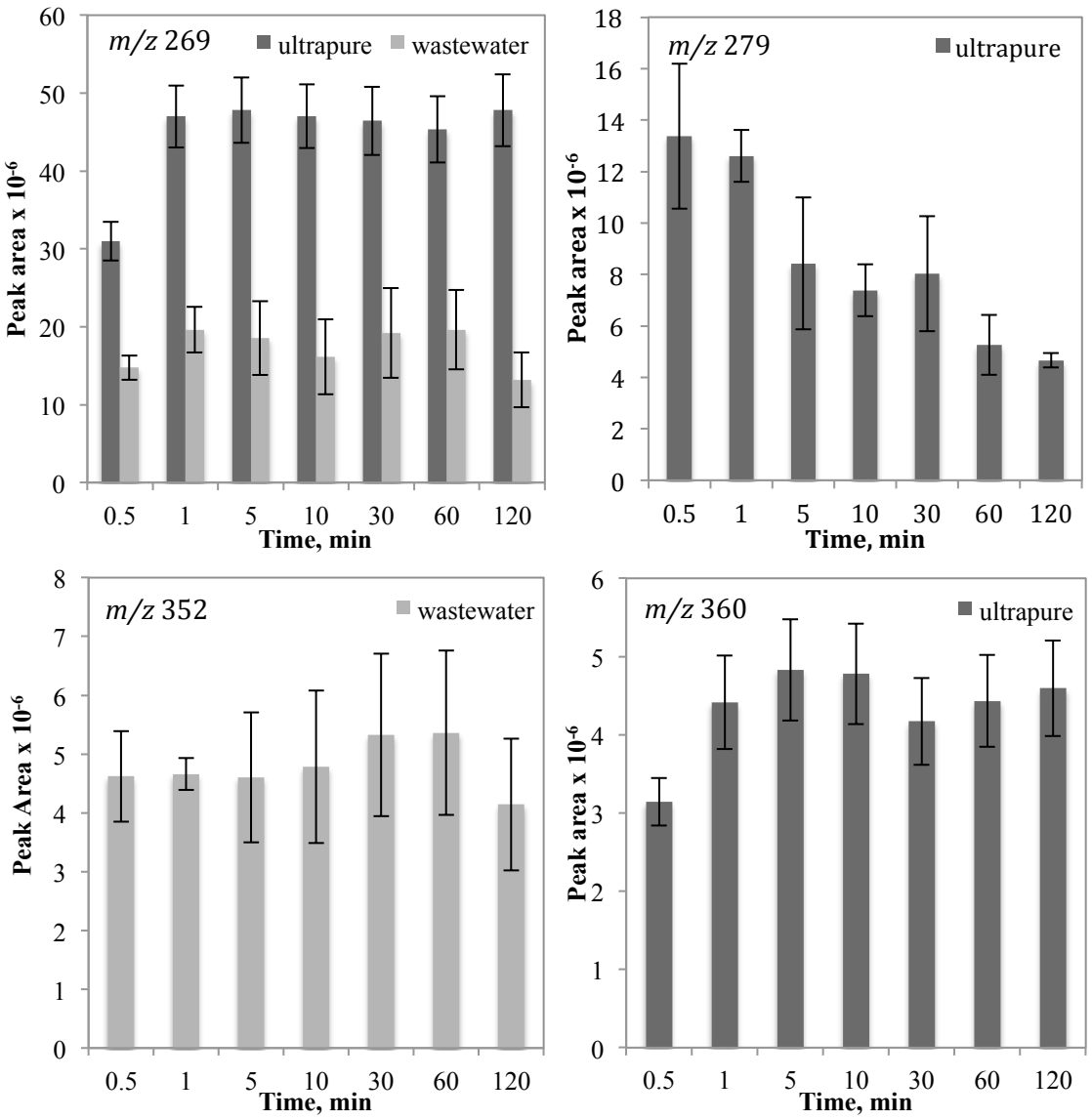


Figure 28. Transformation products of levofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

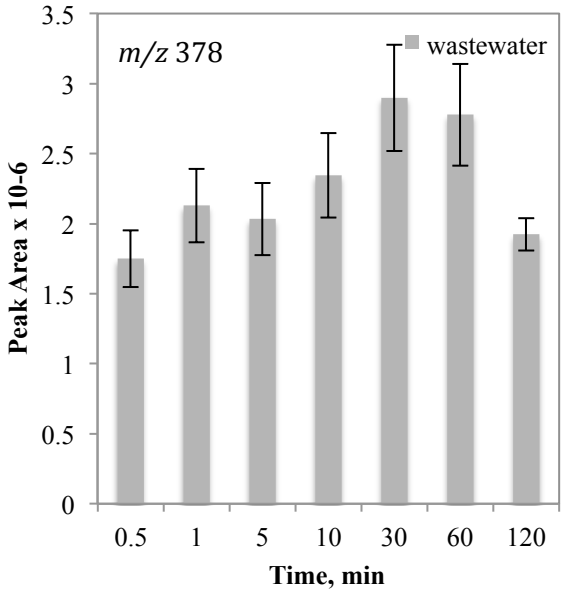


Figure 28. Transformation products of levofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

The TP abundance for ofloxacin from experiments in ultrapure water and wastewater can be seen in Figure 29. The m/z 269 (mono-chlorinated), the only TP to appear in both LVF and OFL in ultrapure water and wastewater, had higher abundance in ultrapure water. The m/z 352 (mono-chlorinated), appeared in ultrapure water. The m/z 304 (mono-chlorinated) had highest formation at 5 and 10 min, otherwise remained stable throughout time. The m/z 326 and 360 (both mono-chlorinated), appeared in wastewater only and had steady formation over time.

The piperazinyl group was most frequently affected in the proposed structures. This group is important to fluoroquinolone class antibiotics as it controls the activity for gram-negative bacteria.[141] The carboxyl group was the second most affected group in the proposed structures and is important for transport of the antibiotic into bacterial cells.[141] .

For these reasons, the likely proposed products retaining antibacterial activity is *m/z* 389 in ciprofloxacin, *m/z* 352 and 378 in levofloxacin, and *m/*z 352 in ofloxacin.

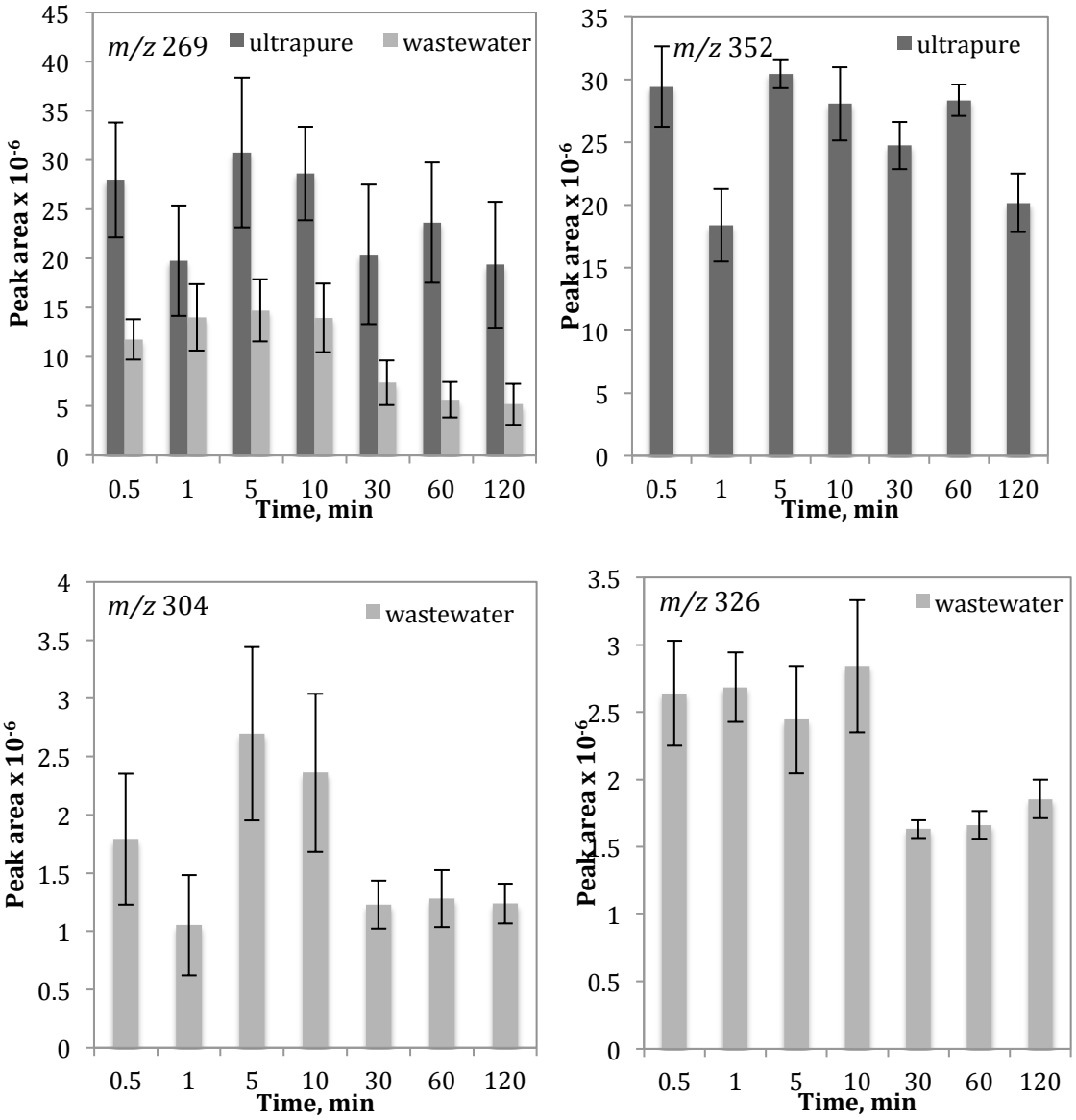


Figure 29. Transformation products of ofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

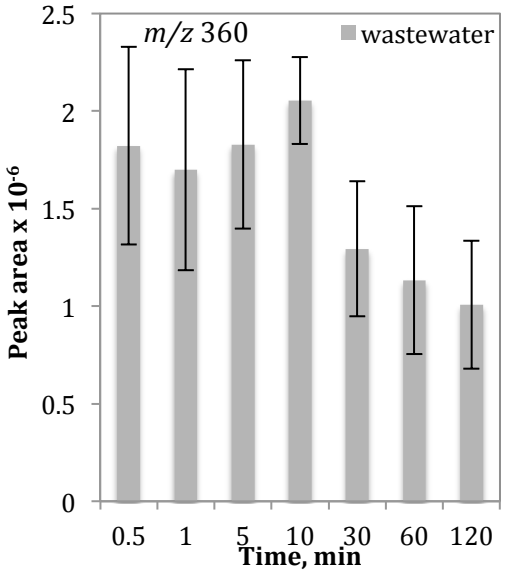


Figure 29. Transformation products of ofloxacin as mass spectrum peak area over time. Results are based on three sets of replicated data. Error bars represent the standard deviation from three repeated experiments.

CHAPTER 4: CONCLUSIONS

The goal of the research conducted was to identify major unrecognized sources of pharmaceutically active compounds in the environment. The sources considered included waste disposal from healthcare facilities, landfill leachates, and transformation product formation during disinfection of wastewater.

4.1 Pharmaceutical disposal in healthcare settings

The main objective in evaluating healthcare facilities load was to explore their pharmaceutical waste handling practices and determine whether they present a substantial load through sewage disposal or through landfill leachate. This was accomplished by understanding the disposal methods utilized by healthcare facilities for non-regulated pharmaceutical waste.

The results showed that most healthcare providers utilize special pharmaceutical waste collection programs and are disposing of non-regulated pharmaceutical waste in a responsible manner. However, the volume of pharmaceutical waste generated by medical facilities is very large (upwards of hundreds of pounds per week by a single facility). If even a few medical facilities did not segregate pharmaceutical waste, it could have a major impact on the receiving landfill or WWTP. More outreach and information campaign efforts may be useful to encourage these special collection programs.

Special pharmaceutical waste collection programs vary greatly depending on the facility and program they are using. It may be beneficial to have standardized waste collection programs to decrease the variability between facilities. Some healthcare providers were unable to report an overall accumulated amount of waste generated at

their facility. This implies that there may be a need for better organization and classification of waste. The waste should be recorded weekly or monthly depending on how much is generated and broken down into the different categories of waste. Although this can be done by an outside consultant, the facilities should be able to know the amount generated and have record of their accumulation over time.

Furthermore, some employees are not aware of what comprises the waste described on the receptacle so they may discard waste into the wrong receptacle. This is a problem because every type of waste has different costs of disposal. For example, hazardous waste is very expensive to dispose of and if non-hazardous waste is combined with hazardous waste, the total volume will incur a higher fee. This is also a concern as healthcare systems are billed for every pound disposed, and the disposal charges can comprise a significant percentage of the overall budget. With better employee education about disposal procedures money can be saved and utilized where it may be needed more. Also, expenses can be discouraging for facilities from participating in voluntary pharmaceutical separation programs. Better management of these waste streams to minimize the unnecessary expenses can lead to broader participation.

From the data collected, there was about 10% (4 out of 37 facilities surveyed) of healthcare facilities that disposed portions of pharmaceutical waste through local municipal utilities (landfill or WWTP). The other 90% utilize special pharmaceutical waste collection programs or discard non-regulated pharmaceuticals with DEA regulated medical waste. This small percentage of non-participating facilities is still a concern as discarded pharmaceuticals may lead to water resources contamination, and in the case of

antibiotics (which is incidentally one of the highest volume class of discarded pharmaceuticals), lead to development of antibiotic resistance within the environment.

4.2 SPE techniques

Pharmaceuticals have been reported in leachate and typically methods involve using HLB cartridges for extraction. However, leachate has a high organic load and can quickly coat the cartridge media and prevent pharmaceuticals from being extracted. For this reason cartridges were tested with size exclusion capacity to understand if improved pharmaceutical detection could be obtained compared to traditional methods. Improved extraction could reveal that higher concentrations of pharmaceuticals are present in leachates than what has been shown in previous research.

Results showed variance amongst the cartridges for recovery of chosen compounds in leachate. The best recovery among the four cartridges was seen with PPL (61-95% recovery) and the lowest recovery was with HLB (50-89% recovery). This shows that leachate can have a significant effect on SPE recovery.

All the cartridges performed reasonably well compared to each other (recoveries of 61-95% PPL, 50-89% HLB, 68-92% PLEXA, and 63-93% ENV), although the three alternative cartridges outperformed the current industry standard HLB. Though there was no statistical significance between the four cartridges used, the extraction results for complex leachate matrices can be improved by introducing a size-exclusion element to the extraction. CBZ was likely lost due to its adsorption to leachate humic substances that are not well retained by the cartridges.

The study found improved extraction techniques for leachate, enhancing the detection capabilities. However, the improvement was subtle and does not suggest that leachates are an underappreciated source of pharmaceuticals.

4.3 Pharmaceuticals in WWTPs

It is known that transformation products form during wastewater disinfection but what is more important is the properties of environmental relevance of the transformation products (e.g. toxicity, endocrine disrupting potential, etc.). If transformation products retain their pharmacological activity, they become an unaccounted for source of pharmaceuticals in the environment. Transformation happens mainly in chemical disinfection processes, although some pharmaceuticals can be biodegradable. The focus for this work was on the antibiotic class of pharmaceuticals because of their major public health significance. Chlorine disinfection was chosen because it is the most common method for disinfecting wastewater before discharge into the environment. Microbial assays were used to evaluate the antibacterial activity of the products that formed and mass spectrometry was used to propose product structures, if antibacterial activity was detected.

The results showed that during chlorine disinfection some of the transformation products of common antibiotics retained antibiotic properties. The formation of these products was observed in ultrapure water and in wastewater effluent matrix. Some of the products, in regards to doxycycline, were inhibited in the presence of wastewater. This inhibition was not seen in the fluoroquinolone class antibiotics (CIP, LVF, and OFL).

The fluroquinolones had some TPs that formed better in ultrapure water and some that formed better in wastewater. For SMX and TMP no active products were detected.

With these antibiotics having pK_a's so close to neutral, examination of the formation of active products for doxycycline and fluoroquinolones at a range of pH may be desirable to determine whether ionized and non-ionized forms of the pharmaceutical form different products or if they form different products in the reaction with OCl^- vs HOCl.

The results highlight the potential for antibacterially active products of antibiotics forming during chlorine disinfection and emphasizes the need to evaluate the properties of other common antibiotics. Minimum concentrations that exert selective pressure for many antibiotics are very close to environmental concentrations, and thus the same could be true for the transformation products. Antibacterially active transformation products may still select for antibiotic resistant microorganisms in the environment. Chlorine incorporation into the structure was an important transformation pathway which may affect the toxicity of products of other, non-antibiotic pharmaceuticals as well.

A continued concern is the amount and range of pharmaceuticals entering the environment. Increased variability of pharmaceuticals and antibacterially active transformation products enhance the concern for resistant bacteria and adverse effect on human health. It is the hope that this study emphasizes the need for proper disposal of pharmaceuticals and for additional efforts in understanding the TPs forming in treatment processes and ways to mitigate the potential effects.

REFERENCES

1. Daemmrich, A.; Bowden, M., Emergence of pharmaceutical science and industry: 1870–1930. *Chem Eng News* **2005,** *83*.
2. Dailey, J., Pharmaceutical industry. *Encyclopedia Britannica. Np* **2016,** *9*.
3. Drug Classes. https://www.drugs.com/drug-classes.html (accessed July 7, 2017).
4. Smallpox. http://www.who.int/csr/disease/smallpox/en/ (accessed July 7, 2017).
5. Ghosh, P., Rinderpest virus has been wiped out, scientists say. *BBC News* **2010,** *14*.
6. World Health Organization, W., Antimicrobial resistance. Fact sheet 194. *World Health Organization (2012b). Safe abortion: technical and policy guidance for health systems* **2012**.
7. Control, C. f. D.; Prevention, Antibiotic/antimicrobial resistance. CDC website. 2017.
8. Faure, P.; Rizzo, P. N. In *Hospital and environment: waste disposal*, Annales pharmaceutiques francaises, 2003; pp 373-377.
9. Pedersen, C. A.; Schneider, P. J.; Scheckelhoff, D. J., ASHP national survey of pharmacy practice in hospital settings: dispensing and administration--2008. *American Journal of Health-System Pharmacy* **2009,** *66* (10).
10. Voudrias, E.; Goudakou, L.; Kermenidou, M.; Softa, A., Composition and production rate of pharmaceutical and chemical waste from Xanthi General Hospital in Greece. *Waste management* **2012,** *32* (7), 1442-1452.
11. Braund, R.; Peake, B. M.; Shieffelbien, L., Disposal practices for unused medications in New Zealand. *Environment International* **2009,** *35* (6), 952-955.
12. Organization, W. H.; Association, I. P.; Association, I. S. W.; UNICEF, Guidelines for safe disposal of unwanted pharmaceuticals in and after emergencies. **1999**.
13. Masoner, J. R.; Kolpin, D. W.; Furlong, E. T.; Cozzarelli, I. M.; Gray, J. L., Landfill leachate as a mirror of today's disposable society: pharmaceuticals and other contaminants of emerging concern in final leachate from landfills in the conterminous United States. *Environmental toxicology and chemistry* **2016,** *35* (4), 906-918.
14. Clarke, B. O.; Anumol, T.; Barlaz, M.; Snyder, S. A., Investigating landfill leachate as a source of trace organic pollutants. *Chemosphere* **2015,** *127*, 269-275.
15. Behr, R.; Stahler, D.; Pistell, A., Preliminary Characterization of the Pharmaceuticals Content of Municipal Solid Waste Landfill Leachate from Three Landfills in Maine. *Unpublished document* **2010**.
16. Rizzo, L.; Manaia, C.; Merlin, C.; Schwartz, T.; Dagot, C.; Ploy, M.; Michael, I.; Fatta-Kassinos, D., Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the total environment* **2013,** *447*, 345-360.
17. Kim, S.; Aga, D. S., Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. *Journal of Toxicology and Environmental Health, Part B* **2007,** *10* (8), 559-573.
18. Schlüter, A.; Szczepanowski, R.; Pühler, A.; Top, E. M., Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides

evidence for a widely accessible drug resistance gene pool. *FEMS microbiology reviews* **2007,** *31* (4), 449-477.
19. Auerbach, E. A.; Seyfried, E. E.; McMahon, K. D., Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water research* **2007,** *41* (5), 1143-1151.
20. Timraz, K.; Xiong, Y.; Al Qarni, H.; Hong, P.-Y., Removal of bacterial cells, antibiotic resistance genes and integrase genes by on-site hospital wastewater treatment plants: surveillance of treated hospital effluent quality. *Environmental Science: Water Research & Technology* **2017,** *3* (2), 293-303.
21. Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T., Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance. *Environmental science & technology* **2002,** *36* (6), 1202-1211.
22. Bengtsson-Palme, J.; Larsson, D. J., Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environment International* **2016,** *86*, 140-149.
23. Kemper, N., Veterinary antibiotics in the aquatic and terrestrial environment. *Ecological indicators* **2008,** *8* (1), 1-13.
24. Kümmerer, K., Resistance in the environment. *Journal of Antimicrobial Chemotherapy* **2004,** *54* (2), 311-320.
25. Kümmerer, K., Antibiotics in the aquatic environment–a review–part I. *Chemosphere* **2009,** *75* (4), 417-434.
26. Neth, N. L. K.; Carlin, C. M.; Keen, O. S., Doxycycline transformation and emergence of antibacterially active products during water disinfection with chlorine. *Environmental Science: Water Research & Technology* **2017,** *3* (6), 1086-1094.
27. Harhay, M. O.; Halpern, S. D.; Harhay, J. S.; Olliaro, P. L., Health care waste management: a neglected and growing public health problem worldwide. *Tropical Medicine & International Health* **2009,** *14* (11), 1414-1417.
28. Chaerul, M.; Tanaka, M.; Shekdar, A. V., A system dynamics approach for hospital waste management. *Waste Management* **2008,** *28* (2), 442-449.
29. Birpınar, M. E.; Bilgili, M. S.; Erdoğan, T., Medical waste management in Turkey: A case study of Istanbul. *Waste Management* **2009,** *29* (1), 445-448.
30. George, S. A., Medical waste segregation apparatus with moveable floor. Google Patents: 2002.
31. Marinković, N.; Vitale, K.; Holcer, N. J.; Džakula, A.; Pavić, T., Management of hazardous medical waste in Croatia. *Waste management* **2008,** *28* (6), 1049-1056.
32. Almuneef, M.; Memish, Z. A., Effective medical waste management: it can be done. *American journal of infection control* **2003,** *31* (3), 188-192.
33. Yong, Z.; Gang, X.; Guanxing, W.; Tao, Z.; Dawei, J., Medical waste management in China: a case study of Nanjing. *Waste management* **2009,** *29* (4), 1376-1382.
34. Oweis, R.; Al-Widyan, M.; Al-Limoon, O., Medical waste management in Jordan: A study at the King Hussein Medical Center. *Waste management* **2005,** *25* (6), 622-625.
35. Mathur, V.; Dwivedi, S.; Hassan, M.; Misra, R., Knowledge, attitude, and practices about biomedical waste management among healthcare personnel: A cross-

sectional study. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine* **2011,** *36* (2), 143.
36. Keenan, J. D.; Steiner, R. L.; Fungaroli, A., Landfill leachate treatment. *Journal (Water Pollution Control Federation)* **1984**, 27-33.
37. Christensen, T. H.; Cossu, R.; Stegmann, R., Landfill leachate: an introduction. *landfilling of waste: leachate* **1992**, 3-14.
38. Barnes, K. K.; Christenson, S. C.; Kolpin, D. W.; Focazio, M. J.; Furlong, E. T.; Zaugg, S. D.; Meyer, M. T.; Barber, L. B., Pharmaceuticals and other organic waste water contaminants within a leachate plume downgradient of a municipal landfill. *Groundwater Monitoring & Remediation* **2004,** *24* (2), 119-126.
39. Benfenati, E.; Pierucci, P.; Fanelli, R.; Preiss, A.; Godejohann, M.; Astratov, M.; Levsen, K.; Barcelo, D., Comparative studies of the leachate of an industrial landfill by gas chromatography–mass spectrometry, liquid chromatography–nuclear magnetic resonance and liquid chromatography–mass spectrometry. *Journal of Chromatography A* **1999,** *831* (2), 243-256.
40. Silva, M. J.; Malek, N. A.; Hodge, C. C.; Reidy, J. A.; Kato, K.; Barr, D. B.; Needham, L. L.; Brock, J. W., Improved quantitative detection of 11 urinary phthalate metabolites in humans using liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry. *Journal of Chromatography B* **2003,** *789* (2), 393-404.
41. Buszka, P.; Yeskis, D.; Kolpin, D.; Furlong, E.; Zaugg, S.; Meyer, M., Waste-indicator and pharmaceutical compounds in landfill-leachate-affected ground water near Elkhart, Indiana, 2000–2002. *Bulletin of environmental contamination and toxicology* **2009,** *82* (6), 653-659.
42. Choi, K.-J.; Kim, S.-G.; Kim, C.-w.; Kim, S.-H., Determination of antibiotic compounds in water by on-line SPE-LC/MSD. *Chemosphere* **2007,** *66* (6), 977-984.
43. Kamal, N.; Galvez, R.; Buelna, G., Application of a solid phase extraction-liquid chromatography method to quantify phenolic compounds in woodwaste leachate. *Water Quality Research Journal* **2014,** *49* (3), 210-222.
44. Carbonell-Martin, G.; Pro-Gonzalez, J.; Aragonese-Grunert, P.; Babin-Vich, M.; Fernandez-Torija, C.; Tarazona-Lafarga, J., Targeting the environmental assessment of veterinary drugs with the multi-species-soil system (MS· 3) agricultural soil microcosms: the ivermectin case study. *Spanish Journal of Agricultural Research* **2011,** *9* (2), 433-443.
45. Shelver, W. L.; Hakk, H.; Larsen, G. L.; DeSutter, T. M.; Casey, F. X., Development of an ultra-high-pressure liquid chromatography–tandem mass spectrometry multi-residue sulfonamide method and its application to water, manure slurry, and soils from swine rearing facilities. *Journal of Chromatography A* **2010,** *1217* (8), 1273-1282.
46. UEBORI, M.; IMAMURA, K.; ISHII, Y.; HASEGAWA, A.; YOSHIDA, Y.; SUZUKI, S., Study on the screening method of chemicals in leachate samples by solid-phase extraction and LC/MS analysis. *Journal of Environmental Conservation Engineering* **2006,** *35* (8), 588-595.
47. Hu, H.; Jiang, C.; Ma, H.; Ding, L.; Geng, J.; Xu, K.; Huang, H.; Ren, H., Removal characteristics of DON in pharmaceutical wastewater and its influence on the

N-nitrosodimethylamine formation potential and acute toxicity of DOM. *Water research* **2017,** *109*, 114-121.
48. Karthikeyan, K.; Meyer, M. T., Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Science of the Total Environment* **2006,** *361* (1), 196-207.
49. Renew, J. E.; Huang, C.-H., Simultaneous determination of fluoroquinolone, sulfonamide, and trimethoprim antibiotics in wastewater using tandem solid phase extraction and liquid chromatography–electrospray mass spectrometry. *Journal of Chromatography A* **2004,** *1042* (1), 113-121.
50. Ben, W.; Qiang, Z.; Adams, C.; Zhang, H.; Chen, L., Simultaneous determination of sulfonamides, tetracyclines and tiamulin in swine wastewater by solid-phase extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A* **2008,** *1202* (2), 173-180.
51. Fatta, D.; Achilleos, A.; Nikolaou, A.; Meric, S., Analytical methods for tracing pharmaceutical residues in water and wastewater. *TrAC Trends in Analytical Chemistry* **2007,** *26* (6), 515-533.
52. Sousa, M.; Gonçalves, C.; Cunha, E.; Hajšlová, J.; Alpendurada, M., Cleanup strategies and advantages in the determination of several therapeutic classes of pharmaceuticals in wastewater samples by SPE–LC–MS/MS. *Analytical and bioanalytical chemistry* **2011,** *399* (2), 807-822.
53. Babić, S.; Ašperger, D.; Mutavdžić, D.; Horvat, A. J.; Kaštelan-Macan, M., Solid phase extraction and HPLC determination of veterinary pharmaceuticals in wastewater. *Talanta* **2006,** *70* (4), 732-738.
54. Vajda, A. M.; Barber, L. B.; Gray, J. L.; Lopez, E. M.; Woodling, J. D.; Norris, D. O., Reproductive disruption in fish downstream from an estrogenic wastewater effluent. *Environmental science & technology* **2008,** *42* (9), 3407-3414.
55. Goñi-Urriza, M.; Capdepuy, M.; Arpin, C.; Raymond, N.; Caumette, P.; Quentin, C., Impact of an Urban Effluent on Antibiotic Resistance of Riverine Enterobacteriaceae andAeromonas spp. *Applied and environmental Microbiology* **2000,** *66* (1), 125-132.
56. Hussain, S.; Naeem, M.; Chaudhry, M. N., Estimation of residual antibiotics in pharmaceutical effluents and their fate in affected areas. *Polish Journal of Environmental Studies* **2016,** *25* (2).
57. Goñi-Urriza, M.; Capdepuy, M.; Arpin, C.; Raymond, N.; Caumette, P.; Quentin, C., Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp. *Applied and Environmental Microbiology* **2000,** *66* (1), 125-132.
58. Zhang, Y.; Marrs, C. F.; Simon, C.; Xi, C., Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp. *Science of The Total Environment* **2009,** *407* (12), 3702-3706.
59. Akiyama, T.; Savin, M. C., Populations of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream. *Science of The Total Environment* **2010,** *408* (24), 6192-6201.
60. Sidrach-Cardona, R.; Hijosa-Valsero, M.; Marti, E.; Balcázar, J. L.; Becares, E., Prevalence of antibiotic-resistant fecal bacteria in a river impacted by both an antibiotic production plant and urban treated discharges. *Science of The Total Environment* **2014,** *488*, 220-227.
61. Joss, A.; Zabczynski, S.; Göbel, A.; Hoffmann, B.; Löffler, D.; McArdell, C. S.; Ternes, T. A.; Thomsen, A.; Siegrist, H., Biological degradation of pharmaceuticals in

municipal wastewater treatment: proposing a classification scheme. *Water research* **2006,** *40* (8), 1686-1696.
62. Simazaki, D.; Fujiwara, J.; Manabe, S.; Matsuda, M.; Asami, M.; Kunikane, S., Removal of selected pharmaceuticals by chlorination, coagulation-sedimentation and powdered activated carbon treatment. **2008**.
63. Negreira, N.; de Alda, M. L.; Barceló, D., Degradation of the cytostatic etoposide in chlorinated water by liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry: Identification and quantification of by-products in real water samples. *Science of The Total Environment* **2015,** *506*, 36-45.
64. Negreira, N.; Regueiro, J.; de Alda, M. L.; Barceló, D., Transformation of tamoxifen and its major metabolites during water chlorination: Identification and in silico toxicity assessment of their disinfection byproducts. *Water research* **2015,** *85*, 199-207.
65. de Jesus Gaffney, V.; Cardoso, V. V.; Benoliel, M. J.; Almeida, C. M., Chlorination and oxidation of sulfonamides by free chlorine: Identification and behaviour of reaction products by UPLC-MS/MS. *Journal of environmental management* **2016,** *166*, 466-477.
66. Rizzo, L.; Fiorentino, A.; Anselmo, A., Advanced treatment of urban wastewater by UV radiation: effect on antibiotics and antibiotic-resistant E. coli strains. *Chemosphere* **2013,** *92* (2), 171-176.
67. Wammer, K. H.; Lapara, T. M.; McNeill, K.; Arnold, W. A.; Swackhamer, D. L., Changes in antibacterial activity of triclosan and sulfa drugs due to photochemical transformations. *Environmental toxicology and chemistry / SETAC* **2006,** *25* (6), 1480-6.
68. Keen, O. S.; Linden, K. G., Degradation of Antibiotic Activity during UV/H2O2 Advanced Oxidation and Photolysis in Wastewater Effluent. *Environmental Science & Technology* **2013,** *47* (22), 13020-13030.
69. Dodd, M. C.; Kohler, H.-P. E.; von Gunten, U., Oxidation of Antibacterial Compounds by Ozone and Hydroxyl Radical: Elimination of Biological Activity during Aqueous Ozonation Processes. *Environmental Science & Technology* **2009,** *43* (7), 2498-2504.
70. Simazaki, D.; Fujiwara, J.; Manabe, S.; Matsuda, M.; Asami, M.; Kunikane, S., Removal of selected pharmaceuticals by chlorination, coagulation-sedimentation and powdered activated carbon treatment. *Water science and technology : a journal of the International Association on Water Pollution Research* **2008,** *58* (5), 1129-35.
71. Acero, J. L.; Benitez, F. J.; Real, F. J.; Roldan, G.; Rodriguez, E., Chlorination and bromination kinetics of emerging contaminants in aqueous systems. *Chemical engineering journal* **2013,** *219*, 43-50.
72. Buth, J. M.; Ross, M. R.; McNeill, K.; Arnold, W. A., Removal and formation of chlorinated triclosan derivatives in wastewater treatment plants using chlorine and UV disinfection. *Chemosphere* **2011,** *84* (9), 1238-1243.
73. Li, Z.; Fenet, H.; Gomez, E.; Chiron, S., Transformation of the antiepileptic drug oxcarbazepine upon different water disinfection processes. *Water research* **2011,** *45* (4), 1587-1596.
74. Bulloch, D. N.; Nelson, E. D.; Carr, S. A.; Wissman, C. R.; Armstrong, J. L.; Schlenk, D.; Larive, C. K., Occurrence of halogenated transformation products of selected pharmaceuticals and personal care products in secondary and tertiary treated wastewaters from southern California. *Environ Sci Technol* **2015,** *49* (4), 2044-51.

75. Bulloch, D. N.; Lavado, R.; Forsgren, K. L.; Beni, S.; Schlenk, D.; Larive, C. K., Analytical and Biological Characterization of Halogenated Gemfibrozil Produced through Chlorination of Wastewater. *Environmental Science & Technology* **2012,** *46* (10), 5583-5589.
76. Volkmann, H.; Schwartz, T.; Bischoff, P.; Kirchen, S.; Obst, U., Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). *Journal of microbiological methods* **2004,** *56* (2), 277-286.
77. Li, M.; Wei, D.; Du, Y., Genotoxicity of quinolone antibiotics in chlorination disinfection treatment: formation and QSAR simulation. *Environmental Science and Pollution Research* **2016,** *23* (20), 20637-20645.
78. El Najjar, N. H.; Deborde, M.; Journel, R.; Leitner, N. K. V., Aqueous chlorination of levofloxacin: kinetic and mechanistic study, transformation product identification and toxicity. *Water research* **2013,** *47* (1), 121-129.
79. Li, M.; Wei, D.; Du, Y., Acute toxicity evaluation for quinolone antibiotics and their chlorination disinfection processes. *Journal of Environmental Sciences* **2014,** *26* (9), 1837-1842.
80. Li, L.; Wei, D.; Wei, G.; Du, Y., Transformation of cefazolin during chlorination process: Products, mechanism and genotoxicity assessment. *Journal of hazardous materials* **2013,** *262*, 48-54.
81. Gudaganatti, M. S.; Hanagadakar, M. S.; Kulkarni, R. M.; Malladi, R. S.; Nagarale, R. K., Transformation of levofloxacin during water chlorination process: kinetics and pathways. *Progress in Reaction Kinetics and Mechanism* **2012,** *37* (4), 366-382.
82. Dodd, M. C.; Huang, C.-H., Aqueous chlorination of the antibacterial agent trimethoprim: Reaction kinetics and pathways. *Water Research* **2007,** *41* (3), 647-655.
83. Göbel, A.; Thomsen, A.; McArdell, C. S.; Joss, A.; Giger, W., Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental science & technology* **2005,** *39* (11), 3981-3989.
84. Lindberg, R. H.; Wennberg, P.; Johansson, M. I.; Tysklind, M.; Andersson, B. A., Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environmental science & technology* **2005,** *39* (10), 3421-3429.
85. Wu, Z.; Fang, J.; Xiang, Y.; Shang, C.; Li, X.; Meng, F.; Yang, X., Roles of reactive chlorine species in trimethoprim degradation in the UV/chlorine process: Kinetics and transformation pathways. *Water research* **2016,** *104*, 272-282.
86. Eliopoulos, G.; Wennersten, C., Antimicrobial activity of quinupristin-dalfopristin combined with other antibiotics against vancomycin-resistant enterococci. *Antimicrobial agents and chemotherapy* **2002,** *46* (5), 1319-1324.
87. Ternes, T. A., Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. *TrAC Trends in Analytical Chemistry* **2001,** *20* (8), 419-434.
88. Hartig, C.; Storm, T.; Jekel, M., Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A* **1999,** *854* (1), 163-173.

89. Adams, C.; Wang, Y.; Loftin, K.; Meyer, M., Removal of antibiotics from surface and distilled water in conventional water treatment processes. *Journal of environmental engineering* **2002,** *128* (3), 253-260.
90. Huber, M. M.; Canonica, S.; Park, G.-Y.; Von Gunten, U., Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. *Environmental science & technology* **2003,** *37* (5), 1016-1024.
91. Dodd, M. C.; Huang, C.-H., Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways. *Environmental science & technology* **2004,** *38* (21), 5607-5615.
92. Golet, E. M.; Xifra, I.; Siegrist, H.; Alder, A. C.; Giger, W., Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental science & technology* **2003,** *37* (15), 3243-3249.
93. Hartmann, A.; Golet, E.; Gartiser, S.; Alder, A.; Koller, T.; Widmer, R., Primary DNA damage but not mutagenicity correlates with ciprofloxacin concentrations in German hospital wastewaters. *Archives of environmental contamination and toxicology* **1999,** *36* (2), 115-119.
94. Wang, H.; Hu, C.; Liu, L.; Xing, X., Interaction of ciprofloxacin chlorination products with bacteria in drinking water distribution systems. *Journal of Hazardous Materials* **2017,** *339*, 174-181.
95. Yassine, M. H.; Rifai, A.; Hoteit, M.; Mazellier, P., Study of the degradation process of ofloxacin with free chlorine by using ESI-LCMSMS: Kinetic study, by-products formation pathways and fragmentation mechanisms. *Chemosphere* **2017,** *189*, 46-54.
96. Esomeprazole, O.; Levocetirizine, C.; Levofloxacin, O.; Escitalopram, C.; Destroketoprofen, K.; Destroibuprofen, I., RACEMIC DRUGS AND ENANTIOMERS Identifying the really useful innovations. *trials 2*, 3.
97. Odenholt, I.; Löwdin, E.; Cars, O., Bactericidal effects of levofloxacin in comparison with those of ciprofloxacin and sparfloxacin. *Clinical microbiology and infection* **1998,** *4* (5), 264-270.
98. Wendelken, S. C., EPA Method 542: Determination of Pharmaceuticals and Personal Care Products in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). **2016**, 542-1-542-35.
99. Jones, O.; Voulvoulis, N.; Lester, J., Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water research* **2002,** *36* (20), 5013-5022.
100. Zeng, G.; Zhang, C.; Huang, G.; Yu, J.; Wang, Q.; Li, J.; Xi, B.; Liu, H., Adsorption behavior of bisphenol A on sediments in Xiangjiang River, Central-south China. *Chemosphere* **2006,** *65* (9), 1490-1499.
101. Torniainen, K.; Tammilehto, S.; Ulvi, V., The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin. *International journal of pharmaceutics* **1996,** *132* (1-2), 53-61.
102. Gauthier, H. Biodegradation of pharmaceuticals by microorganisms. McGill University Montreal, 2008.
103. Finney, D. J., Probit Analysis. Cambridge University Press, 1977.
104. Henze, M.; van Loosdrecht, M. C.; Ekama, G. A.; Brdjanovic, D., *Biological wastewater treatment*. IWA publishing: 2008.

105. Stumm, W.; Morgan, J. J., *Aquatic chemistry; an introduction emphasizing chemical equilibria in natural waters*. 1970.
106. Boreen, A. L.; Arnold, W. A.; McNeill, K., Photochemical fate of sulfa drugs in the aquatic environment: sulfa drugs containing five-membered heterocyclic groups. *Environmental science & technology* **2004,** *38* (14), 3933-3940.
107. Perrin, D. D., *Dissociation constants of organic bases in aqueous solution: supplement 1972*. Butterworths: 1972.
108. Nowara, A.; Burhenne, J.; Spiteller, M., Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. *Journal of Agricultural and Food Chemistry* **1997,** *45* (4), 1459-1463.
109. Ahmad, I.; Bano, R.; Sheraz, M. A.; Ahmed, S.; Mirza, T.; Ansari, S. A., Photodegradation of levofloxacin in aqueous and organic solvents: a kinetic study. *Acta Pharmaceutica* **2013,** *63* (2), 223-229.
110. Tolls, J., Sorption of veterinary pharmaceuticals in soils: a review. *Environmental science & technology* **2001,** *35* (17), 3397-3406.
111. Lennernäs, H.; Palm, K.; Fagerholm, U.; Artursson, P., Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo. *International journal of pharmaceutics* **1996,** *127* (1), 103-107.
112. Trimethoprim. https://pubchem.ncbi.nlm.nih.gov/compound/5578 (accessed Jan. 15).
113. Jewell, K. S.; Castronovo, S.; Wick, A.; Falås, P.; Joss, A.; Ternes, T. A., New insights into the transformation of trimethoprim during biological wastewater treatment. *Water research* **2016,** *88*, 550-557.
114. sulfamethoxazole and trimethoprim, B., Bactrim DS https://www.medicinenet.com/sulfamethoxazole_and_trimethoprim/article.htm (accessed Jan. 15).
115. Calderón, C. B.; Sabundayo, B. P., 2 Antimicrobial Classifications. *Antimicrobial Susceptibility Testing Protocols* **2007**, 7.
116. Wan, Y.; Jia, A.; Zhu, Z.; Hu, J., Transformation of tetracycline during chloramination: Kinetics, products and pathways. *Chemosphere* **2013,** *90* (4), 1427-1434.
117. Yang, J.; Lin, Y.; Yang, X.; Ng, T. B.; Ye, X.; Lin, J., Degradation of tetracycline by immobilized laccase and the proposed transformation pathway. *Journal of Hazardous Materials* **2017,** *322*, 525-531.
118. Wang, P.; He, Y.-L.; Huang, C.-H., Reactions of tetracycline antibiotics with chlorine dioxide and free chlorine. *water research* **2011,** *45* (4), 1838-1846.
119. Jeong, J.; Song, W.; Cooper, W. J.; Jung, J.; Greaves, J., Degradation of tetracycline antibiotics: mechanisms and kinetic studies for advanced oxidation/reduction processes. *Chemosphere* **2010,** *78* (5), 533-540.
120. Vartanian, V. H.; Goolsby, B.; Brodbelt, J. S., Identification of tetracycline antibiotics by electrospray ionization in a quadrupole ion trap. *Journal of the American Society for Mass Spectrometry* **1998,** *9* (10), 1089-1098.
121. Snieckus, V., Directed ortho metalation. Tertiary amide and O-carbamate directors in synthetic strategies for polysubstituted aromatics. *Chemical Reviews* **1990,** *90* (6), 879-933.
122. Clive, D., Chemistry of tetracyclines. *Quarterly Reviews, Chemical Society* **1968,** *22* (4), 435-456.

123. Søeborg, T.; Ingerslev, F.; Halling-Sørensen, B., Chemical stability of chlortetracycline and chlortetracycline degradation products and epimers in soil interstitial water. *Chemosphere* **2004,** *57* (10), 1515-1524.
124. Dürckheimer, W., Tetracyclines: Chemistry, Biochemistry, and Structure‐Activity Relations. *Angewandte Chemie International Edition in English* **1975,** *14* (11), 721-734.
125. Chen, W.-R.; Huang, C.-H., Transformation kinetics and pathways of tetracycline antibiotics with manganese oxide. *Environmental Pollution* **2011,** *159* (5), 1092-1100.
126. Abia, L.; Armesto, X.; Canle, M.; Garcia, M.; Santaballa, J., Oxidation of aliphatic amines by aqueous chlorine. *Tetrahedron* **1998,** *54* (3), 521-530.
127. DeLaat, J.; Merlet, N.; Dore, M., Chlorination of Organic Compounds: Chlorine Demand and Reactivity in Relationship to the Trihalomethane Formation. Incidence of Ammoniacal Nitrogen(Chloration De Composes Organiques: Demande En Chlore Et Reactivite Vis-a-Vis De La Formation Des Trihalomethanes. Incidence De L'Azote Ammoniacal). *Water Research* **1982,** *16* (10).
128. Nelson, M.; Hillen, W.; Greenwald, R. A., *Tetracyclines in Biology, Chemistry and Medicine*. Birkhäuser Basel: 2012.
129. Hooper, D.; Wolfson, J.; Ng, E.; Swartz, M., Mechanisms of action of and resistance to ciprofloxacin. *The American journal of medicine* **1987,** *82* (4A), 12-20.
130. Hooper, D. C.; Wolfson, J. S., The fluoroquinolones: pharmacology, clinical uses, and toxicities in humans. *Antimicrobial agents and chemotherapy* **1985,** *28* (5), 716.
131. Paul, T.; Dodd, M. C.; Strathmann, T. J., Photolytic and photocatalytic decomposition of aqueous ciprofloxacin: transformation products and residual antibacterial activity. *Water Research* **2010,** *44* (10), 3121-3132.
132. Haddad, T.; Kümmerer, K., Characterization of photo-transformation products of the antibiotic drug Ciprofloxacin with liquid chromatography–tandem mass spectrometry in combination with accurate mass determination using an LTQ-Orbitrap. *Chemosphere* **2014,** *115*, 40-46.
133. Dodd, M. C.; Shah, A. D.; von Gunten, U.; Huang, C.-H., Interactions of fluoroquinolone antibacterial agents with aqueous chlorine: reaction kinetics, mechanisms, and transformation pathways. *Environmental Science & Technology* **2005,** *39* (18), 7065-7076.
134. ZHOU, H.; OUYANG, Q.; PENG, M.; LI, W., Investigation on the Reaction of Ciprofloxacin and Chlorine [J]. *Acta Scientiarum Naturalium Universitatis Sunyatseni* **2011,** *1*, 019.
135. Hooper, D. C., Expanding uses of fluoroquinolones: opportunities and challenges. *Annals of internal medicine* **1998,** *129* (11_Part_1), 908-910.
136. Dooley, K. E.; Obuku, E. A.; Durakovic, N.; Belitsky, V.; Mitnick, C.; Nuermberger, E. L.; Efficacy Subgroup, R.-T., World Health Organization group 5 drugs for the treatment of drug-resistant tuberculosis: unclear efficacy or untapped potential? *The Journal of infectious diseases* **2012,** *207* (9), 1352-1358.
137. Kaur, A.; Salunke, D. B.; Umar, A.; Mehta, S. K.; Sinha, A.; Kansal, S. K., Visible light driven photocatalytic degradation of fluoroquinolone levofloxacin drug using Ag 2 O/TiO 2 quantum dots: a mechanistic study and degradation pathway. *New Journal of Chemistry* **2017,** *41* (20), 12079-12090.

138. Hapeshi, E.; Fotiou, I.; Fatta-Kassinos, D., Sonophotocatalytic treatment of ofloxacin in secondary treated effluent and elucidation of its transformation products. *Chemical engineering journal* **2013,** *224*, 96-105.
139. Tay, K. S.; Madehi, N., Ozonation of ofloxacin in water: by-products, degradation pathway and ecotoxicity assessment. *Science of the Total Environment* **2015,** *520*, 23-31.
140. El Najjar, N. H.; Touffet, A.; Deborde, M.; Journel, R.; Leitner, N. K. V., Levofloxacin oxidation by ozone and hydroxyl radicals: kinetic study, transformation products and toxicity. *Chemosphere* **2013,** *93* (4), 604-611.
141. Asif, M., A Mini Review on the Study of New Broad-Spectrum Antimicrobial Fluoroquinolone JNJ-Q2. *J Chem Applications* **2014,** *1* (1), 5.