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Detection of Pharmaceuticals, Personal Care Products (PPCPS) and Illicit Drugs in Wastewater Treatment Plants and Urban River Systems

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DETECTION OF PHARMACEUTICALS, PERSONAL CARE PRODUCTS (PPCPs) AND ILLICIT DRUGS IN WASTEWATER TREATMENT PLANTS AND URBAN RIVER SYSTEMS

by

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ABSTRACT

DETECTION OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS (PPCPS) AND ILLICIT DRUGS IN WASTEWATER TREATMENT PLANTS AND URBAN RIVER SYSTEMS

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Pharmaceuticals, personal care products (PPCPs) and illicit drugs are a threat to human health and the aquatic environment globally. Their usage and consumption is rapidly increasing potentially due to an aging population, the development of new drugs, the overprescribing of prescription drugs and easier accessibility of drugs legally and illegally prescribed. These compounds enter wastewater treatment plants influent through urine or feces, pass through the stages of treatment with some compounds not being removed and ending up in the effluent and ultimately in the aquatic environment. In addition to human consumption, PPCPs are introduced into the environment through veterinary use from livestock, and where concentrated animal feeding operations (CAFOs) are located. Wastewater treatment plants (WWTPs) effluent are the major source of these compounds into the environment. The wastewater effluent is discharged into rivers, streams, or lake systems, and the biosolids are spread on fields for fertilizer. WWTPs are known to not adequately remove these compounds and as a result there is a continuous supply of these compounds to the environment. There are few studies on the long-term effects of these compounds in the environment. The contaminants most often detected in wastewater

treatment effluent are antibiotics, epileptic drugs, analgesics, herbicides, opioids, and recreational drugs. This dissertation uses an alternative extraction method compared to the standard solid phase extraction (SPE) method used in most of the literature. In Chapters 2 and 3, sixty diverse compounds were analyzed from samples taken from influent, effluent, surface water and sludge from two WWTPs that discharge their effluent into the largest freshwater lake in the State of Wisconsin. Data were further analyzed by percent remaining, removal efficiency and seasonality. Chapter 4 surveyed PPCPs and illicit drugs from six urban river systems, using the alternative extraction method from Chapters 2 and 3. In addition, removal efficiency, percent remaining, and seasonality were also studied. There is an increasing trend towards urbanization and lifestyle changes, increasing health ailments such as cardiovascular disease, diabetes, and illicit drug use. With rapid urbanization, brings about an increase in contamination of water. The PPCPs and illicit drugs may be transferred to the water by rainfall, climate changes, infrastructure breakdown of private sceptic systems, and release of gray water. Stressors in the surrounding water environment may be channel form, cement encasements, the type of community surrounding the water whether it be industrial, manufacturing, agricultural, retail, etc.

The alternative extraction method was successful in detecting the 60 diverse compounds but there were limitations on percent recovery values for certain compounds.

Dedication

I dedicate this dissertation to my late husband Charles “Chuck” F Seaman. He supported me 100% in this undertaking. It is bittersweet that he could not be present for my graduation. He would have been so proud.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

PPCPs	pharmaceuticals and personal care products
WWTPs	wastewater treatment plants
DAFT	dissolved air flotation thickeners
WAS	waste activated scum
UV	ultraviolet
COD	chemical oxygen demand
F	Fahrenheit
ECs	emerging contaminants
CAFOs	concentrated animal feeding operations
RAS	return activated sludge
BOD	biochemical oxygen demand
NH_3	ammonia nitrogen
NO_3-N	nitrate nitrogen
NO_2–N	nitrate nitrogen
N_2	nitrogen gas
PO_4^{-3}	ortho phosphate
$(NH_4)MgPO_4$-$6H_2O$)	struvite

DBPs	disinfection by-products
THMs	trihalomethanes
HAAs	haloacetic acids
NaCIO	sodium hypochlorite
EBPR	enhanced biological phosphorus removal
PAO	polyphosphate accumulating organisms
PHB	poly-hydroxybutyrate
nm	nanometer
PAA	peracetic acid
mg/L	milligrams per liter
NSAIDs	non-steroidal anti-inflammatory drugs
OCI^-	hypochlorite ion
OCI_3^-	chlorate
OCI_2^-	dichlorine monoxide
OH	hydroxyl
O_3	ozone
USGS	United States Geological Survey
LC-MS/MS	liquid chromatography mass spectrometry

pK_a	acid-base dissociation constant
Log K_{ow}	octanol-water partition coefficient
$\mu g/L^{-1}$	micrograms per liter
THC-COOH	11-nor-delta9-THC-9-carboxy
BE	benzoylecgonine
AM	amphetamine
MET	methamphetamine
K_H	Henry's Law constant
VOCs	volatile organic compounds
UV/H_2O_2	ultraviolet hydrogen peroxide
H_2O_2	hydrogen peroxide
mm	millimeter
O_3/UV	ozone/ultraviolet radiation
DNA	deoxyribonucleic acid
K_d	dissociation constant
CNS	central nervous system
6MAM	6-monoacetylmorphine
Km	kilometer

^{15}N	isotope of nitrogen
PVC	polyvinyl chloride
CDC	Center for Disease Control
UNODC	United Nations Office on Drugs & Crime
SPE	solid phase extraction
ANOVA	analysis of variance
CMS	Centers for Medicare & Medicaid Services
DWTP	drinking water treatment plant
C	celsius
LC	liquid chromatography
MRM	multiple reaction monitoring
m/z	mass to charge ratio
R^2	correlation coefficient
ng/mg	nanogram per milligram
4-ANPP	4-anilino-N-phenethylpiperidine
IQVIA	IMS Quintiles VIA
m	meter
ACN	acetonitrile

mgd	million gallons per day
µL/sec	microliters per second
US	United States
µm	micrometer
mg	milligrams
HRT	hydraulic retention time
TP	total phosphorus
TN	total nitrogen
P	phosphorus
SH_5	sulfur
NH_2	amino group
-COOH	carboxyl group

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Chapter 1:

Pharmaceuticals and personal care products (PPCPs), opioids and recreational drugs are entering the environment through wastewater treatment plant effluent.

Introduction

The presence of pharmaceuticals and personal care products (PPCPs), opioids and recreational drugs in the environment represent a growing problem globally and impacts economic, ecological, and human and animal health-related issues. Most wastewater treatment plants use technologies that focus on removing bulk solids and returning the liquid components to a non-infectious condition. They are not required to test for the presence of PPCPs, opioids or recreational drugs in the waste stream. Consequently, these chemicals may end up in effluent and ultimately surface water.

General Overview of Wastewater Treatment Plants

Wastewater treatment plant (WWTP) effluent from domestic, livestock, industrial and hospitals is the major pathway for pharmaceuticals, personal care products (PPCPs), opioids and recreational drugs to enter surface waters. WWTP processes are not designed to remove these emerging contaminants (ECs) from their effluent. ECs cover a broad spectrum of compounds such as PPCPs, organic wastewater compounds, antimicrobials, antibiotics, animal and human hormones, endocrine disrupting compounds, and detergents which all have the potential to impact the environment. Not all PPCPs, opioids and recreational drugs are fully metabolized by the liver (Jones et al., 2005). Excretion of these drugs through urine and/or feces results in them entering the WWTP where they may enter the environment through discharge of effluent (Jones et al., 2005). An indirect route for PPCPs, opioids and recreational drugs is flushing them down the toilet which eventually proceeds to the sewer system and into the WWTP (Daughton and

Ruhoy, 2009). Other ways that these drugs may enter the aquatic environment are from agricultural practices, disposal of sewage sludge, veterinary purposes, private septic systems or leaching to groundwater after a rain event (Kümmerer, 2009). Unfortunately, today these drugs may be found in sediment, medical sewage, WWTPs, surface water, groundwater, drinking water, run off from concentrated animal feeding operations (CAFOs), and in the Arctic and Antarctic environments (Fatta-Kassinos et al., 2011b; Gonzalez Alonso et al., 2017; Stroski et al., 2020). CAFOs are on the increase and present a greater risk to water quality because of more waste being produced, as well as the contaminants found in the waste such as antibiotics. Contaminants can enter the surface waters through leakage from poorly constructed manure lagoons, overflow of lagoons during rain events or runoff from applications of the waste to farm fields (Aneja, 2003, Bradford, 2008). Antibiotics are commonly used to prevent infections in animals as well are used to promote rapid growth in livestock (Cromwell, 2002, Gaskins, 2002, Liu, 2005). Approximately one-third of the antibiotics used in the United States yearly is added to animal feed to increase growth. Safe treatment and disposal of sludge is an environmental concern. Land application of sludge is becoming more frequent instead of landfilling or incineration (Hoang, 2022). Sludge is a by-product of wastewater treatment and exists in a semi-solid or insoluble solid form (Zhang, 2017). Sludge contains a large amount of water (97 to 98%) which complicates the disposal (Kacprzak, 2017). Contaminants such as heavy metals, pathogens, persistent organic pollutants and other toxic elements may be found in sewage sludge, as well as valuable nutrients such as phosphorus and nitrogen (Fijałkowski, 2017). These toxic substances are a major concern for land application of sewage sludge (Hoang, 2022, Ye, 2022). Proper sanitation is a global problem. This may be due to poor design or lack of commitment to maintain infrastructure which leads to pollution discharge into surface and groundwaters (Wear,

2021). More than 1.2 trillion gallons of untreated sewage, stormwater, and industrial waste flow into United States rivers annually because of overloaded treatment systems (Ahuja, 2014). This can impact natural habitats and biodiversity.

Modern sewage systems came about in the mid-nineteenth century because of the advent of unsanitary conditions from heavy industrialization and urbanization. The cholera outbreaks that took place in 1832, 1849, and 1855 in London killed tens of thousands of people due to contaminated water supplies (Angelakis, 2018). Sewage farms, in which wastewater was applied to land for agricultural use first began in Poland in 1531 and later in Scotland in 1650 (Angelakis, 2018, Tzanakakis, 2020). Eventually large sewage farms were established in Europe and the United States at the end of the eighteenth century and in Australia at the end of the nineteenth century (Tzanakakis, 2014). The twentieth century brought about WWTPs that could handle large volumes of wastewater that could be discharged into waterways (Angelakis, 2018), and were soon adopted globally in major urban areas since they were compact and did not require large areas for treatment compared to sewage farms (Jiménez, 2015, Lazarova, 2013).

The purpose of wastewater treatment is to remove contaminants in water that may pose a threat to human health and the environment if discharged into surface waters without the proper treatment (Jasim, 2020). Conventional wastewater treatment consists of a combination of physical and biological processes to remove solids, organic matter and nutrients from wastewater comprised of primary, secondary and tertiary processes and treatment of the sludge (grégorio, 2018, Kesari, 2021). Characteristics of the wastewater depend on community use, type of industries present, content of the solids which may consist of floating matter, sediment, suspended material and soluble matter. Wastewater is generated from many different sources such as toilet water, washing clothes, bathing, rainwater, etc. Typical influent is usually less than

95% water, usually gray in color and has a foul-smelling odor (Jasim, 2020, grégorio, 2018). The diversity of the wastewater generates different types of contaminants. These contaminants may be organic matter, salts, ammonia, pesticides, pharmaceuticals and personal care products, metabolites, etc. Heavy metals and pathogens are the most serious of the pollutants (Jasim, 2020). The wastewater treatment process starts with preliminary treatment which is a physical and mechanical process. This initial step removes gross, suspended, and floating solids in which the influent flows through a bar screen. These large items (sticks, plastics, diapers, broken bottles, large stones, etc.) may clog pipes, obstruct water flow, or damage pumps and valves (Khiewwijit, 2015). After the screening process, the smaller particles enter primary treatment which consists of an aerated grit chamber and primary clarifiers where heavy solids settle out (gravel, sand) by gravity and are removed by large mechanical scrapers into a hopper, then pumped to a sludge processing area where oil, grease and other floating materials are skimmed from the surface. The preliminary and primary treatment processes remove about 25% of organic matter load and inorganic solids in addition to the water containing industrial materials (Jasim, 2020). The overall objective of the preliminary and primary treatment processes is to obtain a homogeneous liquid and to transfer this liquid to the next stage of treatment which is secondary treatment and is a biological treatment. The purpose of secondary treatment is to remove soluble organics that aren't removed in primary treatment and to remove suspended solids. Approximately 85% of the organics is removed by secondary treatment (Jasim, 2020, Kesari, 2021). The wastewater entering secondary treatment is called "mixed liquor" which is a combination of primary effluent and return activated sludge (RAS). The secondary biological treatment (activated sludge) process involves aeration and seeding the wastewater with microorganisms which will consume the organic matter. A portion of the organic material is

oxidized by microorganisms to produce carbon dioxide and other end products, and the remainder provides the energy and materials to sustain the microorganism community (Trikoilidou, 2016). The microorganisms present in secondary treatment are comprised of bacteria, Protozoa and Metazoa. Bacteria dominate all other groups in number and biomass and affect the process of mineralization and elimination of organic and inorganic nutrients (Madoni, 2011). The Protozoa help maintain balance by grazing excess bacteria and stimulate growth and promote flocculation (Motta, 2001). The Protozoans'major role in wastewater treatment is the clarification of the effluent (Curds, 1970, Madoni, 2003). The three major categories of Protozoa are the flagellates, sarcodines and ciliates (Amaral, 2004). The ciliates are the most numerous and feed on bacteria, while other ciliates may feed on other ciliates or flagellates. The ciliates are divided into three groups: free swimming (swim in the mixed liquor phase), crawling (live on the surface of the sludge flocs) and sessile (attach to the sludge via a stalk structure) (Amaral, 2004, Motta, 2001, Madoni, 2011). Examples of Metazoas are rotifers and nematodes primarily *Gastrotrichia* and *Oligotricihia* respectively (Motta, 2001). The succession of Protozoa in secondary treatment are that flagellates predominate in the early stages because they have low energy requirements, and as they decrease, the free-swimming ciliates take over and then are replaced by the crawling and attached ciliates (Madoni, 2011).

Nitrogen and Phosphorus Removal in Secondary Treatment

The secondary treatment process involves the removal of nitrogen and phosphorus to prevent eutrophication in surface water that may affect the biodiversity of aquatic ecosystems and promote algal blooms. Algae that form algal blooms release algal toxins that may harm fish and illness in human and other animals. When algal blooms decay, this results in oxygen deficiency in the aquatic environment and may result in decreased biodiversity (Sowmya, 2013).

Secondary treatment removes most nitrogen and phosphorus, but some may remain in the effluent in which case tertiary or advanced treatment removes the excess. Nitrogen in *P. Fluorescnes* wastewater may be in the forms of organic nitrogen, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. Ammonia nitrogen and organic nitrogen are the main forms (Bouraoui, 2014). The process of nitrogen removal involves 3 stages: ammonification, nitrification, and denitrification (Rout, 2021, Semerci, 2016, Liu, 2020). Ammonification is a fast process where organic nitrogen is converted to ammonia nitrogen by ammonifying bacteria (Rout, 2021). Nitrification involves aerobic gram-negative microorganisms such as *Nitrosomonas* and *Nitrobacter* that use the energy released in the nitrification process for growth (Liu, 2020). Ammonia nitrogen (NH_3) is converted to nitrite nitrogen (NO_2^--N) by nitrobacteria, and then nitrite nitrogen is converted into nitrate nitrogen (NO_3^--N) (Kumar, 2010, Rout, 2021, Semerci, 2016). Microorganisms get energy from oxidation of ammonia nitrogen and nitrite nitrogen, and carbon from inorganic carbon compounds (Kumar, 2010). Denitrification involves enzymes such as nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase which catalyze nitrate to nitrogen gas (Ren, 2020, Ding, 2019). This process involves facultative heterotrophic bacteria, such as *Pseudomonas, P. denitrificans, Bacillus and Paracoccus* (Rout, 2021) and uses organic carbon as an electron donor (Winkler, 2011, Semedo, 2018) and nitrate or nitrite as electron acceptors (Semerci, 2016) to convert nitrite nitrogen to N_2 gas by anaerobic respiration under anoxic conditions (dissolved oxygen < 0.5 mg/L) (Winkler, 2011).

Phosphorus effluent release into the aquatic environment promotes eutrophication. As a result, there are strict phosphorus discharge standards (>0.5-1 mg/L) to reduce the phosphorus load entering the aquatic environment (Bowes, 2015, Parsons, 2008, Xiong, 2011, Chen, 2018).

Phosphorus is the limiting nutrient for algae in surface waters. The discharge of phosphorus into receiving waters increases the potential for algal growth which initializes eutrophication (Gu, 2011, Xiong, 2011, Parsons, 2008, Pinelli, 2022, Yeoman, 1988). Chemical precipitation is the most common method to remove phosphorus from wastewater and then through sludge discharge (Qin, 2015, Peng, 2018, Munir, 2019, Gu, 2011, Parsons, 2008, Yeoman). Chemical precipitation removes inorganic forms of phosphate by the addition of coagulants such as metal salts (calcium, aluminum, and iron) (Chu, 2018, Munir, 2019, Gu, 2011, Parsons, 2008, Jupp, 2021, Yeoman, 1988). Primary precipitation entails the addition of chemicals before sedimentation and the precipitate ends up in primary sludge. Secondary precipitation is where the iron or aluminum salts are directly added into the aeration tank of the activated sludge process with phosphorus ending up in secondary sludge (Jupp, 2021). Total phosphorus in secondary effluent can be divided into ortho-phosphate ($PO4^{-3}$), metal bound phosphorus, dissolved organic phosphorus and particulate organic phosphorus (Scherrenberg, 2008). Scherrenberg studied the composition of phosphorus in secondary effluent and found ortho-phosphate, dissolved organic phosphorus, metal bound phosphorus and particulate organic phosphorus accounted for 75.4%, 16.4%, 4.1% and 4.1%, respectively in secondary effluent, which indicated that dissolved phosphorus was the dominate form of phosphorus in secondary effluent. Dissolved phosphorus precipitate is converted to solids which are removed with the sludge (Gu, 2011). Chemical precipitation methods generate up to 95% more sludge and contain more inert material such as Fe phosphate and less soluble phosphorus (Yeoman, 1988). This composition may have an impact on the downstream sludge treatment processes, mainly anaerobic digestion (Yeoman, 1988). The anaerobic digestion process converts sludge into biogas and methane. The sludge generated from chemical precipitation results in approximately 12% less biogas and 8% less

methane compared to sludge not chemically treated (Parsons, 2008). Chemical precipitation produces phosphorus bound as a metal salt within the sludge and is applied to agricultural fields as fertilizer (Morse, 1998). Lime is the most common calcium salt used for phosphorus precipitation. Calcium precipitates bicarbonate from carbon dioxide and any excess calcium (Parsons, 2008). Biological methods, such as enhanced biological phosphorus removal (EBPR), may also be used for phosphorus removal from wastewater. This method is considered environmentally friendly, cost-effective and used globally. It is achieved through the uptake of polyphosphate, orthophosphate, and organically bound phosphorus into cell biomass and then wasting the biomass by desludging (Rout, 2021). EBPR depends on polyphosphate accumulating organisms (PAOs) such as *Accumulibacter*, and *Acinetobacter* sp.) in secondary treatment to remove phosphorus from the water (de-Bashan, 2004). This process requires the combination of both anaerobic and aerobic stages to encourage phosphorus uptake (Parsons, 2008, Gu, 2011). Anaerobic conditions lack dissolved oxygen and oxidized forms of nitrogen (nitrate and nitrite). PAOs are not able to grow under anaerobic conditions but can accumulate and store organic substrate by converting organic acids to poly-hydroxy butyrate (PHB) and other energy rich organic compounds (Gu, 2011, Rashed, 2014, Zahed, 2022). The bacteria gain energy under anaerobic conditions from the conversion of stored energy rich polyphosphate to dissolved phosphate which is released to the water (Gu, 2011). The substrate storage process under anaerobic conditions is controlled by the energy stored in the bacteria in the form of polyphosphates (Gu, 2011). The polyphosphate energy is recharged under aerobic conditions (Zahed, 2022). Wastewater is usually reported as to its organic strength (total concentration of organic material) as either biological oxygen demand (BOD) or as chemical oxygen demand (COD) (Parsons, 2008). Conventional biological treatment may remove only 20% of the

phosphorus present, whereas PAOs can sequester phosphate as intracellular polyphosphate in excess (luxury uptake) of their biological need and 90% of the phosphorus may be removed (Jupp, 2021, Parsons, 2008, Morse, 1998). Sludge usually contains about 2 to 3% of phosphorus, in contrast to post-EBPR phosphorus in which contents range from 4% up to12% (Jupp, 2021).

Other phosphorus recovery methods are the production of recyclable compounds such as struvite, and calcium phosphate (Le Corre, 2007). Struvite [$(NH_4)MgPO_4$-$6H_20$)] is a salt comprised of magnesium, ammonium and phosphate in equal molar concentrations (Jupp, 2021, Parsons, 2008). Precipitation of struvite can remove 80 to 90% of soluble phosphate (Le Corre, 2009). Struvite produces a good fertilizer because it has low solubility and prolongs nutrient release during the growing season (Johnston, 2003, Talboys, 2016). One disadvantage of using struvite for the recycling of phosphorus is the cost of producing struvite compared to its fertilizer value (Parsons, 2008). The advantage of using struvite is the savings attained because of decreased costs of sludge handling and removal (Shu, 2006). The challenge of using struvite in for phosphorus recovery in wastewater occurs when conditions are less than 50 mg/L and suspended solids are above 2000 mg/L (Mehta, 2015, Wong, 2013).

Tertiary or advanced wastewater treatment is used when receiving water conditions or other uses require higher quality effluent than what is produced by secondary wastewater treatment. Secondary treatment removes most of the organic content from wastewater, residual pollutants such as inorganic nutrients like nitrogen and phosphorus. Increased microbial content and emerging contaminants (e.g., pharmaceuticals and illicit drugs) may have a significant impact on the final treated water (Zagklis, 2022). Disinfection is the most common type of tertiary treatment. This phase may involve chlorination, ultraviolet irradiation, and ozonation, to

name a few processes because of their low economical costs and high efficiency (Zagklis, 2022, Jasim, 2020, Kesari, 2021).

Disinfectants

Disinfection is the final step in treatment of wastewater. This step will render inactive any microorganisms or viruses that have continued to exist after the wastewater treatment (Núñez-Núñez et al., 2020). Chlorination or ultraviolet (UV) light are the most widely used disinfectant processes for WWTP effluents (Block et al., 2014; Chhetri et al., 2019; Collivignarelli et al., 2017; Krasner et al., 2009; Turtoi, 2013). Chlorination is effective in the removal of endocrine disrupting compounds and non-steroidal anti-inflammatory drugs (NSAIDs) including ibuprofen (Noutsopoulos, 2015). Chlorine inactivates microorganisms by damaging their cell membranes (Ghernaout, 2017). Once the cell membrane is in a weakened state, the chlorine enters the cell and disrupts respiration and DNA activity (Ghernaout, 2017). Chlorine is a strong oxidizer that reacts with organic compounds and may form mutagenic/carcinogenic disinfection by-products (DBPs) (Amin, 2013, Zagklis, 2022, Núñez-Núñez, 2020). These by-products are formed by the reaction between organic and inorganic matter and disinfectants (Doederer et al., 2014; Kozari et al., 2020) which can be toxic to aquatic organisms and human health (Chhetri et al., 2019; Collivignarelli et al., 2017; Krasner et al., 2009; Watson et al., 2012). Trihalomethanes (THMs) and haloacetic acids (HAAs) are associated with bladder, colon, stomach and rectal cancer (Amin et al., 2013b; Collivignarelli et al., 2017; Dell'Erba et al., 2007; Hajenian, 1981; Núñez-Núñez et al., 2020)Noutsopoulos, 2015). Chlorate can accumulate in chlorine treated wastewater effluents as a degradation by product. Chlorine, in the form of sodium hypochlorite (NaCIO) breaks down to hypochlorite ion (OCI^-) and the degradation of OCI^- results in the formation of chlorate (OCL_3^-) via an intermediate state

(OCl_2^-) (Zhong, 2019, Deborde, 2008). The toxicity of chlorination may be decreased somewhat due to dechlorination prior to releasing the effluent into the receiving waters, but this does not remove the disinfection by-products (Amin et al., 2013a; Watson et al., 2012) Dechlorination of wastewater effluent is more complex because the amount of total chlorine residual needs to be <0.01 $mgCl_2/L$ to decrease toxicity to the receiving waters (Sathasivan, 2017). The most common compounds used in dechlorination is sulfur dioxide, but other compounds such as sulfite compounds, activated carbon and hydrogen peroxide are used (Sathasivan, 2017). Chlorination is cost-effective compared to UV radiation or ozone disinfection. Also, chlorine residual that remains in the effluent can prolong disinfection after the initial treatment.

In a large part of the United States and Canada, ultraviolet radiation (UV) for disinfection of wastewater has become an accepted alternative to chlorination/dechlorination and does not produce by-products (Das, 2001, Lazarova, 1998). UV is emitted from the region of the spectrum beyond visible light and before X-rays (Das, 2001). The upper wavelength limit is 400 nm and the lower wavelength limit is 100 nm. The UV band of wavelengths between 200 and 300 nm is the germicidal region because this light is lethal to microorganisms, including bacteria (fecal coliforms, *Salmonella*, *Shigella* and *Escherichia coli*), protozoa, viruses, mold, yeasts, fungi, and algae (Aghalari et al., 2020; Antonelli et al., 2008; Ashok and Khedikar, 2016; Turtoi, 2013). A UV lamp contains an inert gas (e.g. argon) and a small amount of liquid mercury (Ashok and Khedikar, 2016). A voltage is applied to the lamp and some of the liquid mercury vaporizes which causes free electrons and ions to collide with the mercury atoms exciting the mercury atoms into a higher energy state (Ashok and Khedikar, 2016). Excited mercury atoms return to their normal energy state by discharging energy in the form of UV light (Ashok and Khedikar, 2016). UV radiation inactivates microorganisms' cells by damaging their nucleic acid so

replication cannot take place (Das, 2001; Turtoi, 2013). The nucleotide bases: adenine, guanine, thymine, and cytosine are strong UV absorbers, the nucleotides differing in their ability to absorb UV light (Das, 2001). The pyrimidines (thymine and cytosine) are ten times more sensitive to UV light than the purines (adenine and guanine) (Das, 2001). UV reacts with two adjacent thymine molecules to produce a thymine dimer. New bonds are formed between adjacent nucleotides, creating double molecules, or dimers particularly with thymine which is the most common form of photochemical damage (Das, 2001). Even though nucleic acid is damaged, the microorganisms can still undergo metabolism and other cell functions. This damage can be repaired by enzymes, allowing the microorganisms to repair themselves and become infectious again after a certain time from the UV treatment which may affect disinfection efficiency (Ali, 2016; Turtoi, 2013). The UV dosage must be high enough (maximum dosage of UV-C at 254 nm) to completely destroy the nucleic acid so it cannot be repaired (Amin et al., 2013a; Das, 2001; Kim and Tanaka, 2009; Turtoi, 2013). In comparison, when chemical disinfectants (such as chlorine) are used, they will destroy and damage the cellular structures that interfere with metabolism, biosynthesis and growth (Koutchma et al., 2009). Suspended solids generally are comprised of bacteria particles of varying number and size. Some suspended solids will absorb or reflect the UV light before it can penetrate the solids to kill any microorganisms (Das, 2001; Turtoi, 2013). UV light can penetrate suspended solids using longer contact times and higher lamp intensities but still not all microorganisms are destroyed (Ali, 2016; Andreadakis et al., 1999; Das, 2001). The advantage of UV radiation in addition to the inactivation of most viruses, bacteria and spores is that it is easy to operate the UV equipment , no chemicals are needed, disinfection is very fast with contact times in the range of a few seconds, no danger of overdosing, and low maintenance requirements (Ashok and Khedikar, 2016). Disadvantages of

using UV may be costly, breakage of the lamps may cause a mercury hazard, power interruptions, and the possible reactivation of pathogenic microorganisms (Aghalari et al., 2020). In addition, turbidity, suspended solids concentration, UV absorbing inorganic and organic compounds may affect disinfection efficiency modifying the radiation penetration (Jolis et al., 2001; Madge and Jensen, 2006). Wastewater treatment plant #2 studied in Chapter 2 of this dissertation is an example of one plant that uses UV radiation for disinfection.

Ozone for disinfection of wastewater is becoming a promising technology to inactivate pathogens from the wastewater treatment process. This process involves the production of highly reactive oxygen species, which attack organic compounds and microbes. Ozone is a strong oxidizing agent, effective in destroying bacteria, viruses and parasites (Lazarova et al., 2013; Paucar et al., 2019). Ozone destroys microorganisms by totally or partially lysing the cell wall. Ozone can break chromosomes, nitrogen-carbon bonds between sugar and bases, DNA hydrogen bonds, as well as phosphate sugar bonds (Lazarova et al., 2013). Ozonation produces hydroxyl (OH) radicals through the decomposition of ozone (O_3) (Rosal et al., 2010; Shahid et al., 2021). Ozone disinfection has two mechanisms: 1. a direct oxidation of compounds by the ozone molecule and 2. a reaction involving the hydroxyl radical (Lazarova et al., 2013; Reungoat et al., 2012). This radical is highly reactive and only has a life span of a few microseconds in water so the reaction depends on the wastewater characteristics (Lazarova et al., 2013). A study by Paucar (Paucar et al., 2019) on 37 PPCPs detected found 11 antibiotics were degraded by ozone at 9 mg/L and 15 minutes, trimethoprim, lincomycin and sulfadimethoxine were found to be sensitive to O_3 and sulfamethoxazole, azithromycin and clarithromycin were found to be insensitive to O_3 requiring an ozone dose of 6-9 mg/L to degrade to their limit of detection . Another study (Dodd et al., 2006) found trimethoprim and ciprofloxacin were degraded quickly

with O_3. The anticonvulsant carbamazepine was susceptible to O_3 as it was degraded at an ozone dose of 2 mg/L in 10 minutes (Ikehata et al., 2006). The PPCPs that are poorly removed by ozonation usually contain electron-withdrawing functional groups such as fluoro, nitro, chloro, amide and carboxyl groups (Hey et al., 2012; Hollender et al., 2009; Nakada et al., 2007). Carbamazepine contains an electron-withdrawing functional group (amide) but remains reactive because of its C=C double bond (Nakada et al., 2007). Ozonation creates by-products because it is a highly reactive oxidant and reacts with organic matter in the water (Park et al., 2016). Examples of DBPs from ozonation are formaldehyde and acetaldehyde and (Richardson et al., 2007) categorized them as having some or all of the toxicological features of human carcinogens. Some researchers (Rosal et al., 2010) combine ozone with hydrogen peroxide (O_3/H_2O_2) and found mineralization of dissolved carbon was improved from 15% to over 90% after one hour. Ozone decomposition into radicals is promoted by electron transfer or by the reaction of ozone with H_2O_2 (Liu et al., 2021). A combination of UV with ozone can accelerate the ozone decomposition and improve OH generation (Chen et al., 2016). The UV/ H_2O_2 process degraded carbamazepine very effectively while UV alone was not as effective for decreasing carbamazepine concentration (Vogna et al., 2004a; Vogna et al., 2004b).

Peracetic acid (PAA) is starting to be used as an alternative disinfectant in place of chlorine due to its lack of disinfection by-products formation because PAA breaks down to oxygen, water and acetic acid (Block et al., 2014; Collivignarelli et al., 2017; Dell'Erba et al., 2007). Currently, PAA is used in different industries, such as the medical and food safety areas, as a disinfectant and a sterilizing agent (Block et al., 2014). The effectiveness of PAA depends on the type of microorganisms, disinfection contact time and the quality of the wastewater effluent (Block et al., 2014). One study (Dell'Erba et al., 2007) looked at PAA in secondary

effluent to observe any formation of disinfectant by-products. They detected aldehydes, but at negligible concentrations, and no halogenated phenols were observed. Another study (Collivignarelli et al., 2017) compared sodium hypochlorite and PAA from two WWTPs in northern Italy. Results showed using the same disinfectant dosage and comparable initial *E. coli* concentration, PAA exhibited the greatest microbial removal (up to 99.99%). Acute toxicity was apparent at higher doses and with higher residuals of PAA (2.68 mg L^{-1}) compared to free residual chlorine (0.17 mg L^{-1}).

Treatment of sludge or biosolids

Sludge is a solid, semisolid, or slurry material produced as a by-product of wastewater treatment processes (Aziz and Mustafa, 2022; Fijałkowski et al., 2017a; Ye et al., 2022). Biosolids are generated from the treatment of sludge and are higher in solids concentration and lower in pathogen content and is often referred to as the cake product from the dewatering process (Aziz and Mustafa, 2022). Biosolids are the sewage sludge that has been treated using processes such as anaerobic digestion, aerobic composting and incineration (Wijesekara et al., 2021).

Sludge is comprised of a mixture of organic compounds, inorganic compounds, pathogens (Fijałkowski et al., 2017a), and nutrient-rich in phosphorus and nitrogen and contains over 90% water (Kacprzak et al., 2017). Sludge results from the primary sludge which contains settleable solids and secondary treatment processes, consists of biological solids and extra settleable solids (Aziz and Mustafa, 2022). The focus of the sludge treatment is to decrease moisture or volume content, to remove organic matter, to destroy microorganisms, and eliminate toxic materials (Aziz and Mustafa, 2022).

Anaerobic Digestion

Sludge has become an issue for WWTPs because it contains water, organics, pathogens sometimes heavy metals and other hazardous materials (Li et al., 2018). Anaerobic digestion is a process to eliminate the risk of sludge entering the environment. Anaerobic digestion is comprised of three biochemical reactions of fermentation, acetogenesis (reduction of carbon dioxide and organic acids takes place, acetate is formed by the oxidation of the products of acidogenesis by microorganisms called acetogens), and methanogenesis ((Li et al., 2018). Prior to anaerobic digestion, the sludge is thickened for the purpose of biogas production (Appels et al., 2008) to produce heat and electricity (Neves et al., 2018). Biogas is made up of 50 to 70% methane and 20 to 40% carbon dioxide (Appels et al., 2008; Neves et al., 2018). Anaerobic digestion involves microorganisms that digest the leftover organic matter without using oxygen (Forster-Carneiro et al., 2008). Four trophic groups are important to anaerobic digestion, mainly hydrolytic, acidogenic, acetogenic, and methanogenic bacteria (Kwietniewska and Tys, 2014). The first phase is the hydrolytic process which involves the breakdown of carbohydrates, lipids, and proteins into sugars, long-chain fatty acids, and amino acids by *Clostridia, Micrococci, Bacteroides, Streptococcus* etc. (Meegoda et al., 2018; Neves et al., 2018). The second phase is by the action of acidogenic fermentative microorganisms (*Streptococcus, Lactobacillus, Bacillus, Escherichia coli, Salmonella*) which convert the products of hydrolysis into fatty acids, alcohols, lactic acid, carbon dioxide, hydrogen ammonia, and hydrogen sulfide (Christy et al., 2014; Meegoda et al., 2018). The third phase involves acetogenic bacteria which convert the compounds from the second phase by producing hydrogen, carbon dioxide, and acetate (Christy et al., 2014; Meegoda et al., 2018). The final phase the methanogenic archaea breakdown organic compounds from the third phase in which either degradation to acetic acid or methanol to

promote methane and/or hydrogenotrophic which uses hydrogen and carbon dioxide to produce methane (Christy et al., 2014; Meegoda et al., 2018). Four temperature schemes may be used in anaerobic digesters for biogas formation which are 1) psychrophilic (10-20 degrees C), 2) mesophilic (20-45 degrees C), 3) thermophilic (50 to 65 degrees C), and extremely thermophilic (between 65 and 70 degrees C) (Forster-Carneiro et al., 2013; Kothari et al., 2014; Meegoda et al., 2018). Chemical and biological reactions occur slowly under psychotropic conditions and the metabolic rate of microorganisms increases under thermophilic conditions (Kothari et al., 2014; Kwietniewska and Tys, 2014). Thermophilic temperatures are commonly used in large-scale anaerobic digesters and higher rates of pathogen destruction are promoted (Kothari et al., 2014; Kwietniewska and Tys, 2014). Mesophilic temperatures are the most popular for biogas formation, allowing good digestion with lower energy costs (Kothari et al., 2014). Anaerobic digesters operate at around a pH of 7, and a temperature of around 96 degrees to 98 degrees F (Kwietniewska and Tys, 2014). Hydraulic retention time (HRT) is the amount of time required for complete breakdown of organic matter or the time the organic matter remains in the digester (Kothari et al., 2014). The HRT for mesophilic microorganisms ranges from 10 to 40 days, whereas hydrogen producing bacteria have short retention times because produce volatile fatty freely between the particles acids and hydrogen in the exponential phase and alcohols in the stationary phase (Kothari et al., 2014). After anaerobic digestion, the sludge is then called biosolids. The biosolids are made up of a slurry in which water moves (Kanda et al., 2011). The free water can be separated by mechanical methods such as centrifugation (Chen et al., 2006; Chu et al., 2005; Kamizela and Kowalczyk, 2021). Dewatering aids in minimizing sludge volume, and concentrates the solids by decreasing the water content (Wu et al., 2020). Dewatering is affected by factors such as moisture distribution, surface charge and pore structure

(Rao et al., 2022; Wu et al., 2020). The moisture distribution has four forms: free water, interstitial water, surface water and bound water (Rao et al., 2022). Free water is easy removeable water, interstitial water which can be dewatered by mechanical methods, water that adheres to the surface of the organic components and adsorbed on the surface of the particles due to surface tension is surface water, and bound water is enclosed in solid particles or in cell membranes of microorganisms and is difficult to remove by mechanical dewatering (Rao et al., 2022; Wu et al., 2020). The sludge is sent to a centrifuge that dewaters the sludge increasing it from approximately 2% solids to 27 to 30% solids and is then called a "cake" which is not free-flowing and instead forms lumps (Rao et al., 2022) and it is this from that is usually trucked to agricultural fields.

Biosolids Used as Fertilizer

Biosolids are valuable organic fertilizers that improve soil but are also a main source of chemical pollutants (Jechalke et al., 2014; Rizzo et al., 2013; Verlicchi and Zambello, 2015). Biosolids contain pathogens such as bacteria (Salmonella), viruses (enterovirus), protozoa, trematodes and nematodes that can spread diseases if there is direct contact with the biosolids (Fijałkowski et al., 2017b; Marguí et al., 2016; Uggetti et al., 2012). Biosolids contain a mixture of nitrogen-rich organic compounds and a low carbon-to-nitrogen ratio (Liu et al., 2016; Torri and Cabrera, 2017). Inorganic phosphorus is the predominant form in biosolids (O'Connor et al., 2004; Torri and Cabrera, 2017) and is influenced by the wastewater treatment process used (Torri and Cabrera, 2017). When phosphorus is applied it often exceeds crop removal and more than 95% of phosphorus in biosolids remains in the soil (Correa, 2004). In addition, biosolids contain metals such as zinc, copper, nickel, cadmium, lead, mercury and chromium which are present in low concentrations in domestic wastewater, but found in high concentrations in

industrial wastewater (Mora et al., 2016; Torri and Cabrera, 2017). Biosolids contain these metals in varying concentrations resulting from business effluents (car washes, dental uses, etc.), traffic emissions (asphalt wear, brake linings, vehicle exhausts, tires, etc.), and household effluents (Torri and Cabrera, 2017) which end up in WWTPs and into biosolids (Bergbäck et al., 2001). Treated sewage and industrial effluents are used for irrigation of crops in developing countries and may contain heavy metals (Hussain et al., 2019). Athamneh et al. (Athamneh et al., 2014) studied land application of treated wastewater and biosolids to enhance crop production and soil fertility. At harvest, crop yield and yield components were determined, and soil samples were analyzed for physical, chemical, and microbiological parameters. Crop yield and plant uptake of macronutrients and micronutrients increased the same with the application of biosolids, commercial fertilizer or manure. They determined that the combined application of treated wastewater and biosolids improved crop production and enhanced soil fertility without any significant impact on the environment.

Removal Mechanisms

WWTPs are not designed to completely remove PPCPs, opioids and recreational drugs. They are unable to completely reduce the drug load because they are primarily designed to handle moderately degradable organics in the mg/L range (Chiavola et al., 2019; Patel et al., 2019). A study by Kwon and Rodriguez (Kwon and Rodríguez, 2014) 4 PPCPs from 3 WWTPs and reported carbamazepine showed the lowest removal rate of -99 to -100%, whereas sulfamethoxazole was removed from 62 to 97%. Baalbaki et al. (Baalbaki et al., 2017) observed low removal rates of <40% for carbamazepine during activated sludge treatment and negative removals were observed for tramadol. Subedi et al. (Subedi et al., 2017) studied PPCPs,

antibiotics and psychoactives from two WWTPs in Southern India and found trimethoprim and diphenhydramine were removed at >50% and carbamazepine had negative or no removal.

Average removal rates for miconazole, acetaminophen, diltiazem, caffeine and paraxanthine ranged from 82 to 99%. There are several removal pathways of emerging contaminants in conventional activated sludge wastewater treatment systems They include biodegradation, sorption, hydrolysis, photolysis and volatilization (Simon et al., 2021; Younes et al., 2018). These removal mechanisms are dependent on the physical/chemical properties of the compound. Biodegradation is the most common process in which organic matter are broken down by microorganisms in WWTPs (Hu et al., 2015). For acidic PPCPs, such as ibuprofen and naproxen, biodegradation is the mechanism responsible for removal (Hu et al., 2015). Some influencing factors in the biodegradation of these compounds include biomass concentration, sludge retention time, temperature and pH (Chen, 2016). Sludge retention time is an important parameter in biodegradation (Patel et al., 2019). Partial biodegradation occurred between 10 to 15 days, but after only 4 days no biodegradation occurred (Patel et al., 2019). The biodegradation time for PPCPs depend on the structure and properties of the compounds (Chen, 2016; Patel et al., 2019). For example sulfamethoxazole degradation time is 2 to 5 days, while roxithromycin is 5 to 15 days, and carbamazepine is unchanged at >20 days (Patel et al., 2019). Chen et al. (Chen, 2016) focused on three PPCPs (carbamazepine, triclosan and non-steroidal anti-inflammatory drugs (NSAIDs). They found the NSAIDs to be rapidly degraded, followed by triclosan, while carbamazepine was not degraded at all (Chen, 2016).

Sorptive removal involves substances moving from a dissolved phase to adhere onto a solid phase such as sludge in a conventional activated sludge WWTP. This is quantified as the soil/water distribution coefficient, K_d, which is the concentration of the compound in the solid

phase relative to its concentration in the aqueous phase. As such, the removal fate of these compounds depends on their chemical properties, including the octanol-water partition coefficient (log K_{ow}), aqueous solubility, polarity, and the acid-base dissociation constant (pK_a) (Das et al., 2017; Manallack, 2007). The octanol serves as a well-defined analog for the organic/solid phase in the system. The K_{ow} increases as the compound's aqueous solubility decreases. As such, (accumulation of a compound at the solid/water interface) to sludge is insignificant for compounds with log K_{ow}<2.5, moderate sorption for log K_{ow} between 2.4 and 4, and high sorption for log K_{ow}>4.0 (Das et al., 2017). The pK_a is an equilibrium constant used to measure the strength of a weak acid (Manallack, 2007). The lower the pK_a value, the stronger the acid, meaning it is more easily ionized (Manallack, 2007). The pK_a of a drug may influence its total charge, which will impact its lipophilicity, solubility, protein binding and permeability which affects absorption, distribution, metabolism and excretion (Manallack, 2007).

Min et al. (Min et al., 2018) investigated six PPCPs (ibuprofen, sulfamethoxazole, carbamazepine, ciprofloxacin, bezafibrate and metronidazole) and found biodegradation and sorption to be the dominant pathways for removal, with volatilization and hydrolysis having little effect. Hirsch et al. (Hirsch et al., 1999) examined 18 antibiotics belonging to the macrolide, sulfonamide, penicillin, and tetracycline groups. The effluent and surface waters contained sulfamethoxazole and roxithromycin at concentrations up to 6 μgL^{-1}. Penicillin's were found at a concentration of 20 ngL^{-1}. These researchers (Chiavola et al., 2019), studied the fate of amphetamine (AM), methamphetamine (MET), 11-nor-delta9-THC-9-carboxy (THC-COOH) and benzoylecgonine (BE) in a conventional activated sludge WWTP. They observed partial removal of AM and MET; BE showed limited removal, and THC-COOH displayed almost

complete removal. Removal occurred through biodegradation and adsorption, while volatilization did not play a significant role in removal.

Hydrolysis is another non-biotic pathway for elimination of PPCPs, opioids and recreational drugs. PPCPs containing the functional groups (esters and amides) are the most common ones to undergo hydrolysis (Patel et al., 2019). Persistent PPCPs that accumulate in the environment exhibit resistance to hydrolysis, such as the fluoroquinolone antibiotics (Patel et al., 2019).

Volatilization plays a minor role in removal of PPCPs, opioids and recreational drugs. Henry's law constant (k_H) or the air-water partition coefficient may be used to characterize the volatility of a compound (Lin and Chou, 2006). It is an important equilibrium factor in the transfer process of volatile organic compounds (VOCs) (Lin and Chou, 2006). Lin and Chou (Lin and Chou, 2006) studied samples of hydrophilic VOCs (methanol, isopropanol and acetone) and hydrophobic VOCs (toluene and p-xylene) between deionized water and deionized water diluted with pasteurized wastewater (called mixed liquor). They found that a medium to high concentration of activated sludge can enhance water solubility of hydrophobic compounds. In addition, these results gave information for selecting suitable scrubbing liquor to remove VOCs by the bioscrubber. For example, for hydrophilic VOCs, water and organic-rich wastewater could be the scrubbing liquor, and for the hydrophobic VOCs, high biomass-activated sludge could be the scrubbing liquor (Lin and Chou, 2006). Volatilization occurs along with the aeration process and usually removal is less than 10% (Wang and Wang, 2016). Suarez et al. (Suárez et al., 2008) found volatilization to be negligible (3 to 16%) for removal of pharmaceuticals.

To enhance the removal efficiency of PPCPs some WWTPs use advanced oxidation processes for tertiary treatment such as ozonation and/or ultraviolet radiation (UV) (Das et al., 2017; Sui et al., 2014). Ozonation and O_3/UV or UV/hydrogen peroxide (UV/H_2O_2) were found to be effective for the degradation of recalcitrant organic compounds in aqueous solution (Wang and Wang, 2016). Ozone depends on the formation of hydroxyl radicals to eliminate these compounds (Wang and Wang, 2016). UV treatment destroys the chemical bonds of these drugs by absorbing UV light (photolysis) (Wang and Wang, 2016). UV alone was found to not be successful in reducing the concentration of carbamazepine, except when hydrogen peroxide was added (Wang and Wang, 2016) . The removal of 13 PPCPs was studied using sequential UV and ozonation processes in a conventional activated sludge WWTP in Beijing, China (Sui et al., 2014). They found both combined processes to be effective in removing carbamazepine and trimethoprim, among others, with a median removal efficiency above 80% (Sui et al., 2014). Caffeine displayed poor removal and in some cases the concentration of caffeine increased after ozonation (Sui et al., 2014). Another study (Kim et al., 2009) examined 41 PPCPs and compared removal efficiency between UV (wavelength: 254mm) alone and UV and UV/H_2O_2. UV alone removed only a few compounds (>90%) and had removals of macrolides between 24% and 34%. With the addition of UV/H_2O_2 to UV alone efficacy and removal efficiency increased up to 90% for 39 out of the 41 compounds (Kim et al., 2009). A later study (Kim, 2012) measured 62 PPCPs from six WWTPs in Korea. Clarithromycin had a removal efficiency of 30% with ozonation and sulfamethoxazole and lincomycin had a removal efficiency of 20% with UV with limited removal efficiency with chlorination.

Transformation Products

Most PPCPs undergo metabolic transformation in human and/or animal bodies. These products are excreted through urine or feces and most enter WWTPs (Han and Lee, 2017) where parent compounds and their metabolites can undergo structural changes by the processes of biodegradation, hydrolysis and/or photolysis in which new chemical entities may be formed having different properties (Aymerich et al., 2016). The transformation products or metabolites cannot be completely removed in WWTPs, and with the parent compounds may end up in the effluent and then surface water (Yin et al., 2017). These transformed products enter surface water or groundwater through effluent where these metabolites are found at higher concentrations than their parents in the environment.

Several studies have examined illicit drugs and their metabolites in wastewater effluent and surface water. Miao and Metcalfe (Miao and Metcalfe, 2003b) examined Canadian WWTP influent, effluent and surface water samples for the five metabolites of carbamazepine and the parent, carbamazepine. Carbamazepine and the metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine was found to have 3 times higher concentrations in the surface water (2.2 ng/L) than the parent compound (0.7 ng/L) from the Otonabee River in Canada (Miao and Metcalfe, 2003b). These studies (Castiglioni et al., 2006; Zuccato et al., 2005) found in the River Po, Italy, cocaine and its metabolite, BE at concentrations of 0.0012 µg/L and 0.025 µg/L, respectively. In addition, this study (Hummel et al., 2006) found cocaine and BE in three German rivers at concentrations of 3 ng/L. Bones et al. (Bones et al., 2007a) found cocaine and BE at concentrations of 31 ng/L and 138 ng/L respectively from effluent from three United Kingdom WWTPs. Li et al. (Li et al., 2019b) examined the fate of PPCPs and their metabolites in different wastewater treatment processes in the Yangtze River Delta, China. Five wastewater

treatment plants were studied for the removal and distribution of acetaminophen, ibuprofen, and naproxen. They found acetaminophen and ibuprofen to have 100% removal in the biological treatment stage, while naproxen needed the addition of flocculants for removal.

Some PPCPs, opioids and recreational drugs have higher concentrations in effluent than in the influent, resulting in net addition or increase in concentration which is called negative removal. This may be due to the release of organic matter adsorbed in particulate matter or the conversion of conjugated metabolites by enzymatic reaction (Kosma et al., 2013; Kosma et al., 2014) where glucuronic acid reverts back to the precursor compounds or fecal particles may release PPCPs as the feces are being broken down by the microbes (Göbel et al., 2007). Almashaqbeh et al. (Almashaqbeh et al., 2020) examined removal efficiencies of 18 PPCPs from a WWTP in Jordan. Of the 18 compounds, thiabendazole had a negative removal efficiency of -17.2% possibly due to transformation or recombination. Additionally Gao et al. (Gao et al., 2016) studied the fate and removal of PPCPs in a Beijing, China, WWTP and found negative removal efficiency of – 46% for carbamazepine. Gemfibrozil, carbamazepine and the macrolide clarithromycin were detected in higher concentrations in effluent than in the influent possibly due to the formation of transformation products (i.e. glucuronide conjugate or methylates) converting back to the parent compounds (Jelic et al., 2011). Blair et al. (Blair et al., 2015) found negative mass balances for 7 PPCPs (carbadox, carbamazepine, ciprofloxacin, clarithromycin, enrofloxacin, norfloxacin, ofloxacin) may be due to the PPCPs enclosed in fecal particles and which are released when the fecal particles break down or the PPCPs are being retransformed into the parent compounds.

Human Metabolites

Most drugs in the human body undergo transformation with the site of metabolism occurring in the liver. These conversions frequently have some loss of pharmacological activity and an increase in hydrophilicity to promote elimination (Celiz et al., 2009). Fluoroquinolones, penicillin and some beta-blockers are commonly excreted unchanged while analgesics undergo different degrees of metabolism There are two stages to metabolism of drugs in the human body: Phase I involves oxidation, reduction or hydrolysis, and Phase II metabolites (conjugates) result from biochemical reactions where a molecule (i.e. glucuronic acid) is added to the parent compound (Celiz et al., 2009; Kosma et al., 2019; Writer et al., 2013). In Phase I, enzymes convert lipophilic organic molecules to more polar compounds by the addition of reactive functional groups into the molecule (Daughton and Ternes, 1999). The superfamily of enzymes (cytochrome P450) which is found in all forms of life, such as prokaryotes, yeast, fungi, plants, and insects convert lipophilic molecules to water soluble compounds by the addition of functional groups such as -OH, -SH, -NH_2, or -COOH during Phase I transformation (Danielson, 2002). Phase II involves conjugation with either sugars (glucuronidation) or peptides to aid in solubility and enable excretion (Daughton and Ternes, 1999). These metabolites may deconjugate back to the parent (Polesel et al., 2016) compound following cleavage of the conjugated portion form (Golovko et al., 2014b; Subedi and Kannan, 2015; Writer et al., 2013) and end up in WWTPs (Celiz et al., 2009). Carbamazepine, an antiepileptic drug is metabolized in the liver and one of its major metabolites is 2-hydroxycarbamazepine and 2-hydroxyiminostilbene (Ju and Uetrecht, 1999). These metabolites have been detected in influent, effluent and biosolids and occur in higher concentrations than the parent compound (Miao and Metcalfe, 2003a; Miao et al., 2005; Zhao and Metcalfe, 2008). Miao and Metcalfe (Miao and

Metcalfe, 2003b) measured concentrations of carbamazepine in influent (8.5 to 1,572 ng/L) in influent, (9.3 to 1,325 ng/L) in effluent, and 2.2 ng/L in surface water. Carbamazepine was detected in effluent at a concentration of 800 ng/L and in surface water at a concentration of 20 ng/L (Batt et al., 2008). Fluoxetine (Prozac), a serotonin reuptake inhibitor and its main human metabolite, norfluoxetine, have been detected in effluents in concentrations from 3.9 to 25 ng/L (Vanderford and Snyder, 2006).

Conjugated metabolites can undergo deconjugation and transform back to the parent compound. For example, The metabolite of sulfamethoxazole (acetylsulfamethoxazole) was detected in effluent at a concentration of 82 ng/L in Switzerland (Göbel et al., 2004) and detected in rivers and streams at a concentration of 70 ng/L in the United Kingdom (Ashton et al., 2004).

Microbial Metabolites

Pharmaceuticals and their human metabolites can be microbially transformed by biotic processes which aid in reducing the release of these compounds into the aquatic environment. The biotic treatment of wastewater, which includes the activated sludge process involves the transformation of dissolved and suspended organic contaminants by microorganisms (mainly bacteria and protozoa) (Michael et al., 2014).

Excretion of human compounds into WWTPs contain a mixture of parent compounds and metabolites which can undergo structural changes by processes such as biodegradation from bacteria and fungi and/or non-biotic processes such as hydrolysis and photolysis (Kern et al., 2010; Michael et al., 2014). The new structural entities contain different properties and transformation products are formed (Kern et al., 2010). Microbial deconjugation of human

metabolites to parent forms has been observed for carbamazepine. Eight transformation products of cocaine were detected in wastewater, including benzoylecgonine (BE), norbenzoylecgonine, norcocaine, cocaethylene, ecgonine methyl, ecgonine, anhydroecgonine and anhydroecgonine methyl ester (Evgenidou et al., 2015). BE and ecgonine methyl ester are the primary metabolites of cocaine and BE was the most abundant metabolite found in most of the influent and effluent samples at concentrations ranging from 3701 ng/L to 4003 ng/L respectively (Evgenidou et al., 2015). Three metabolites of morphine were detected (6-acetylmorphine, normorphine and morphine-3a-D-glucuronide) (Gilart et al., 2013). Concentrations of 6-AM in influent ranged up to 715 ng/L, while in effluent the concentrations were the lowest concentration at which the analyte could be detected (Gilart et al., 2013). The metabolite of methadone (2-ethylene-1,5-dimethyl-3,3-diphenylpyrrolidine) was detected higher in effluent than influent (206 ng/L) (Terzic et al., 2010).

PPCPs, Opioids, and Recreational Drugs in aquatic environments

PPCPs, opioids and recreational drugs can enter the aquatic environment due to population growth, agricultural runoff, urbanization, and industrialization (Gerbersdorf et al., 2015; Kiani and Rahimpour, 2020). In addition, wastewater effluent, leaking septic system pipes, production/manufacturing sites, aging infrastructure and/or combined sewer overflows contribute to these compounds in surface waters (Gerbersdorf et al., 2015; Rosi-Marshall et al., 2015). In a 2015 survey by aus der Beek et al. (aus der Beek et al., 2016) found that pharmaceuticals were detected in 70 countries with over 600 different pharmaceuticals measured over the detection limit. In Asia, antibiotics were found with the highest maximum concentrations in surface waters (e.g., 6.5 mg/L ciprofloxacin in India) (aus der Beek et al., 2016). Golovko et al. (Golovko et al., 2021) investigated surface water samples upstream or downstream of WWTPs

and observed the highest concentrations for metformin (19,000 ng/L), and caffeine (3600 ng/L), in addition, tramadol and codeine were detected at >95% frequency in high concentrations. Lindim et al. (Lindim et al., 2016) found in Swedish surface waters high concentrations of metformin and tramadol. Chemicals of emerging concern are usually found at lower concentrations in surface water than in WWTP effluent because of dilution, sorption, biodegradation, and photodegradation (Ferguson et al., 2013; Kasprzyk-Hordern et al., 2008a; Vieno et al., 2005). Golovko et al. (Golovko et al., 2021) determined that concentrations of these emerging compounds in surface water at downstream locations were on average, 50% higher than those upstream of the WWTP concluding that WWTP effluent is a major source of input into surface waters. Some investigators have measured the concentrations of illicit drugs in surface waters receiving effluent and found trace concentrations of amphetamine, methamphetamine morphine, cocaine and cocaine metabolites (Bartelt-Hunt et al., 2009; Bones et al., 2007a; Jones-Lepp et al., 2004; Zuccato and Castiglioni, 2009; Zuccato et al., 2005). Bartelt-Hunt et al. (Bartelt-Hunt et al., 2009) detected illicit drugs and PPCPs from samplers placed upstream and downstream of WWTP effluent discharges in the Midwest and found methamphetamine concentrations downstream ranging from approximately 2 to 350 ng/L with few detections upstream and no detection of amphetamine. Berset et al. (Berset et al., 2010) conducted a study in Switzerland and found cocaine, BE, amphetamine, methamphetamine, morphine present in WWTP effluent as well as in several lakes and streams that receive effluent. Rosi-Marshall et al. (Rosi-Marshall et al., 2015) found that illicit drugs may not be persistent, because their half-lives are relatively short, but may exhibit pseudo-persistence, wherein continual use results in persistent occurrence.

Sewer overflow is the release of raw or poorly treated wastewater and fecal-derived pathogens into water bodies such as rivers, lakes, seas, etc. Heavy rainfalls can induce sewage overflow. Surface runoff, and sanitary and industrial sewage are sending untreated contaminated water directly into receiving water bodies (Luo et al., 2014). Stormwater runoff is rainfall that flows over the ground surface including roads, streets, developed and undeveloped lands, rooftops and other paved surfaces, where wastewater exceeds the capacity of the WWTP, and the untreated wastewater ends up in the receiving water bodies. Urban streams may be affected by contaminants from stormwater effluents and sewer overflows. During rain events, WWTPs that are connected to a combined sewer system face not only PPCPs or industrial chemicals, but also incoming pesticides used in lawn fertilizer, organo-phosphorous compounds and compounds from tire abrasion and road wear (Koeleman et al., 1999; Singer et al., 2010). Buerge et al. (Buerge et al., 2006) used caffeine as a chemical marker to estimate the fraction of sewer overflows in the catchment area of Lake Greifensee, Switzerland and found caffeine loads higher (0.1-1.6 mg person/day) than the loads in the WWTP effluents (<0.15 mg person/day). Buerge et al. (Buerge et al., 2006) concluded that combined sewer overflows were the most likely source of caffeine because the loads correlated with precipitation during sampling. Madoux-Humery et al. (Madoux-Humery et al., 2013) found median concentrations of caffeine, carbamazepine in two combined sewer overflows from 1.2 to 51.4 times lower than dry weather wastewater, and Del Rio et al. (Del Río et al., 2013) observed mean concentrations of carbamazepine in combined sewer wastewater to be 1.3 to 7.9 times greater during wet weather than dry weather conditions. Wastewater effluent, wastewater compounds urban streams and Lake Champlain were studied from March to August 2006 and found the highest concentrations of wastewater compounds were 10-100 µg/L in WWTP samples and combined sewer overflow samples (Phillips and

Chalmers, 2009). Wastewater compounds from urban stream samples ranged from 0.1 to 10 µg/L, and urban stream storm runoff samples had higher concentrations than baseflow samples because of wastewater compounds from combined sewer overflows and leaking sewer pipes (Phillips and Chalmers, 2009).

Marine ecosystems may be affected by PPCPs and illicit drug contamination (Klosterhaus et al., 2013). Coastal zones are under increased pressure due to an increase in human activities (Arpin-Pont et al., 2016). PPCPs are released into marine water through submarine or marine sewage treatment outfalls (Fenet et al., 2014) or in runoff via rivers and streams (Farré et al., 2008). Other sources of PPCPs in the marine environment are fish farming, for antibiotics (Zou et al., 2011), antiparasitic drugs (Rico and Van den Brink, 2014), and recreational activities (Bachelot et al., 2012). A worldwide study of PPCPs was conducted and half of the 100 compounds investigated were detected in marine water (Arpin-Pont et al., 2016). The most frequently detected compounds were erythromycin, sulfamethoxazole, and trimethoprim (Minh et al., 2009). The highest concentration was for erythromycin (1900 ng/L) located adjacent to an effluent discharge and located in the typhoon shelter in Victoria Harbor, Hong Kong, China (Minh et al., 2009). Non-steroidal anti-inflammatory drugs were detected in marine water at concentrations ranging from 0.7 to 6100 ng/L (Jiang et al., 2014; Togola and Budzinski, 2008; Weigel et al., 2004). Acetaminophen was detected (230,000 ng/L) less than 500 m from a WWTP outfall in Marseille, France (Togola and Budzinski, 2008). Concentrations of PPCPs in marine water are generally lower than in freshwater due to higher dilution factors (Arpin-Pont et al., 2016). For example, carbamazepine was detected at concentrations ranging from 9 to 2000 ng/L in surface waters (Fent et al., 2006), whereas in marine waters,

carbamazepine concentrations ranged from 3.89 ng/L along the coastline (Jiang et al., 2014) to 185 ng/L in estuaries (Wille et al., 2010).

Wastewater treatment as a source of these compounds in aquatic environments

There are three major sectors of wastewater: domestic, industrial, and agricultural. Domestic wastewater encompasses water from day-to-day household activities, such as water from the kitchen, shower, toilet, and laundry (Manasa and Mehta, 2020). Classifications of domestic wastewater include yellow water, which is in the form of urine, blackwater which is in the form of fecal matter and high in organic matter and pathogens, and lastly grey water which is generated from showers, baths, and laundry (Manasa and Mehta, 2020). Grey water contains fewer pathogens than blackwater and can be treated and reused within 48 hours for crop irrigation and non-potable uses (Tilley et al., 2014). Industrial comes from chemical, paper, pulp pharmaceuticals and food, etc. (Manasa and Mehta, 2020). This type of wastewater is rich in organic and inorganic contents, but excessive release of these compounds can cause increased nutrients in the water leading to excessive growth of plants and algae (Manasa and Mehta, 2020). Some common food industries that produce wastewater are the beverage, brewery, and dairy industries. The beverage industry encompasses manufacturing non-alcoholic drinks, which are juices, soft drinks, tea, coffee, and water and alcoholic drinks such as beer, wine and spirits (Manasa and Mehta, 2020). This type of wastewater contains carbohydrates such as sugar, pectin, and flavorings (Ait Hsine et al., 2004). The brewery industry mainly produces beer. Brewery waste includes suspended solids, organic components such as volatile fatty acids, sugar, soluble starch and ethanol (Arantes et al., 2017). The dairy industry's main staple is the production of milk. The effluent from the dairy industry contains soluble organics, suspended solids and trace organics which produces high BOD and COD (Slavov, 2017). Agricultural

wastewater is the excess water that runs off from fields. Farms and agricultural fields are contaminated with chemicals, fertilizers, pesticides, crop remains and animal waste (Farré et al., 2008; Manasa and Mehta, 2020). This type of wastewater contains high concentrations of excreted pathogens, such as viruses, bacteria and fecal coliforms (Hussain et al., 2002). Antibiotics are usually discharged by industrial, urban, and agricultural sewage into the aquatic environment (Adams et al., 2002; Kiani and Rahimpour, 2020). WWTPs do not completely remove antibiotics or other compounds, therefore they are continually released into the aquatic environment (Hirsch et al., 1999; Kümmerer, 2009; Ma et al., 2016). Liu and Wong (Liu and Wong, 2013) conducted a study in the Pearl River Delta located in South China and found high detection frequency and concentrations for macrolides (roxithromycin and erythromycin), fluoroquinolones (ofloxacin and norfloxacin), and sulfonamides (sulfamethoxazole). In eastern China, antibiotics were present in WWTPs in the Yangtze River Delta (Liu and Wong, 2013). Veterinary antibiotics (sulfamethazine, sulfadiazine and sulfamethoxazole) were monitored in the wastewater of 27 animal farms from the Jiangsu Province with the highest concentration up to 211 ug/L (Wei et al., 2011) implying that livestock wastewater is an important source of antibiotic input into the environment.

Concentrated Animal Feeding Operations (CAFOS)

Over the past few decades, concentrated animal feeding operations (CAFOs) have increased and have become a growing environmental concern (Long, 2018). CAFOs are defined as small, medium or large operations that do not store or grow crops but have over 1,000 animal units onsite that are contained indoors at high densities for a minimum of forty-five days per year (Raff, 2021, Kast, 2019) until they are transported to slaughterhouses (Burkholder, 2007). An animal unit is the equivalent of 1,000 pounds live weight of animals (Raff, 2021). A thousand

animal units equates to 700 dairy cows, 1,000 meat cows, 2,500 pigs weighing more than 55 pounds (25 kg), 10,000 pigs weighing under 55 pounds, 10,000 sheep, 55,000 turkeys, 125,000 chickens, or 82,000 egg laying hens or pullets (Long, 2018).

CAFOs generate large volumes of manure and runoff water (Bradford, 2008, Brown, 2020, Pagliari, 2020, Burkholder, 2007, Raff, 2021, Miralha, 2022, Centner, 2011) which introduces problems of disposal of the waste. Cattle produce the greatest amount of manure, followed by pigs, poultry, sheep and goats worldwide (Sommer, 2013). Burkholder et al. (Burkholder, 2007) found that CAFOs yield over 130 million tons of manure annually and this manure is applied to surrounding fields. Problems with mismanagement of manure can lead to reduced water quality because of precipitation events that result in overflow of lagoons, runoff from recent applications of waste to fields, overapplications, and accidental spills causing pollutants to enter surface water (Centner, 2011, Aneja, 2003, Scanes, 2018). Gerba and Smith (Gerba, 2005) state that CAFOs generate approximately 100 times more manure as wastewater treatment plants produce biosolids in the United States. The manure produced by CAFOs is used to fertilize cropland, but often there may not be enough nearby cropland to receive all the manure. Then the manure may be applied on cropland far from the CAFO barns, or stored on site, or over-applied on nearby cropland (Long, 2018). Nutrients found in manure can accumulate in the soil or run off fields and contaminate surface water (Long, 2018, Miralha, 2022). Contaminants such as pathogens, veterinary pharmaceuticals, heavy metals (ex.: zinc and copper), hormones and nutrients are present in livestock waste (Gerba, 2005, Boxall, 2004, Jongbloed, 1998). The major sources of human and animal pathogens in the environment originate from CAFOs, septic tanks, wastewater effluents and biosolids (Gerba, 2005). The transmission route of pathogens from animals to humans could be when manure is used as a

fertilizer for food crops, by storm water runoff or percolation to ground water. Some of the pathogens found in cattle waste are the bacteria pathogens *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7, and the protozoan parasites *Cryptosporidium parvum* and *Giardia lamblia* (Gerba, 2005). Some viruses of concern are poliovirus, coxsackie virus, echovirus, hepatitis A, rotavirus and Norwalk virus (Gerba, 2005). Hubbard et al. (Hubbard, 2020) investigated poultry litter, groundwater and surface water from 9 CAFO locations in Iowa and one in Wisconsin from May and June 2016. They found detectable bacteria (Salmonella spp., enterococci, staphylococci, lactobacilli) in poultry litter, groundwater and surface water samples as a likely source of environmental contamination. Miralha et al. (Miralha, 2022) examined spatial organization of CAFOs and its relationship to water quality. They looked at 16 United States CAFO locations for total phosphorus (TP) and total nitrogen (TN) concentrations. Their results showed that watershed with significant clustering patterns had higher TP and TN concentrations. These data were also found for spring and summer which are the seasons manure is land applied.

In addition, improper disposal of animal carcasses and abandoned livestock facilities can impact water quality (Burkholder, 2007). CAFOs in regions prone to flooding or have a shallow water table also have the potential to impact water quality (Burkholder, 2007).

Veterinary pharmaceuticals are used in CAFOs for rapid growth purposes, to maintain good health (Kümmerer, 2004, Liu, 2003, Kumar, 2005) and allowing the animals to be brought to market faster and at lower cost (Boxall et al., 2003). Antibiotics are the major group of pharmaceuticals (Boxall, 2004) with tetracyclines and sulfonamides the two most heavily used antibiotics (Thiele-Bruhn, 2003). How the drug is given to the animals depends on whether the animal received the treatment topically, in feed, as an injection or bolus (Boxall et al., 2003).

Most of the antibiotics are not metabolized completely and are excreted from the animal shortly after receiving the medication and about 80% of the antibiotics were found as parent compounds in the animal wastes (Boxall et al., 2003; Kumar et al., 2005; SHORE et al., 1988). Routes of entry into the environment can be direct discharge of aquaculture products, excretion in urine and/or feces, and through topical treatments washing off of the animals (Boxall et al., 2003). Multiple veterinary medicines may be used to treat a herd exposing aquatic and terrestrial organisms to a combination of medicines and chemicals (Boxall et al., 2003).

Excess metals are often added to animal feeds to promote growth (Bradford, 2008). For example, arsenic is fed to chickens to promote growth, kill parasites, and improve pigmentation of the chicken meat (Hileman, 2007, ŽIvkov BaloŠ, 2019, Schaefer, 2007). Copper and zinc are added to swine feeds to promote growth (Pérez, 2011). Swine manure is mostly applied near to the swine CAFOs which may lead to accumulation of copper and zinc in the soil (Jongbloed, 1998).

Nutrient content of animal waste can be advantageous for land application, but over applied can saturate the soils with macronutrients such as nitrogen and phosphorous, as well as the micronutrients of heavy metals (Penha, 2015) and therefore runoff into receiving waters (Burkholder, 2007, Richards, 2011). Excess nutrients and organic materials that enter surface waters can cause algal blooms that increase turbidity and biochemical oxygen demand (BOD) (Richards, 2011, Long, 2018). As a result, noxious odors and fish kills can result if the dissolved oxygen falls below critical thresholds. Excess manure remaining on the fields can cause nitrate-nitrogen (NO3-N) and phosphorus (P) to accumulate in the soil (He, 2014). Long et al. (Long, 2018) examined 13 CAFOs in southeastern Michigan within a 15 km (9.3 mile) radius to each other in the River Raisin and Maumee River watersheds, both which discharge to western Lake

Erie and found over-application of manure and found soil phosphorus levels >50 ppm (42% of all cases), over-application to soybean fields (7% of all cases), and over exceeding state permits (26% of all cases). Algal blooms and hypoxia in Lake Erie have increased due to elevated P loadings from these watersheds that drain into the lake. Farm fertilizers and manure are the primary sources of the Maumee River's P load, and it has been estimated that 12% of phosphorus applied to cropland throughout western Lake Erie watersheds stems from manure (Scavia, 2017). Brown et al. (Brown, 2020) studied nutrient pollution from CAFOs located on the Coastal Plain of North Carolina, where the Cape Fear River basin is located. They found enriched ^{15}N signatures in sites nearby to CAFOs as well as point-source wastewater discharge areas, in addition to higher nitrate concentrations compared to the control stream and two estuarine sites. A study by Raff and Meyer (Raff and Meyer, 2021) examined the relationship between surface water quality and the size and location of CAFOs using longitudinal data. They used a dataset from 1995 to 2017 that links CAFO intensity with nearby surface water quality in Wisconsin. In 2019, about 3.5% of all dairy operations in Wisconsin were CAFOs. They found that increasing CAFO intensity increases the nutrients, specifically total phosphorus and ammonia in surface water. They compared changes in nutrient concentrations among surface waters with large expansions of CAFOs to changes in nutrient concentrations in control water with no or low expansions in CAFOs. In a similar study by Miralha et al. (Miralha et al., 2022) they hypothesized that clustered CAFOs are likely to be associated with higher concentrations of total phosphorus (TP) and total nitrogen (TN) in the United States. They looked at CAFO locations in 16 states and found that watersheds with significant clustering patterns were associated with higher TP and TN concentrations. Bernot et al. (Bernot et al., 2013) investigated the spatial and temporal variability of human and veterinary PPCPs in a rural, central Indiana

stream (Sugar Creek) affected by CAFOs. Sample sites were located before and after CAFO influence, totaling 102 water samples. There were 50 animal agricultural sites in the watershed and 18 swine and cattle CAFOs in close vicinity to Sugar Creek. Detection frequencies of veterinary PPCPs were lincomycin (57%), sulfamethazine (59%), while sulfathiazole, sulfadimethoxine, and sulfamerazine were not detected in any samples. Spatially, lincomycin and sulfamethazine concentrations were approx. 30% higher about 10 km downstream of the uppermost sampling site which is located immediately adjacent to swine CAFOs. Expectations were that veterinary PPCP concentrations would be highest in fall following manure application to fields, but the highest concentrations were measured in July.

Use and abuse of opioids

The United States Department of Health and Human Services declared the opioid epidemic a public health emergency in 2017 (Jones, 2018, Singh, 2019). The opioid epidemic has impacts across age, gender, racial/ethnic, socioeconomic, and geographic/rural-urban groups (Singh, 2019, Schuckit, 2016). Opioids are natural or synthetic compounds that bind to opioid receptors in the central nervous system and can decrease sensations of pain (Hoffman, 2019, Lyden, 2019). Opioids include oxycodone, hydrocodone, codeine, fentanyl, tramadol, morphine, and heroin (Salmond, 2019). Opioids are used to treat moderate to severe pain in the short-term (Chou, 2009, Singh, 2019, Hagemeier, 2018) in addition to diminishing cough and relieving diarrhea. These drugs generate feelings of euphoria, and tranquility that can lead to addiction (Schuckit, 2016). There are risks involved with using opioid medication including misuse (Hoffman, 2019), dependence and deaths due to overdose (Chou, 2009, Singh, 2019). Overdose fatalities have increased due to the synthetic opioids such as fentanyl and carfentanil which is 50 and 5000 times as potent as heroin, respectively (Volkow and Collins, 2017). Misuse of or

accidental exposure to these synthetics increase the risk of overdose, and naloxone that can reverse the effects of overdose may not be effective (Volkow and Collins, 2017). Currently, there are only three medications approved for treating overdoses: methadone, buprenorphine, and naltrexone (Volkow and Collins, 2017). From 1999 to 2011, the opioid poisoning death rate in the United States nearly quadrupled due to misuse and abuse of prescription opioid analgesics but increases in opioid poisoning deaths also occurred in Canada, Sweden, Norway, Ireland, and the United Kingdom indicating a growing health crisis (Jayawardana, 2021). Jayawardana et al. (Jayawardana, 2021) studied opioid analgesic consumption for 76 countries between 2009 and 2019 using a database that assessed differences between high-income, upper-middle income, and low and lower middle-income countries. Consumption declined in the United States and Germany, but overall, the high-income category had increased consumption rates. Overall, consumption rates were associated with income, trade, and physician density. Tramadol consumption rate increased during this same study period across all country-income groups. There is a growing concern regarding the misuse of tramadol in Africa (Salm-Reifferscheidt, 2018). Africa is poverty ridden and people working as hard laborers take tramadol to keep and maintain their jobs. For example, one person was taking up to 19 tramadol capsules daily. Tramadol is cheap and easy to obtain, and pharmacists are known to sell painkillers without a prescription. There is also an illegal market for tramadol from Asia, Ghana, or Nigeria. Tramadol producers in China increased the concentrations of tramadol up to 250 mg, where the standard prescription concentration is 50 to 100 mg.

In the United States, the opioid epidemic is a major health care problem. From 1999 to 2019, approximately 500,000 Americans have died from drug overdoses using opioids (Kandil, 2021). The opioid epidemic started in the 1990s when opioids were prescribed for minor injuries,

surgical procedures, or dental procedures (Kandil, 2021). During this time, Americans consumed >80% of the world's opioid supply despite representing only 4.6% of the world's population (Manchikanti and Singh, 2008). Hydrocodone was consumed by Americans to >99% of the world's hydrocodone supply prior to when it was rescheduled as a scheduled 2 medication (Manchikanti and Singh, 2008). The 2006 National Survey on Drug Use and Health found that 7.0 million or 2.8% of persons aged 12 or older had used prescription type psychotherapeutic drugs (pain relievers, stimulants) nonmedically in the past month, 16,387 million, or 6.6% of the population has used in the past year, and 20.3%, or approx. 49.8 million had used prescription psychotherapeutic drugs during their lifetime (Manchikanti and Singh, 2008).

There are large knowledge gaps in understanding abuse and misuse of opioids. Providers of opioids need a better understanding of addiction and what populations are at risk for opioid addiction and to change the belief that opioid addiction is only a psychological problem when in reality it involves psychological and physiological issues related to a chronic, painful disease (Horn et al., 2023).

Future Perspectives

The demand for PPCPs, opioids and recreational drugs continues to grow worldwide. In the United States, the opioid epidemic is the most severe in public health history (Volkow and Blanco, 2021). The two major factors in the opioid crisis are the steady increase in the rate of opioid prescriptions, the decrease in price and the increase in availability of heroin and synthetic opioids (Volkow and Blanco, 2021). The increase in opioid prescriptions generated a surplus of medication that was diverted for non-medical use (Volkow and Blanco, 2021). From 1991 to 2013, the non-medical use of prescription opioids in the United States doubled from 1.5% to 4.1% and the frequency of use increased among nonmedical users (Volkow and Blanco, 2021).

aging population, the development of new drugs, the over prescribing of drugs and easier accessibility to drugs whether legally prescribed or not legally prescribed. Fentanyl, the most recent synthetic opioid is almost twice as commonly involved in overdose deaths as other opioids or heroin (Jones et al., 2018). Fentanyl has low production costs, and its potency (50-fold compared to heroin) makes it easy to mix with heroin and other manufactured prescription opioids (Frank and Pollack, 2017). Overdoses from fentanyl by itself or combined with heroin are much harder to reverse with naloxone possibly due to the high potency mu opioid receptor and how very fast it enters the brain minimizing time for intervention (Suzuki and El-Haddad, 2017).

Demand for these drugs has substantial economic costs, especially in the areas of healthcare and law enforcement. One study (Birnbaum et al., 2011) estimated the overall societal impact of prescription opioid abuse, dependence, and misuse in the United States to be $55.7 billion in 2007. The United Nations Office on Drugs and Crime (UNODC) World Drug Report in 2017 (UNODC, 2019) reported global estimates for illicit drug use for adults aged 15 to 64 years was highest for cannabis, followed by amphetamines, opioids and cocaine. Salmond and Allread (Salmond and Allread, 2019), looked at the current opioid crisis and found it is officially the deadliest drug crisis in American history and is accelerating. In 2016, synthetic opioids have surpassed prescription opioids as the leading cause of drug overdose deaths (Salmond and Allread, 2019). The National Institute of Drug Abuse (NIDA) (NIDA, 2020) reported in 2018 that there were more than 72,000 drug overdose deaths in the United States in 2017 (Salmond and Allread, 2019; Wilson, 2020). The increase in overdose deaths was 13% from 2016 to 2017, attributable to using synthetic opioids (fentanyl alone or combined with other opioids such as heroin (CFDC, 2019). A major public health crisis is the availability of

synthetically carfentanil (10,000 times more potent and can be deadly with the dose the size of a grain of salt) leading to accidental overdoses (Hayes, 2018). These drugs are available and can be purchased on the dark web; they are cheap; and China delivers them through the United States Post Office (Ciccarone, 2019). Healthcare professionals and policymakers need to use their training and skills to help address this problem, to cope with the economic, racial, and social issues related to the opioid epidemic.

This research uses an alternative extraction method. This method is economical, and fast for determination of certain compounds found in water or urine samples. In urine samples for example, one could tell whether a person has used a drug, such as cocaine and its metabolite benzoylecgonine, amphetamines, opioids (natural or synthetics) such as heroin, morphine, oxycodone, and methadone. Drug testing is frequently used in clinical, employment, educational, and legal settings where fast results are needed, and misinterpretation of test results can result in adverse consequences for the individual being tested.

Other uses for this extraction method would be for example if there is a contamination spill of some kind in the aqueous environment that needs to be identified in a speedy manner. Or this extraction method could be used for private wells to detect contaminants in the water supply.

Testing kits or visual testing kits are a fast way to determine results of drug use, but often are not reliable in that false positives happen and in visual test kits often they are difficult to read because of faint color or uncertain color leading to a subjective interpretation. Immunoassays are used in clinical settings but need to also use patient history and other collaborative information to make a judgement. Gas chromatography assessment must be conducted by trained personnel and are time-consuming and costly, whereby liquid chromatography/tandem mass spectrometry is more time efficient.

This purpose of this research was to conduct a survey of a diverse number of compounds entering a wastewater treatment plant's influent, and to determine if the concentration of those compounds changed through the treatment process and what concentration was left in effluent if any, or in the aquatic environment. This change in concentration from influent to effluent was determined by this alternative extraction method.

Chapter 2:

A Survey of Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs from Influent, Effluent and Surface Water from Two Wastewater Treatment Plants

Introduction

Pharmaceuticals and personal care products (PPCPs), opioids and recreational drugs are an emerging pollution concern as their usage and consumption are rapidly increasing, potentially affecting aquatic life, and human health in the United States (US) as well as globally. Global prescription spending on medicine in 2020 was estimated to be approximately $1.3 trillion (Rajkumar, 2020). The United States spending is estimated to be around $350 billion (Rajkumar, 2020). These spending rates are expected to increase at a rate of 3 to 6% annually globally (Rajkumar, 2020; Tichy et al., 2022). The Centers for Medicare and Medicaid Services (CMS) projects United States retail prescription drug spending will be approximately 9% of overall national health expenditures from 2019 to 2028 (Conti et al., 2021). If non-retail prescription drug spending is included with retail spending, then it is estimated the total US drug spending increases by 50%. Drug spending was approximately $500 billion in 2018 and will increase to $863 billion by 2028 (Conti et al., 2021). This increase in consumption of drugs may be due to factors such as an aging population, the development of new drugs, the overprescribing of prescription drugs and easier accessibility of drugs legally and illegally prescribed (Rajkumar, 2020). In addition to human consumption, pharmaceuticals are introduced into the environment through veterinary use from livestock, especially in rural areas and where concentrated animal feeding operations (CAFOs) are located.

Wastewater Treatment Plants (WWTPs) are intended to remove pollutants, but they remain a source of environmental pollutants to the environment. Human excretion, veterinary drugs used for livestock, as well as herbicide run-off are introduced into the environment mainly

through wastewater effluent. The wastewater effluent is discharged into rivers, streams or lake systems, and the sludge is spread on the fields as fertilizer. Wastewater treatment processes do not adequately remove all of these pollutants entering the WWTPs as influent and these pollutants are discharged to the receiving waters having an effect on the environment (Kay et al., 2017; Martín et al., 2012; Vatovec et al., 2016). These compounds are usually detected at very low concentrations in the nanogram or microgram per liter range (Patel et al., 2019). To put this in perspective 1 nanogram is approximately equal to one human cell and 1 microgram would be equal to the dot at the end of a sentence is the dot is made of carbon (answers.com). Furthermore, WWTPs are not required to monitor for these environmental pollutants. This has the potential to become an emerging public health concern for humans and animals because of the increased use of these compounds that result in a continuous supply to the environment (Ebele et al., 2017). There are few studies on the long-term effects of these compounds in the environment.

The environmental contaminants most often found in wastewater treatment effluent are antibiotics, epileptic drugs, ace inhibitors, analgesics, herbicides, opioids, and recreational drugs. Out of 133 studies, 580 unique compounds were found in different matrices (Reyes et al., 2021). The most frequently occurring compounds found in wastewater influent were carbamazepine, caffeine, ibuprofen diclofenac and acetaminophen (Reyes, 2021). Likewise in wastewater effluent, carbamazepine, caffeine, diclofenac, sulfamethoxazole and triclosan were the most frequent compounds detected (Reyes et al., 2021). One study of an eastern Canadian city measured six psychoactive drugs by comparing usage on weekdays and weekends (Palardy et al., 2016a). BE and methamphetamine (both recreational drugs) concentrations ranged from 6.3 ng/L on the weekend to below the limit of detection on weekdays for effluent. Codeine, morphine and methadone (all opioids) concentrations were between 7.5 ng/L and 71.4 ng/L in effluent with no

difference between weekend and weekday sampling (Palardy et al., 2016a). A study of five WWTPs from the largest industrial city in Korea yielded a high percentage of antibiotics (56% to 81% of samples) in effluent with lincomycin present (49% to 81%) in all the WWTPs followed by triclosan (an anti-microbial) and naproxen (an anti-inflammatory) (0.5 to 3%, and 0.2 to 5.6% respectively) (Behera et al., 2011). Blair et al. (Blair et al., 2013a) evaluated 54 pharmaceuticals at various sample sites and a site near an effluent discharge area in Lake Michigan and Milwaukee harbor and found four compounds (metformin 100%, caffeine 97.6%, sulfamethoxazole 83.3% and triclosan 71.4%) detected with greater than 50% occurrence at all sampling sites.

In the Wolf River/Lake Winnebago system, the effluent from WWTP #1 is discharged just a short distance upstream from the mouth of Lake Winnebago (Figure 2.1). At the southern end of Lake Winnebago, WWTP #2 (Figure 2.1) discharges the effluent directly into the lake. In addition, Wisconsin's largest CAFO is located just 17 miles from Oshkosh where there is the potential risk of agricultural PPCPs entering groundwater and surface water upstream from the City of Oshkosh.

The objective of this research was to survey and analyze 24-hour composite influent and effluent and surface water samples using an alternative extraction procedure for the presence of 60 PPCPs, opioids and recreational drugs from two WWTPs discharging into Lake Winnebago, the largest freshwater inland lake in the State of Wisconsin and serving over 250,000 people for drinking water. Samples were analyzed by liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). Data were further analyzed by percent remaining and removal efficiencies.

Sample Sites

Sampling was conducted at two WWTPs that use Lake Winnebago as their receiving water. WWTP #1 (Figure 2.1) effluent discharges into the Fox River which flows approximately 3218 m (2 miles) to the mouth of Lake Winnebago. WWTP #2 (Figure 2.1) is located along the southern shore of Lake Winnebago and their effluent flows directly into the lake. Surface water samples were taken at the end of the intake pipe of the DWTP in Lake Winnebago. Land cover for the Lake Winnebago watershed consists of 52.55% agriculture, 3.99% developed land, 1.63% wetlands, 35.70% water with the remainder in barren land, forest, and grassland (Lake Winnebago, United States Tourist Information, www.touristlink.com).

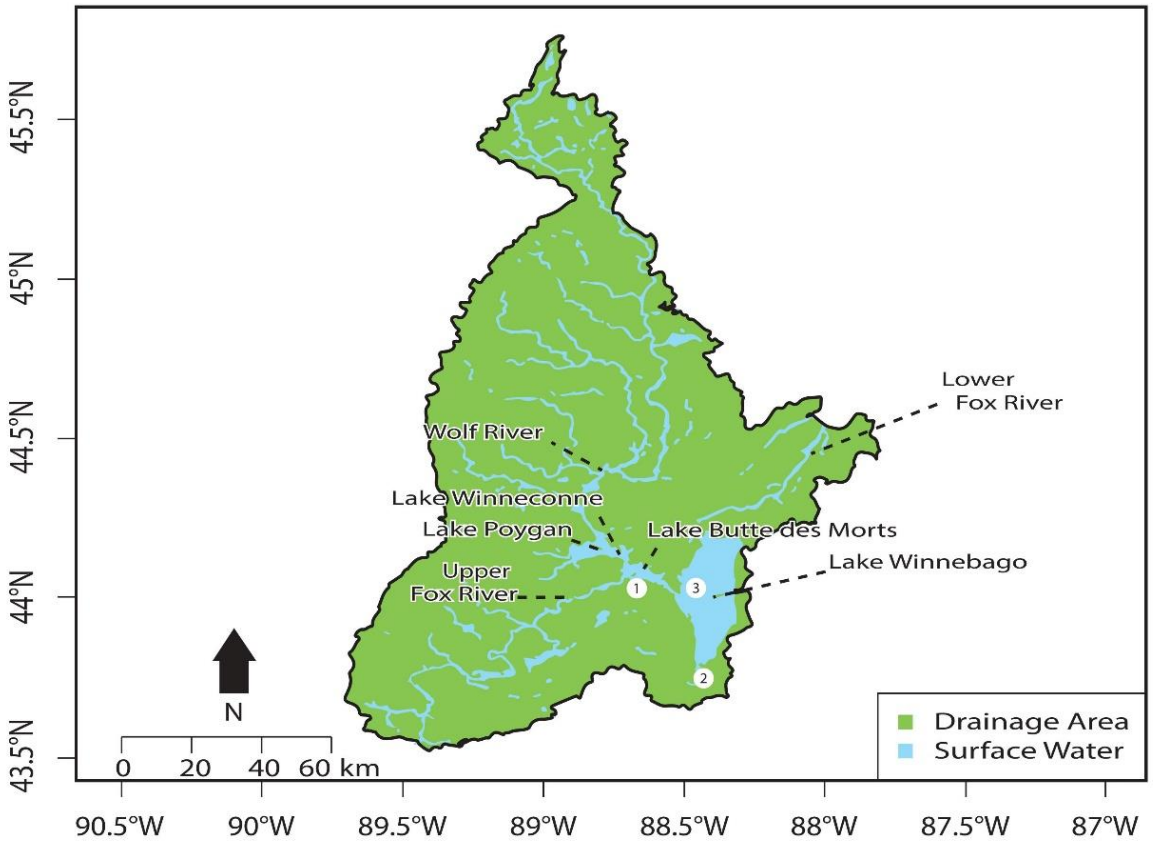


Figure 2.1: Location of both WWTPs and surface water sampling sites. WWTP #1 is (#1) upstream approximately 2 miles from the mouth of Lake Winnebago. WWTP #2 is located (#2) at the south end of Lake Winnebago. Surface water site (3) is located at the drinking water treatment plant intake pipe. Map shows the entire Wolf River Winnebago system which encompasses Lake Poygan, Lake Winneconne, Lake Butte des Morts, the upper Fox River and Lake Winnebago. Lake Winnebago water enters the lower Fox River and ends in the Bay of Green Bay.

Wastewater Treatment Train

Both WWTPs are comprised of pre-treatment, primary and secondary treatment (Figures 2.2a and 2.2 b). Raw wastewater is pumped by the influent pumping station and gravity flows through the screening and grit removal processes before entering the primary clarifiers. At WWTP #1, dissolved air flotation thickeners (DAFT) receives the "scum" and the waste activated scum (WAS) where it is thickened prior to entering the anaerobic digesters. At WWTP #2, heavier, primary solids co-settle to the bottom with WAS as "blended" sludge in the primary clarifiers. Lighter solids or "scum" float on the surface and are collected by surface skimmers. Scum and blended sludge are pumped to the anaerobic digesters. WWTP #1 uses chlorination and WWTP #2 uses the ultraviolet disinfection process.

Blended sludge at WWTP #1 (Figure 2.2a) flows into two anaerobic digesters that are maintained at a pH 7 and a temperature between 96 and 98 º F, then transferred to a third digester where the material remains until it is centrifuged. WWTP #2 (Figure 2.2b) occurs in four temperature-phased anaerobic digesters (two are thermophilic at 130 º F, two are mesophilic at 100 º F). In addition, WWTP #2 receives high strength waste with high chemical oxygen demand (COD) directly into the digesters. Leachate and other hauled substrates are also received at the facility and flow through the headworks. After anaerobic digestion at both plants the sludge is processed by centrifuge dewatering. The liquid portion goes back to the primary clarifier and the solid portion (dewatered sludge, now biosolids) is trucked off each site for final disposal by land application. WWTP #1 is 100% land applied and WWTP #2 is 75% land applied with 25% landfilled.

The differences between the two WWTPs could affect analyte concentrations. They both have different materials entering their plants. WWTP#1 has some industries with heavy metals,

mercury, etc. which must be pretreated prior to entering the sewer system and then the WWTP. WWTP #2 receives high strength waste that is organic in nature such as cheese, ice cream, other dairy products that have high chemical oxygen demand. This type of waste does not enter the headworks but is sent directly to the digesters. Another difference that may affect analyte concentration is the different disinfection regimes. WWTP #1 disinfects with chlorination. Chlorination is very effective against a wide spectrum of pathogenic organisms, plus there is a chlorine residual that remains after the initial disinfection treatment. Chlorination also forms disinfection by-product toxins, such as trihalomethanes which is toxic to aquatic life. WWTP #2 used UV radiation for its disinfection process. UV radiation is effective in inactivating most viruses, bacteria, and spores, but does not have a residual effect after disinfection like chlorination does and total suspended solids in the wastewater may render UV disinfection ineffective. Burch et al. (Burch et al., 2019) reviewed chlorination and UV from WWTPs in terms of antibiotic removal and found chlorination significantly reduces antibiotic concentrations in wastewater effluents. In comparison, UV was less effective. They also found discrepancies across treatment processes such as sampling strategies, specific operating parameters of the WWTPs and deconjugation. The digesters at each plant are also different. WWTP #1 has thermophilic (55-60^{o}) digesters only, while WWTP #2 has 2 thermophilic digesters and 2 mesophilic (35-40^{o}) digesters. Labatut et al. (Labatut et al., 2014) evaluated the influence of organic loading rate and chemical composition on stability between mesophilic and thermophilic digesters and found the stability of the thermophilic co-digestion process is highly dependent on the influent substrate composition. In contrast the mesophilic co-digestion provided a more robust and stable process regardless of influent composition.

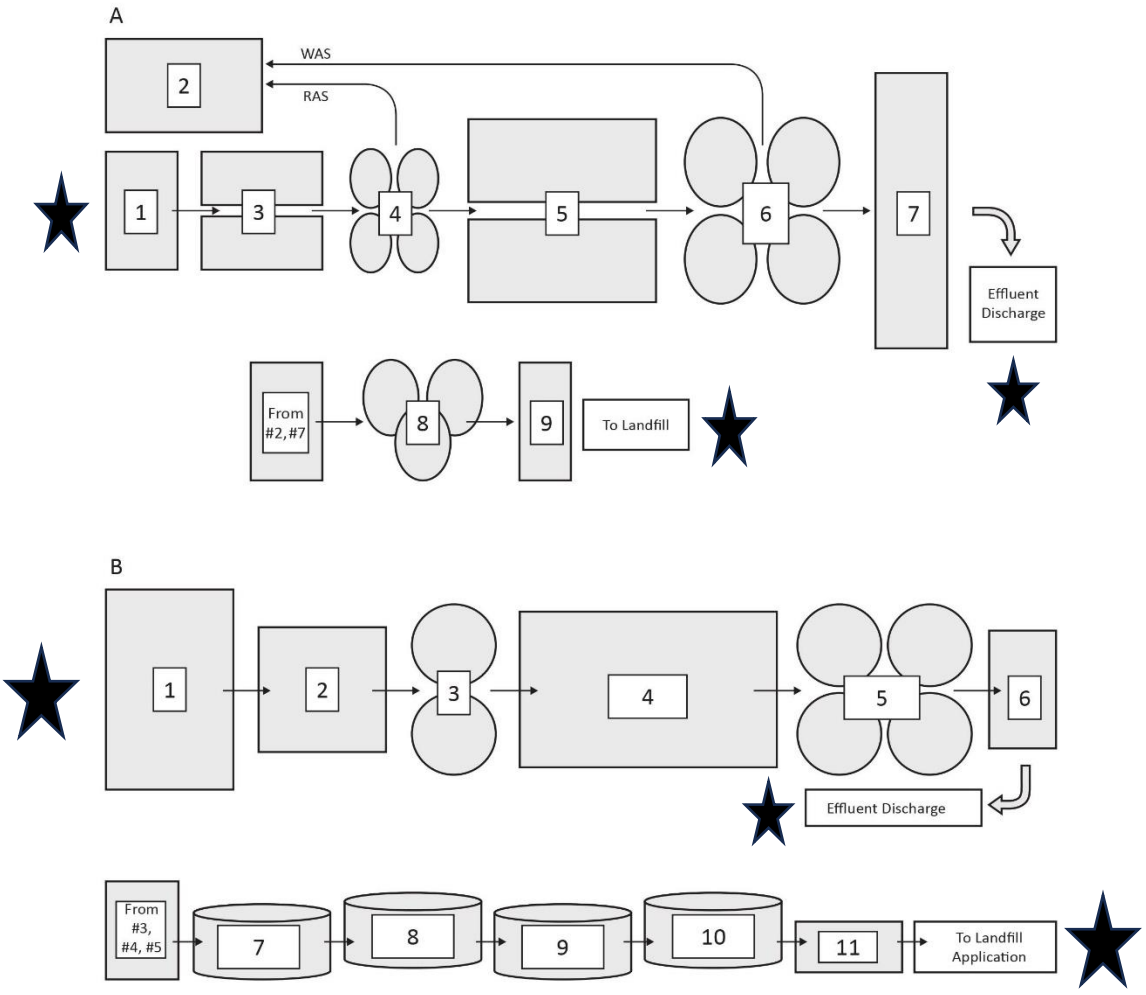


Figure 2.2a: Flow diagram of WWTP #1 showing water treatment train from influent to effluent (1) Influent pump station, (2) Dissolved air flotation thickeners*, (3) Aerated grit chambers, (4) Primary clarifiers, (5) Aeration tanks, (6) Secondary clarifiers, (7) Chlorine contact chamber to effluent discharge. Sludge Treatment: (8) Anaerobic digester complex, (9) Solids handling dewatering building to landfill application. * DAFT handles primary scum and waste activated sludge which are thickened and stored in blended sludge holding tanks until pumped into digesters.

Figure 2.2b: Flow diagram of WWTP #2 showing treatment train from influent to effluent (1) Influent pump station, (2) Aerated grit chamber, (3) Primary clarifiers, (4) Aeration tanks, (5) Secondary clarifiers, (6) UV Disinfection. Sludge treatment (7-8) Thermophilic digesters #1 & #2, (9-10) Mesophilic digesters #3 & #4), (11) Centrifuge sludge dewatering building

Black stars represent the sampling sites.

Chemicals

Sixty standards were analyzed in this research study, and all were of high purity grade (>90%). Chemicals were purchased via Sigma-Aldrich (St. Louis, Missouri), Cayman Chemical (Ann Arbor, Michigan), Acros (Newark, Delaware), Fluka (Belgium), and Cerilliant (Round Rock, Texas). Appendix A, Table 1 shows the targeted compounds, category of each compound and CAS number.

Sample Collection

Samples of influent and effluent were collected from June 18th, 2014, to July 22nd, 2015 on a biweekly or monthly basis from two WWTPs that use Lake Winnebago as their effluent receiving water. Surface water samples were collected from July 16, 2014 to October 16th, 2014 on a biweekly or monthly basis on different dates from the influent and effluent samples. Surface water sampling occurred from an area near the Drinking Water Treatment Plant (DWTP) intake pipe in Lake Winnebago. All samples were collected in amber glass bottles and placed on ice for transport to the laboratory and frozen until extractions could be done. Composite samples were made by combining the duplicate samples from the same date into a beaker. Two-hundred milliliters of each sample were pooled and put into a four-liter plastic container until all dates were added to the container. This procedure was done separately for influent, effluent, and surface water. The remainder of the contents of each sample bottle per date (after the 200 ml was removed) was put back into the proper dated amber bottle and placed in the freezer. PPCPs and/or illicit drugs can fluctuate daily. The use of composite samples helps in alleviating this fluctuation. In this study, samples were collected for an entire year, each sample bottle may contain a totally different combination of PPCPs and illicit drugs. By making composite samples each monthly collection is combined into one getting a diversity of the compounds into one

complex mixture. The composite samples would allow for a better probability of getting the whole picture of compounds over one year.

Alternative Extraction Procedure

Composite samples were thawed. Twenty-five milliliters (ml) of effluent from each WWTP, 10 ml of influent from each WWTP, and 25 ml of surface water were aliquoted in triplicate into 50 ml falcon tubes. Spiked samples contained external standards and a mixed PPCP standard, unspiked samples contained only external standards to examine extraction efficiency. Each falcon tube top was wrapped in parafilm, then 3 holes were poked into the parafilm and put in a -80 ºfreezer until fully frozen. After freezing, the falcon tubes were put on the lyophilizer. After lyophilization, samples were resuspended with 5 ml of 40:40:20 (methanol:acetone:5% glacial acetic acid), vortexed and sonicated in a 50 ºC sonicating water bath for ten minutes. Samples were centrifuged at maximum speed for 15 minutes and the supernatant was transferred to 20 ml scintillation vials. Five ml of solute was added after each centrifugation to the falcon tubes, centrifuged two more times ending with 15 ml of solute in each scintillation vial. Sample extracts were evaporated at 50 ºC. Dried samples were reconstituted with 1 ml of 50% methanol (MeOH), then vortexed, sonicated, and sample material was transferred to 1.5 ml centrifuge tubes and centrifuged for 15 minutes at maximum speed. The supernatant was transferred to 1 ml liquid chromatography (LC) vials, then analyzed by liquid chromatography tandem mass (LC-MS/MS) spectrometry (Appendix A, Figure 1).

LC-MS/MS

Instrumental analysis was performed by LC-MS/MS using a Shimadzu system equipped with an auto sampler and connected in series with a ®4000 Q-Trap triple quadruple ion trap mass spectrometer operating with a Turbo Ion Spray Source in positive scheduled mode. Chromatographic separation was achieved with a Kinetex 1.6 µm C18 100A 50x3 mm column in a 40 º C column oven. Analytes were detected using a multiple reaction monitoring (MRM) mode. Two parent-product transition mass-to-charge transitions (m/z's) were monitored for most analytes (Appendix A, Table 2). Quantitation of peaks was by Analyst® 1.6.3 software by Sciex. Mobile phase A consisted of distilled water, 0.1% formic acid and 5 ml ammonium formate and mobile phase B consisted of 100% acetonitrile. Appendix A, Table 3 shows the elution gradient. The injection volume was 4 µL, flow rate was 3 ml/minute, rinsing volume was 1000 uL, needle stroke 54 mm, rinsing speed 35 µL/sec, sampling speed 15.0 µL/sec and purge time was 25.0 minutes. The total working time was 10 minutes.

Statistical Analyses

Data were analyzed using R Statistical Environment (version 3.0.2 RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL (http://www.rstudio.com/). Actual concentrations to generate the box and whisker plots were calculated by the Analyst software and converted into ng/L for the detected compounds. Removal efficiency and percent remaining was calculated using Equations 1 and 3. Percent recovery was calculated on the spiked samples using Equation 2. Kruskal-Wallis nonparametric tests were used to determine whether there is a statistically significant difference between two independent samples when there is a nonnormal distribution and a small sample size. A significant Kruskal-Wallis test indicates that at least one sample dominates another sample. The

test does not identify where this dominance occurs. Calibration curves were used for quantification (minimum of 7 levels 0.1 to 50 µg/L). A continuous calibration verification sample was run every 12 samples within the batch of samples and calibration curves were run at the beginning and end of each total run. The correlation coefficients (R^2) of each analyte were over 0.99.

Removal Efficiency Equation

Removal efficiencies were calculated for both WWTPs using the percentage of reduction between the compound in influent and the compound in effluent using the following equation:

$$R_{rem}\,(\%) = (\text{Influent} - \text{Effluent})/\text{Influent} \times 100 \qquad \text{(Eq. 1)}$$

Spike Recovery Equation

Spike Recoveries were calculated for all analytes for both WWTPs and surface water samples using the following equation:

$$R_{rec}\,(\%) = (\text{Spiked cal conc} - \text{unspiked cal conc.}) / \text{expected conc} \times 100 \qquad \text{(Eq. 2)}$$

Percent Remaining Equation

Percent remaining was calculated for all analytes for both WWTPs using the following equation:

$$R_{remaining}\,(\%) = \text{Effluent}/\text{Influent} \times 100 \qquad \text{(Eq. 3)}$$

Results and Discussion

Sixty targeted compounds were examined (Table 2.1) from two WWTPs sampled from influent, effluent and surface water. The compounds were grouped into General, Antibiotics,

Opioids and Recreational drugs. Box and whisker plots (Figures 2.3, 2.4, 2.5 and 2.6) show the analyte concentration for both WWTPs.

Table 2.1: Targeted compounds, and metabolites separated by classification. (n=60)

Classification	**Compound**	**Classification**	**Compound**
Ace Inhibitor	Lisinopril	Calcium Channel Blockers	Diltiazem Dehydronifedipine
Anti-acid Reflux	Cimetidine Ranitidine	Anti-lipidemics	Simvastatin
Antibiotics	Ampicillin Azithromycin Carbadox Ciprofloxacin Enrofloxacin Flumequine Lincomycin Lomefloxacin Ofloxacin Oxacillin Penicillin G Penicillin V Roxithromycin Sarafloxacin Sulfachloropyridazine Sulfadiazine Sulfadimethoxine Sulfamerazine Sulfamethazine Sulfamethizole Sulfamethoxazole Sulfamethazine	Opioids	4-ANPP Buprenorphine Carfentanyl Cis-3-methylfentanyl Codeine Fentanyl Hydrocodone Hydromorphone Methadone Meperidine Naloxone Morphine Naltrexone Norfentanyl Norhydrocodone Noroxycodone Oxycodone Oxymorphone Sufentanil Tramadol
		Recreational	6-Monoacetylmorphine Amphetamine Benzoylecgonine Caffeine Cocaine Methamphetamine Paraxanthine
Analgesic	Acetaminophen	Cardiac Glycosides	Digoxigenin
Anti-depressant	Fluoxetine	Fungicide	Thiabendazole Miconazole
Anti-diabetic	Metformin	Herbicide	Atrazine
Anti-epileptic	Carbamazepine	Nicotine Metabolite	Cotinine
Anti-histamine	Diphenhydramine		

At both WWTPs acetaminophen (Figure 2.3) had the highest concentrations in influent, likely due to its prevalent use for pain relief. It has been estimated that billions of doses of acetaminophen are consumed annually (Al-Mashaqbeh et al., 2019; 2020; Nieto-Juárez et al., 2021). Both WWTPs had incomplete elimination of acetaminophen (Figure 2.3). The daily load in influent was 1677 mg/day/1000 inhabitants in summer and 5074 mg/day/1000 inhabitants in winter. Kanama et al. (Kanama et al., 2018) examined 17 PPCPs from two hospital WWTPs in Northwest Province, South Africa and found acetaminophen was the dominant PPCP in the influent with concentrations ranging from 21 to 119 ug/L. The South African WWTPs were able to remove acetaminophen 76 to 98%. Carbamazepine (an antiepileptic drug) and diltiazem (a calcium channel blocker and antihypertensive drug) were not eliminated from effluent at WWTP #1 and miconazole (an antifungal drug) and simvastatin (a cholesterol drug) at WWTP #2, whereas lisinopril was completely removed from effluent at both WWTPs. Carbamazepine is a persistent compound and difficult to eliminate from effluent (Golovko et al., 2021; Zhang et al., 2008b). Loos et al. (Loos et al., 2013) reported a 90% detection of carbamazepine in effluents from European Union WWTPs.

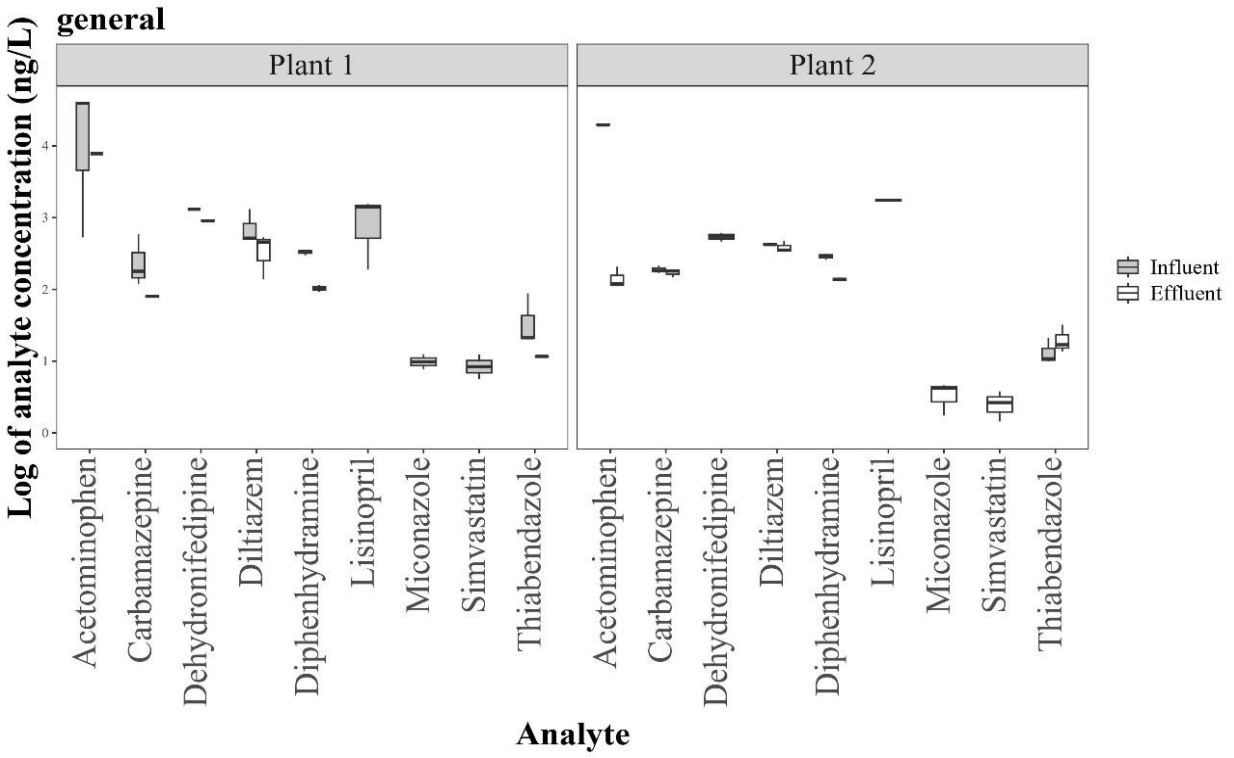


Figure 2.3: Log base 10 Box and Whisker plots showing detected General compounds from WWTPs #1 and #2 from influent and effluent. Gray bars represent influent, white bars represent effluent.

Seven opioids were detected at WWTP #1 and eight opioids were detected at WWTP #2 (Figure 2.4). Oxymorphone and norhydrocodone was not detected at WWTP #1but were detected in the influent at WWTP #2 and eliminated from effluent. Sufentanil was not detected at WWTP #2 (Figure 2.4). Tramadol and methadone had higher concentrations in effluent compared to influent at WWTP #2 indicating it was not completely removed (Figure 2.4). Whereas at WWTP #1 tramadol and methadone had high concentration in influent, but also were not completely removed from effluent. Tramadol is known to have a high risk for dependence and addiction for treatments such as osteoarthritis, gout, etc. Du et al. (Du et al., 2021) collected wastewater samples from 30 cities in 7 geographic regions in China from 2016 to 2019 and found tramadol in most of the samples at concentrations ranging up to 186 ng/L and found

fentanyl detected in only a few samples. In this study, fentanyl was not detected at either WWTP. Cis-3-methylfentanyl, meperidine and norfentanyl had slightly higher concentrations at WWTP#1 compared to WWTP #2 but were eliminated from effluent. Kamika et al. (Kamika et al., 2021) investigated 19 opioids in 4 WWTPs and found some WWTPs were unable to remove methadone (-27.3%), codeine (-21.7%), and hydrocodone (-1.06%). Phillips et al. (Phillips et al., 2010) examined 35 to 38 effluent samples of PPCPs and opioids from three wastewater treatment plants (WWTPs) in New York. Methadone and oxycodone, were detected in NY3 effluent with median concentrations ranging from 3.4 to >400 µg/L and maximum concentrations of oxycodone at 1700 µg/L.

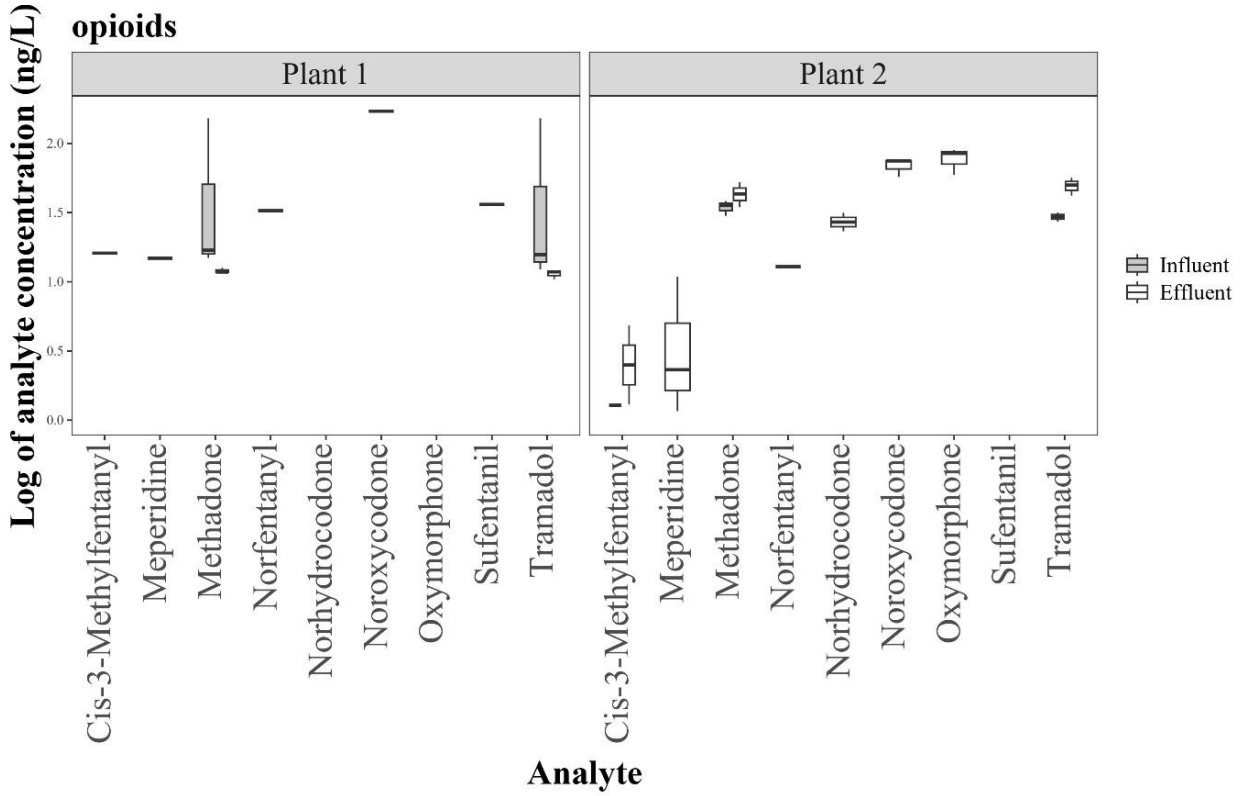


Figure 2.4: Log base 10 Box and Whisker plots showing detected Opioid compounds from WWTPs #1 and #2 from influent and effluent. Gray bars represent influent, white bars represent effluent.

Among the antibiotics, ciprofloxacin, ofloxacin, sulfamethoxazole and trimethoprim antibiotics were detected at both WWTPs (Figure 2.5). Ciprofloxacin, ofloxacin, sulfamethoxazole, and trimethoprim were detected in the influent at WWTP #1 (Figure 2.5). WWTP #1 was unable to completely remove ofloxacin, sulfamethoxazole and trimethoprim from the effluent (Figure 2.5). WWTP #2 was unable to eliminate ciprofloxacin and ofloxacin from the effluent, in addition to trimethoprim. At WWTP #2 there were slightly higher concentrations of ofloxacin and trimethoprim in the effluent indicating incomplete removal. A previous study by Rodriguez-Mozaz et al. (Rodriguez-Mozaz et al., 2020) found ciprofloxacin at concentrations of 1435 ng/L in influent, in addition to ofloxacin at 613 ng/L in effluents of a European wastewater treatment study. Here we found ciprofloxacin in influent at WWTP #2 at just over >5000 ng/L and effluent concentrations at approximately 7000 ng/L. These concentrations are higher than what was observed Martinez-Organiz et al. (Martínez-Orgániz et al., 2021) who observed ciprofloxacin at concentrations ranging from 2,733 to 1,717 ng/L in influent and 494.6 to 444.7 ng/L in effluent. Ofloxacin belongs to the class of quinolone antibiotics and is commonly used for treatment of bacterial infections found in the eye such as pink eye. Shigei et al. (Shigei et al., 2021) found ofloxacin had the highest concentration in wastewater effluent of the 18 targeted antibiotics along with sulfamethoxazole, and ciprofloxacin from the largest treatment facility in the Zarqa River, Jordan. A study by Zhang et al. (Zhang et al., 2017) of 20 antibiotics from WWTP samples from Dalian, China found ofloxacin with the highest influent concentration with average concentration of 609.8 ng/L. In addition, Martinez-Organiz et al. (Martínez-Orgániz et al., 2021) detected ofloxacin at concentrations of 338.9 to 291.5 ng/L in influent, whereas here we found much higher influent concentrations of ofloxacin (>10,000 ng/L). The higher influent concentration of ofloxacin may be that ofloxacin is being prescribed for a wide range of

infections and it is not completely metabolized by the human body so it is eliminated primarily by urinary excretion and discharged into WWTPs influent (Deng et al., 2022; Monk and Campoli-Richards, 1987). The higher ofloxacin influent concentrations in this study may be because there are many hospitals and clinics in the area that may be prescribing this antibiotic more than other antibiotics.

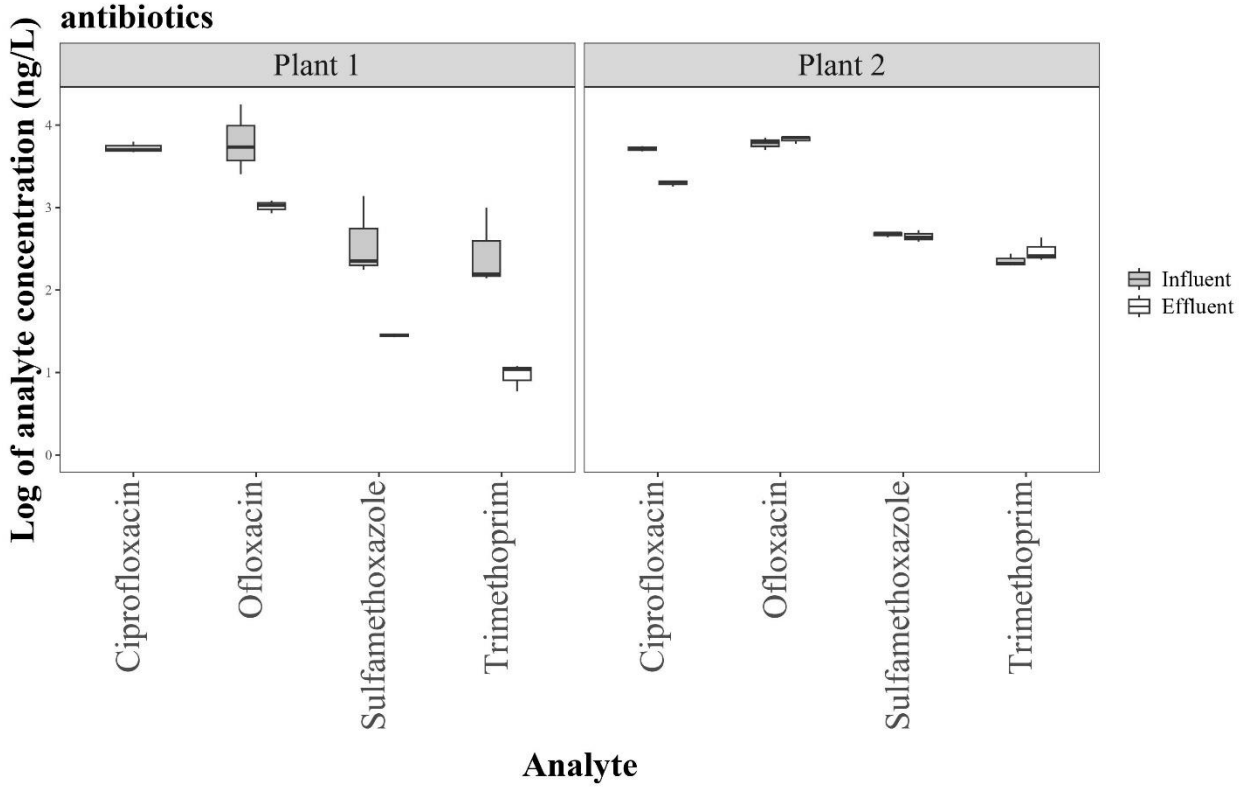


Figure 2.5: Log base 10 Box and Whisker plots showing detected Antibiotic compounds from WWTPs #1 and #2 from influent and effluent. Gray bars represent influent, white bars represent effluent.

Sulfamethoxazole was detected at both WWTPs which represents one of the compounds most frequently found in wastewater due to being a commonly used antibiotic and it has a high excretion rate from humans and animals (de Jesus Gaffney et al., 2017; Martínez-Orgániz et al., 2021; Reyes et al., 2021). Trimethoprim is a sulfonamide antibiotic prescribed for urinary tract

infections. It is commonly found in wastewater (Liu et al., 2021). Kortesmaki et al. (Kortesmäki et al., 2020) observed higher concentrations of trimethoprim in the effluent similar to what was found in this study (Figure 2.5).

Caffeine, and its human metabolite of caffeine called paraxanthine, were detected at both WWTPs (Figure 2.6). Caffeine and paraxanthine had similar concentrations in the influent at WWTP #1 and WWTP #2 but were not completely removed by both WWTPs. A study of influent and effluent samples from the As-Samra Wastewater Treatment Plant in Jordan found caffeine with the highest concentration (155.6 µg/L) and paraxanthine (1,7-dimethylxanthine) to have the third highest concentration (10.49 µg/L) in the influent of the 14 detected compounds (Al-Mashaqbeh et al., 2019). Caffeine had the third highest estimated average concentration (0.086 µg/L) in effluent (Al-Mashaqbeh et al., 2019; 2020). Caffeine found in wastewater influent accounts for disposing of unconsumed caffeinated drinks, or possibly the incomplete metabolism of caffeine in the body found in urine (He et al., 2018; Li et al., 2019a). Guedes-Alonso et al. (Guedes-Alonso et al., 2020) assessed 11 pharmaceutical compounds in three different wastewater treatment plants (WWTPs) in Gran Canaria (Spain) and found caffeine and paraxanthine to have the highest concentrations in influent (45.8 and 95.6 µg/L respectively). Among PPCPs, the highest estimated average concentrations in raw wastewater were caffeine, acetaminophen, 1,7-dimethylxanthine, cotinine, and carbamazepine sampled during the summer, at an estimated concentration of 155.6 µg/L, 36.7 µg/L, 10.49 µg/L, and 1.104 µg/L, respectively.

Benzoylecgonine (BE) and cocaine had lower concentrations in influent at WWTP #1 and were still detected in the effluent. WWTP #2 had higher concentrations of BE and cocaine in effluent (Figure 2.6) compared to influent. In contrast to this study, van Nuijs et al (van Nuijs et

al., 2009) examined cocaine and BE in 37 WWTPs in Belgium and found cocaine and BE detectable in influent concentrations ranging from 10–753 ng/L and 33–2258 ng/L, respectively. Deng et al. (Deng et al., 2020) observed 12 illicit drugs in both influents and effluents from 8 WWTPs where cocaine was the most frequently observed compound in all influent samples with the highest concentration at one WWTP (0.75 ng/L) and the highest concentration in effluent at the same WWTP (0.38 ng/L). Another study by Styszko et al. (Styszko et al., 2021) examined WWTPs along the Wisla River catchment in South Poland and found 68 emerging contaminants were detected in wastewater influent, and 66 emerging contaminants detected in effluent samples. The average concentrations of cocaine and its main metabolite benzoylecgonine were 70 ± 16 ng L−1 and 58 ± 17 ng L−1 in the influents of Plaszow WWTP, and 84 ± 53 ng L−1 and 70 ± 12 ng L−1 in Kujawy WWTP (Styszko et al., 2021).

Amphetamine was detected in the influent at similar concentrations at both WWTPs and was eliminated from the effluent (Figure 2.6). Centazzo et al. (Centazzo et al., 2019) studied 48 wastewater grab samples from six WWTPs from the four boroughs in New York City (Manhattan, The Bronx, Brooklyn and Queens) for one year. Amphetamine was detected in all wastewater samples (n=48) with the highest concentration of amphetamine (265.1 ng/mg) in Newtown Creek, Manhattan before Memorial Day and the lowest concentration was 12.9 ng/mg in Hunts Point (The Bronx) after New Year's. In addition, all 48 samples were positive for cocaine and BE with cocaine's highest concentration (1814.8 ng/mg) in The Bronx and BE's highest concentration (947.2 ng/mg) also in The Bronx.

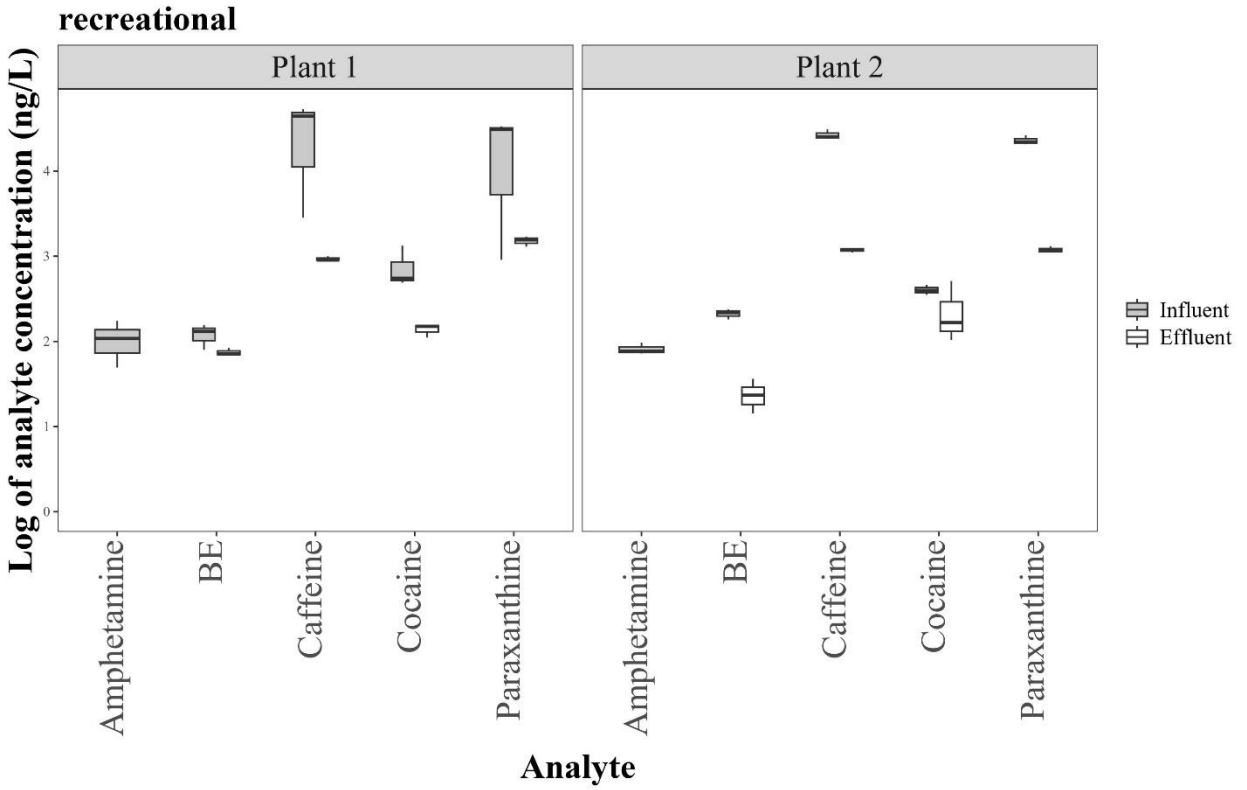


Figure 2.6: Log base 10 Box and Whisker plots showing detected Recreational compounds from WWTPs #1 and #2 from influent and effluent. Gray bars represent influent, white bars represent effluent.

Figure 2.7 shows the targeted analytes detected in lake surface water sampled near the intake pipe of the water treatment plant in Lake Winnebago. Thiabendazole, dehydronifedipine, caffeine, cocaine, BE and amphetamine had the highest concentrations in surface water. Other studies found cocaine in surface water at much lower concentrations of 10 ng/L in Spain (Huerta-Fontela et al., 2011), <1 to 753 ng/L in Belgium (van Nuijs et al., 2009), and 78 ng/L in a South Wales study (Kasprzyk-Hordern et al., 2008b; Khalik et al., 2017; Kolpin et al., 2002). Ferry et al. conducted a study (Ferrey et al., 2018) in the Grand Portage Indian Reservation in northeastern Minnesota found ciprofloxacin in rain samples (10.3 ng/L) suggesting that atmospheric wet deposition may play a role in waters with minimal human impact. These results

indicate that drugs of abuse present in surface waters are impacted by wastewater discharges, though at very low concentrations. Cocaine is easily transformed to BE in the human body so one should see a greater concentration of BE compared to cocaine. This study found higher cocaine concentrations than its metabolite BE. Bones et al. (Bones et al., 2007b) observed similar results whereby cocaine was detected in surface water at higher concentrations (between 25 to 489 ng/L) than BE (22 to 290 ng/L). Bones et al. (2007) explained these concentrations based on a sewage epidemiology approach, using levels of excreted drug residues in wastewater. The amounts of drug residues found in the WWTPs reflected the amounts excreted with urine. These data were used to estimate consumption of the active parent drugs. In their research, Bones et al. (Bones et al., 2007b) used Zucatto et al. (Zuccato et al., 2008) approach which studied cocaine in WWTPs and receiving waters in Dublin and Greater Dublin area of Ireland. Cocaine concentrations were detected in influents, effluents, and surface waters and was used to estimate consumption of cocaine within the community served by those WWTPs. Zucatto et al. (Zuccato et al., 2008) included two assumptions: firstly, that cocaine is relatively stable in wastewater; and secondly, that the source of the cocaine comes from human excretion rather than the dumping of cocaine into the WWTP system.

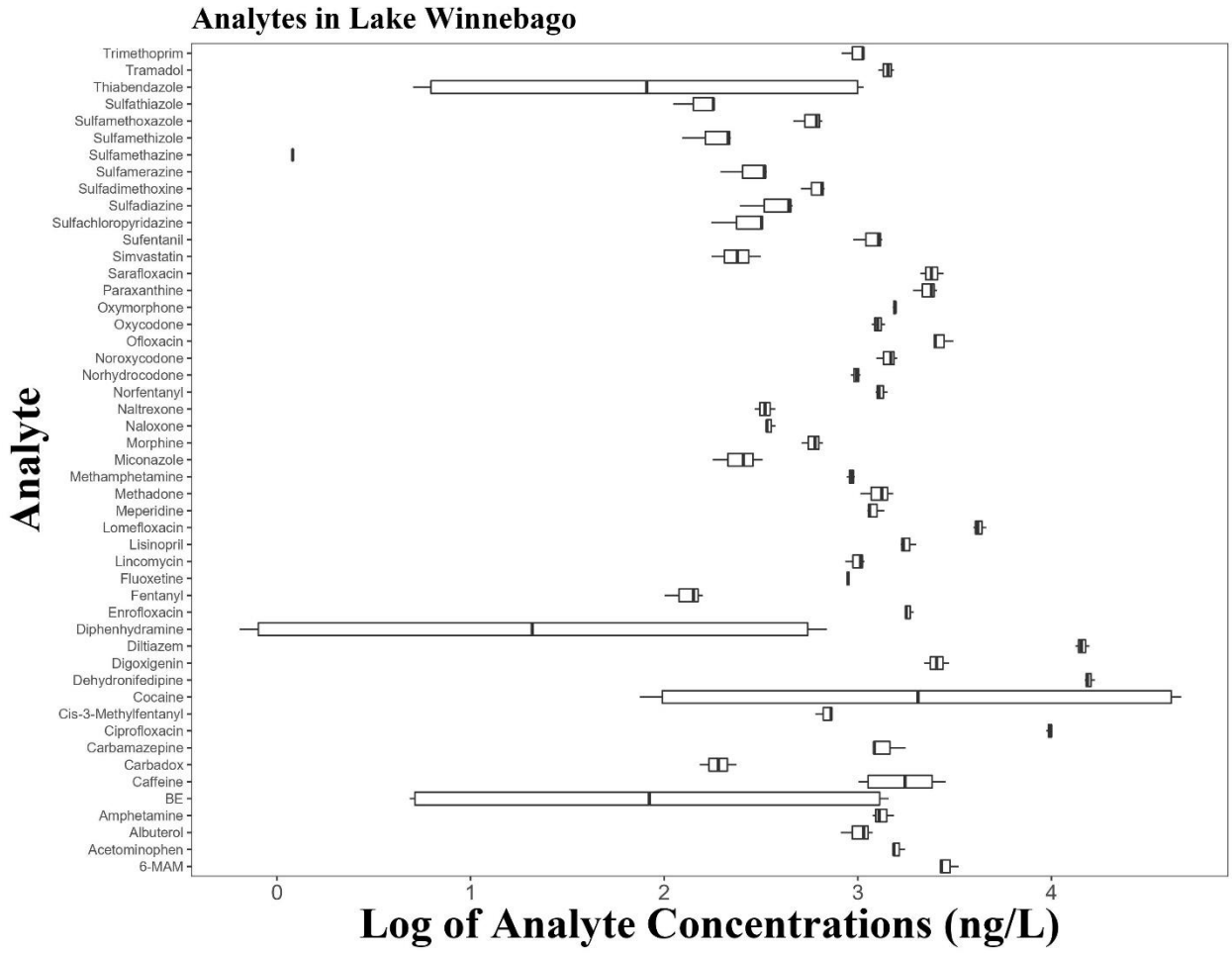


Figure 2.7: Log base 10 Box and Whisker plots showing detected surface water compounds from WWTPs #1 and #2 from influent and effluent.

Removal Efficiency

PPCPs, opioids and recreational drugs are frequently detected in WWTP effluent. Conventional treatment processes do not remove some compounds completely (Gracia-Lor et al., 2012; Kolpin et al., 2002). Though removal concentration rates are low, different removal rates occur because of different physiochemical properties of the compounds (Gracia-Lor et al., 2012). A summary of removal efficiency (Eq. 1) of each analyte and each WWTP is presented in Figure 2.8. Dehydronifedipine was the only compound from WWTP #1 that had negative removal compared to 14 compounds at WWTP #2. WWTP #1 was more efficient at removal of the compounds than WWTP #2. The Kruskal-Wallis non-parametric ANOVA test for removal efficiency between plants was significant (p-value = 0.0003).

However, both WWTPs had 100% removal for amphetamine and lisinopril (Figure 2.8) which corresponds to the box and whisker plots that showed complete elimination of these compounds from the effluent (Figures 2.6 and 2.3 respectively).Caffeine, ciprofloxacin, cis-3-methyfentanyl, meperidine, miconazole, norfentanyl, noroxycodone, simvastatin, sufentanil and trimethoprim all had 100% removal at WWTP #1, whereas acetaminophen had 100% removal at WWTP #2 (Figure 2.8). Carbamazepine, diltiazem, and sulfamethoxazole had the lowest removal efficiency of all the compounds from WWTP #2 (Figure 2.8). Notably, carbamazepine, which is an anti-epileptic drug, and the antibiotic sulfamethoxazole are among the top 5 most frequently detected compounds globally (Zhang et al., 2008a) and was detected in the influents of all seven WWTPs sampled by Khasawneh and Palaniandy (Khasawneh and Palaniandy, 2021). Carbamazepine has been proposed as an anthropogenic marker in water bodies (Clara et al., 2004b). Carbamazepine demonstrated a high degree of persistence due to its low water solubility (Al-Mashaqbeh et al., 2019; Gao et al., 2016; Jelic et al., 2011; Radjenović et al., 2009;

Zhang et al., 2008b). Verlicchi et al. (Verlicchi et al., 2012) found carbamazepine removal efficiency between -67 and 11%. The human body can metabolize 98% to 99% of carbamazepine and transform it to carbamazepine-10,11-epoxide (Doummar et al., 2014).

One study of sulfamethoxazole from two WWTPs observed no removal of sulfamethoxazole but found higher concentrations in the final effluent at +36% in WWTP #1 and +71% in WWTP #2 (Phonsiri et al., 2019). Nas et al. (Nas et al., 2021) found different results from this study for sulfamethoxazole removal efficiency. This study found low removal efficiency at WWTP #2, where Nas et al. found negative removal efficiency (−133.4%) in WWTPs having advanced biological treatment. (Haddaoui and Mateo-Sagasta, 2021)

Negative removal is a commonly found occurrence in all wastewater treatment plants irrespective of the pollutants, amount of wastewater, capacity of the treatment plants, and regions (Kumar et al., 2022). Fourteen analytes had negative removal rates (Figure 2.8). The negative removal of analytes was observed at both WWTPs, though WWTP #2 had the most negative removal compounds compared to only one compound (dehydronifedipine) at WWTP#1 (Figure 2.8). Possible explanations for negative removal are that some compounds have conjugate compounds that are not detected in the influent, but retransform back into the original compound during the treatment process resulting in an enhanced concentration of the parent compound in the effluent (Carmona et al., 2014; Dinh et al., 2017; Göbel et al., 2005; Gulkowska et al., 2008; Kermia et al., 2016; Salgado et al., 2012). This is dependent on factors like the compounds chemical structure or the specific treatment processes by each WWTP and residence time at different WWTPs (Carmona et al., 2014; Gulkowska et al., 2008). Not all metabolites and conjugate forms are easily transformed within wastewater, some compounds, such as tramadol, may partly be caused by a combination of biotic and abiotic events that lead to transformation of

parental compounds and their metabolites (Archer et al., 2017b). Haddaoui and Mateo-Sagasta (Haddaoui and Mateo-Sagasta, 2021) speculated that differences in negative removal may be due to the type of influent wastewater containing the contaminants which may inhibit the effectiveness of biological treatment or favor transformation or degradation, or treatment conditions such as the age of the WWTP, and climate conditions, or operational conditions such as shorter retention times based on overloading beyond the WWTP capacity.

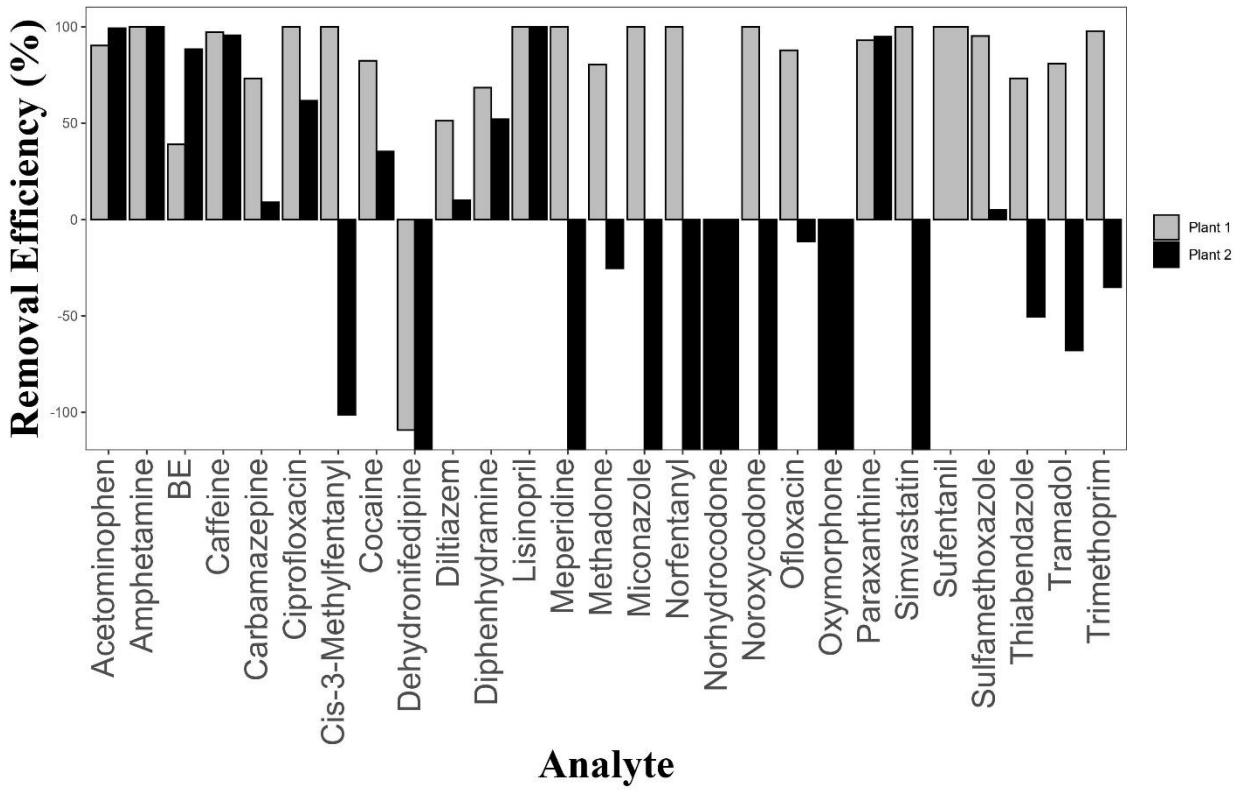


Figure 2.8: Bar graph showing removal efficiency of detected compounds from WWTPs #1 and #2. Gray bars are WWTP #1 and black bars are WWTP #2.

Percent Remaining

Figure 2.9 (Eq. 3) shows the analyte concentration that entered the WWTPs as influent but remained in the effluent. WWTP #1 had less analytes in the effluent than WWTP #2. Fifteen analytes were remaining in the effluent at WWTP #1, whereas WWTP #2 had eighteen analytes remaining in the effluent. Acetaminophen, carbamazepine, caffeine, cocaine, olfloxacin, methadone, paraxanthine, sulfamethoxazole, thiabendazole, tramadol and trimethoprim were found in the effluent at ≤ 25% at WWTP #1. WWTP #1 had no analytes remaining at 100% and only BE (60%), diltiazem (48%), and diphenhydramine (31%) were the highest analytes remaining in effluent. Meperidine, miconazole, norfentanyl, norhydrocodone, noroxycodone, oxymorphone, and simvastatin, had negligible concentrations in effluent or were not detected at WWTP #1 (Figure 2.9). Amphetamine, cis-3-methyfentanyl, lisinopril, and sufentanil were not detected in effluent at either WWTPs. WWTP #2 had eight analytes (Figure 2.9) with concentrations over 100% in the effluent. In addition, carbamazepine (90%), cocaine (64%), diltiazem (90%), diphenhydramine (47%) and sulfamethoxazole (94%) were detected in the effluent at WWTP #2. BE (11%) at WWTP #2, had slightly lower concentrations in the effluent compared to BE (60%) at WWTP #1. Dehydronifedipine, meperidine, miconazole, norfentanyl, norhydrocodone, noroxycodone, oxymorphone, and simvastatin were 100% remaining in the effluent at WWTP #2. The Kruskal-Wallis statistical test for percent remaining was significant between plants ($p = 0.0003$). A comparison of removal efficiency (Figure 2.8) and percent remaining in effluent (Figure 2.9) shows that dehydronifedipine, meperidine, miconazole, norfentanyl, norhydrocodone, noroxycodone, oxymorphone and simvastatin are in unification with one another.

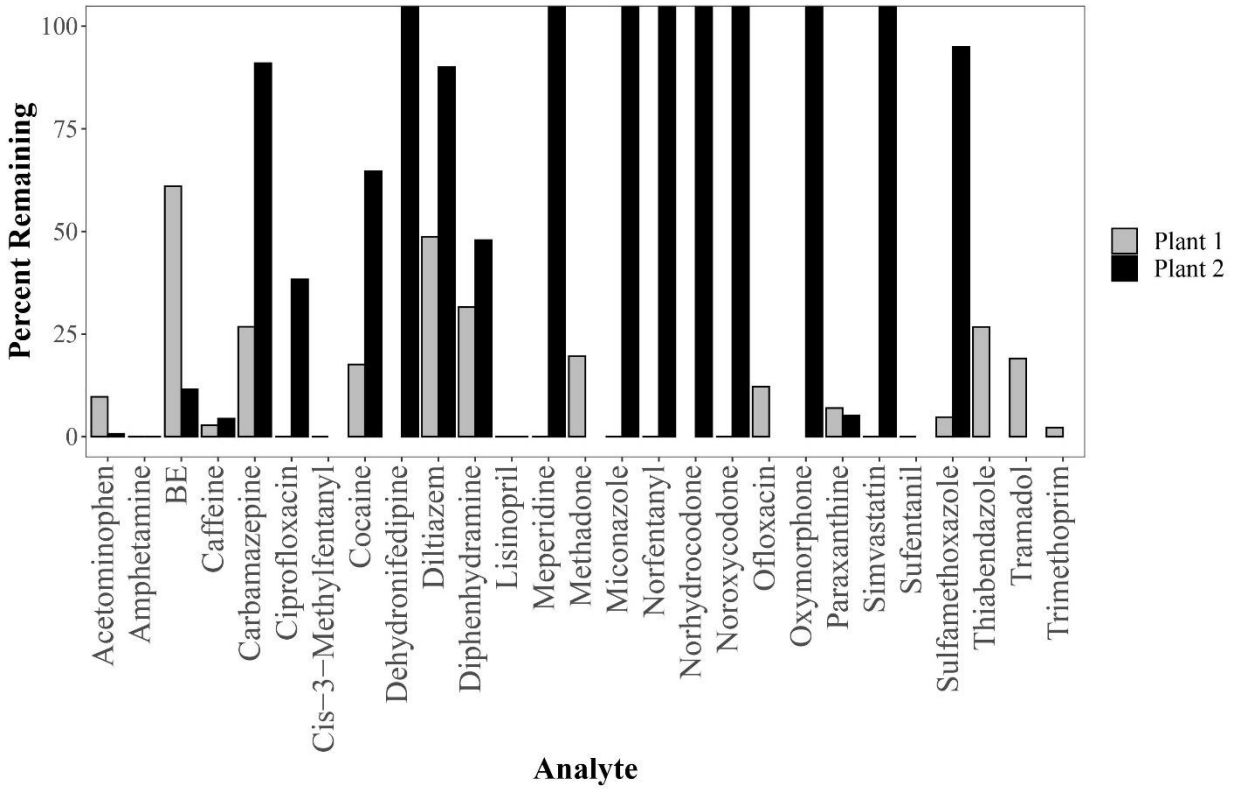


Figure 2.9: Bar graph of percent remaining of detected analytes from WWTP #1 and WWTP #2. Gray bars are WWTP #1 and black bars are WWTP #2. Shown are the analytes that entered the plants as influent but remained in the effluent.

Percent Recovery

Percent recovery is tested by adding a known amount of analyte into samples followed by extraction and analysis of the method being tested. Spike recovery is used to evaluate the accuracy of analytical results. In this study the performance of the alternative extraction method was assessed. Percent recovery was calculated (Eq. 2) for all the analytes from both WWTPs and from lake surface water (Tables 2.2, 2.3, 2.4). The following tables (2.2, 2.3, 2.4) show percent recovery values for analytes with a 50% or higher recovery value for WWTP #1, WWTP #2 and surface water. To view the percent recovery values for all the analytes see Appendix A6, A7, and A8. Percent recovery values for WWTP #1 ranged from 55% for thiabendazole to 198% for ciprofloxacin (Table 2.3) and for WWTP #2 ranged from 50% for sulfamethoxazole to 270% for ciprofloxacin (Table 2.4). Lake water percent recoveries ranged from 0% for 4-ANPP to dehydronipedipine at 782.6%. Cocaine, diltiazem and ciprofloxacin samples showed an abnormally high percent recovery 2155.4% to 2953.6% for cocaine from WWTP #1, WWTP #2 and surface water (Tables A6, A7, A8)), and 684.6% to 722.0% for diltiazem and 753.0% to 489.3% for ciprofloxacin (A6, A7, A8). The samples may have contained contaminates or the solvent was not completely dried off. Other reasons may be there was a bias in the method, the precision of the method is poor.

The alternative extraction method was applied to 60 diverse compounds but there are some limitations on percent recovery as seen by cocaine and ciprofloxacin. The alternative extraction method showed very good percent recovery values for BE (57.7% to 79.5%), caffeine 67.7% to 138.8%), lomefloxacin (66.5% to 298%), meperidine (60.6% to 82.8%), methadone (57.8% to 81.4%), thiabendazole (50.0% to 66.5%) and tramadol(58.8% to 75.6%) for WWTP #1 (Table 2.2, Table A6), in addition to WWTP#1 recovery values, WWTP #2 had good

recovery values for albuterol, amphetamine, carbamazepine, lincomycin, methamphetamine, family of morphine compounds and codone compounds, naloxone, norfentanyl, ofloxacin, sufentanil, and tramadol (Table 2.3, Table A7). Surface water percent recovery values were similar to WWTP #1 and WWTP #2 except for acetaminophen (80.2%) (T able 2.4, Table A8). The alternative extraction method showed limitations with the majority of the sulfa compounds, in addition to carbadox, fentanyl and miconazole (Table 2.2, 2.3. 2.4, A6, A7, A8).

Table 2.2: Average Percent Recovery from analyte samples from WWTP #1 using Equation 2 and having over 50% recovery.

Analyte	WWTP#1 Percent Recovery
Benzoylecgonine	57.7
Caffeine	81.9
Ciprofloxacin	198.5
Cocaine	2706
Dehydronipedipine	161
Diltiazem	436
Enrofloxacin	170.7
Lomefloxacin	66.5
Meperidine	69.6
Methadone	57.8
Ofloxacin	191.7
Thiabendazole	55.5
Tramadol	58.8

Table 2.3: Average Percent Recovery from analyte samples from WWTP #2 using Equation 2 and having over 50% recovery.

Analyte	WWTP#2 Percent Recovery
6-MAM	271
Acetominophen	129
Albuterol	51.6
Amphetamine	93.2
Benzoylecgonine	79.5
Caffeine	138.8
Carbamazepine	54
Ciprofloxacin	753
Cocaine	2953
Dehydronipedipine	215
Diltiazem	684
Enrofloxacin	191
Lincomycin	80
Lisinopril	145
Lomefloxacin	298
Meperidine	82.8
Methadone	81.4
Methamphetamine	59.7
Morphine	93.7
Naloxone	89.5
Norfentanyl	91
Norhydrocodone	73.6
Noroxycodone	88
Ofloxacin	146
Oxycodone	78
Oxymorphone	97.5
Paraxanthine	158.6
Sufentanil	78.8
Sulfamethoxazole	50.1
Thiabendazole	66.5
Tramadol	75.6
Trimethoprim	61.6

Table 2.4: Average Percent Recovery from analyte samples from surface water using Equation 2 and having over 50% recovery.

Analyte	Surface Water Percent Recovery
6-MAM	144.6
Acetominophen	80
Albuterol	51
Amphetamine	67
Benzoylecgonine	65.4
Caffeine	67.7
Carbamazepine	69.7
Ciprofloxacin	489.3
Cocaine	2155
Dehydronipedipine	782.6
Diltiazem	722
Enrofloxacin	91
Lisinopril	89.6
Lomefloxacin	211.6
Meperidine	60.6
Methadone	64.7
Norfentanyl	65.6
Noroxycodone	72
Ofloxacin	134.6
Oxycodone	63.7
Oxymorphone	77
Paraxanthine	114.7
Sufentanil	59
Thiabendazole	50
Tramadol	70.8

Conclusions

A survey was conducted of 60 diverse PPCPs and illicit compounds from two WWTPs using Lake Winnebago as their effluent receiving water. Both WWTPs service different types of industries, manufacturing, medical, etc. WWTPs are a major source of these compounds entering the aquatic environment. Other sources to the aquatic environment are from agricultural practices, veterinary purposes and CAFOs, etc. This study detected a variety of different compounds from general (acetaminophen, lisinopril), opioids (tramadol, methadone), antibiotics (ciprofloxacin and ofloxacin) and recreational drugs (caffeine, cocaine). Surface waters were sampled at the drinking water intake pipe in Lake Winnebago. Lake Winnebago serves over 250,000 people for their drinking water source. Results found cocaine and BE in these water samples. An alternative extraction protocol for PPCPs and illicit drugs was used instead of the SPE method commonly used in the literature. Some limitations to the alternative extraction method were seen for some compounds.

Removal efficiency was examined for PPCPs and illicit drugs from both WWTPs and the Kruskal-Wallis statistical test was significant for removal efficiency between plants (p=0.0003). WWTP #1 was more efficient than WWTP #2 at removing analytes. Negative removal, a common phenomenon occurring at most WWTPs was also seen in this study. WWTP #2 had more negative removal of analytes than WWTP #1.

Many analytes are successfully processed through the WWTP and do not end up in effluent. Attention must be given to those analytes that do end up in effluent and enter the aquatic environment. More research is needed on the long-term effects of these compounds on the aquatic organisms' potential sensitivity to them.

Chapter 3:

Mass balance, removal, and seasonality of sixty pharmaceuticals and personal care products (PPCPs), and illicit drugs from two wastewater treatment plants (WWTPs).

Introduction

Pharmaceuticals and personal care products (PPCPs) and illicit drugs comprise a large and diverse group of compounds. Antibiotics, central nervous stimulants, opioids, and recreational drugs are a few of the categories. They are widely used in many fields such as medicine, industry, livestock farming, aquaculture, and daily life for most people (Barceló and Petrovic, 2007; Ebele et al., 2017; Ziylan-Yavas et al., 2022). Their usage and consumption are increasing due to factors such as the development of new drugs, an aging population, an expanding population, over prescribing of prescription drugs and easier accessibility to drugs whether legally prescribed or not. According to the IQVIA Institute (2022) the biggest contributors to the global growth in prescriptions in the next five years are oncologic, immunology, anti-diabetics, and neurology — the growth being a result of continuous influx of innovative products. In 2021, pharmaceutical expenditures in the United States grew 7.7% compared to 2020 for a total of $576.9 billion (Tichy et al., 2022).

Wastewater Treatment Plants (WWTPs) are a major source of environmental pollutants to the environment through effluent and biosolids. Human excretion is the major influent source (Archer et al., 2017b; Szopińska et al., 2022), along with veterinary drugs used for livestock, herbicide run-off, disposal of expired drugs to name a few. The wastewater effluent is discharged into rivers, streams, or lake systems, and the biosolids are spread on fields as fertilizer (Petrie et al., 2015). Wastewater treatment processes do not remove all these pollutants that are entering the WWTPs as influent and furthermore, the WWTPs are not required to monitor for these environmental pollutants (Gago-Ferrero et al., 2017; Gerbersdorf et al., 2015; Golovko et al.,

2014a; Petrie et al., 2015). These pollutants may enter the receiving waters in un-metabolized forms or as metabolites with little known about the effect on the environment (Kay et al., 2017; Martín et al., 2012; Petrie et al., 2015; Vatovec et al., 2016). This has the potential to become an emerging public health concern for humans and animals because of the increased use of the compounds which results in a continuous supply to the environment (Ebele et al., 2017).

The environmental pollutants most often found in wastewater effluent are antibiotics, epileptic drugs, ace inhibitors, analgesics, herbicides, illicit drugs such as cocaine, and recreational drugs such as methamphetamine. One study of an eastern Canadian city measured six psychoactive drugs by comparing usage on weekdays and weekends (Palardy et al., 2016b). Benzoylecgonine and methamphetamine (both recreational drugs) concentrations ranged from 6.3 ng/L on the weekend to below the limit of detection on weekdays for effluent. Codeine, morphine and methadone (all opioids) concentrations were between 7.5 ng/L and 71.4 ng/L in effluent with no difference between weekend and week day sampling (Palardy et al., 2016b). A study of five WWTPs from the largest industrial city in Korea yielded a high percentage of antibiotics (56% to 81% of samples) in effluent with lincomycin present (49% to 81%) in all the WWTPs followed by naproxen, an anti-inflammatory (3% to 5.6%) (Behera et al., 2011). Blair et al. (Blair et al., 2013) evaluated 54 PPCPs at various sample sites and a site near an effluent discharge area in Lake Michigan and Milwaukee harbor and found three compounds (metformin 100%, caffeine 97.6%, sulfamethoxazole 83.3%) detected with greater than 50% occurrence at all sampling sites.

Most research has focused on the concentrations of PPCPs and illicit drugs in the aqueous phases of influent and effluent. These compounds may also sorb onto suspended particles in wastewater and are found in biosolids (Martín et al., 2012; Yan et al., 2014). Antibiotics, such as

ciprofloxacin and norfloxacin have been found in large amounts in biosolids (Kümmerer, 2009; Martín et al., 2015). Caffeine and some anti-inflammatory drugs (NSAIDS) may be found in biosolids because of high mass loads in WWTPs (Carballa et al., 2007; Jelic et al., 2011; Martín et al., 2015). The octanol/water partitioning coefficient (K_{ow}) indicates a compounds affinity to sludge (Scheytt et al. 2005). A high K_{ow} value for a compound indicates the compound will partition to sludge rather than the aqueous phase. It is important to analyze concentrations of PPCPs and illicit drugs in biosolids because most biosolids are recycled as fertilizer in agriculture. Sewage sludge is rich in nutrients because it contains a mixture of organic, inorganic, and microbiological contaminants provides an economical way to fertilize agricultural lands (Ahmad et al. 2004).

The objective of this research was to assess the distribution of diverse types of PPCPs, and illicit drugs present in influent, effluent and biosolids from two WWTPs. An alternative extraction process was used to extract the PPCPs and illicit drugs from the samples collected from each WWTP. Samples were analyzed by liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). Data were further analyzed by removal efficiencies; seasonality and a mass balance analysis were performed to identify the effective elimination process and to estimate distribution for these PPCPs and illicit drugs.

Sample Sites

Sampling was conducted at two WWTPs that use Lake Winnebago as their receiving water. Plant #1 (Figure 2.1) effluent discharges into the Fox River which flows approximately 3218 m (2 miles) to the mouth of Lake Winnebago. Plant #2 (Figure 2.1) is located along the southern shore of Lake Winnebago and their effluent discharges directly into the lake. Land cover for the Lake Winnebago watershed consists of 52.55% agriculture, 3.99% developed land,

1.63% wetlands, 35.70% water with the remainder in barren land, forest, and grassland (Lake Winnebago, United States Tourist Information, www.touristlink.com).

Both WWTPs are comprised of pre-treatment, primary and secondary treatment (Figures 2.2a and 2.2b). Raw wastewater is pumped by the influent pumping station and gravity flows through the screening and grit removal processes before entering the primary clarifiers. At Plant #1, dissolved air flotation thickeners (DAFT) receive the "scum" and the waste activated scum (WAS) where it is thickened prior to entering the anaerobic digesters. At Plant #2, heavier, primary solids co-settle to the bottom with WAS as "blended" sludge in the primary clarifiers. Lighter solids or "scum" float on the surface and are collected by surface skimmers. Scum and blended sludge are pumped to the anaerobic digesters. Plants #1 and #2 use either chlorination or ultraviolet radiation, respectively for their disinfection process.

Blended sludge at Plant #1 (Figure 2.2a) flows into two anaerobic digesters that are maintained at a pH 7 and a temperature between 96 and 98 º F, then transferred to a third digester where the material remains until it is centrifuged. Plant #2 (Figure 2.2b) occurs in four temperature-phased anaerobic digesters (two are thermophilic at 130 º F, two are mesophilic at 100 º F). In addition, Plant #2 receives high strength waste with high chemical oxygen demand (COD) directly into the digesters. Leachate and other hauled substrates are also received at the facility and flow through the headworks. After anaerobic digestion at both plants the biosolids are processed by centrifuge dewatering. The liquid portion goes back to the primary clarifier and the solid portion (dewatered sludge) is trucked off each site for final disposal by land application. WWTP #1 is 100% land applied and WWTP #2 is 75% land applied.

Biosolids must meet federal and state requirements if applied to agricultural land. Most states require permits to apply biosolids. Class A permits have specified treatment requirements

for requirements for removal of pollutants, and pathogens. Class B permits have reduced pathogens, but not eliminated. The Environmental Protection Agency (EPA) Biosolids Program identifies pollutants found in biosolids through reviews and sewage sludge surveys to assess their potential risk to public health and the environment.

The differences between the two WWTPs could affect analyte concentrations. They both have different materials entering their plants. WWTP#1 has some industries with heavy metals, mercury, etc. which must be pretreated prior to entering the sewer system and then the WWTP. WWTP #2 receives high strength waste that is organic in nature such as cheese, ice cream, other dairy products that have high chemical oxygen demand. This type of waste does not enter the headworks but is sent directly to the digesters. Another difference that may affect analyte concentration is the different disinfection regimes. WWTP #1 disinfects with chlorination. Chlorination is very effective against a wide spectrum of pathogenic organisms, plus there is a chlorine residual that remains after the initial disinfection treatment. Chlorination also forms disinfection by-product toxins, such as trihalomethanes which is toxic to aquatic life. WWTP #2 used UV radiation for its disinfection process. UV radiation is effective in inactivating most viruses, bacteria, and spores, but does not have a residual effect after disinfection like chlorination does and total suspended solids in the wastewater may render UV disinfection ineffective. Burch et al. (Burch et al., 2019) reviewed chlorination and UV from WWTPs in terms of antibiotic removal and found chlorination significantly reduces antibiotic concentrations in wastewater effluents. In comparison, UV was less effective. They also found discrepancies across treatment processes such as sampling strategies, specific operating parameters of the WWTPs and deconjugation. The digesters at each plant are also different. WWTP #1 has thermophilic (55-60^{o}) digesters only, while WWTP #2 has 2 thermophilic digesters and 2

mesophilic (35-40^{o}) digesters. Labatut et al. (Labatut et al., 2014) evaluated the influence of organic loading rate and chemical composition on stability between mesophilic and thermophilic digesters and found the stability of the thermophilic co-digestion process is highly dependent on the influent substrate composition. In contrast the mesophilic co-digestion provided a more robust and stable process regardless of influent composition.

Chemicals

Sixty standards were analyzed in this research study, and all were of high purity grade (>90%). Chemicals were purchased via Sigma-Aldrich (St. Louis, Missouri), Cayman Chemical (Ann Arbor, Michigan), Acros (Newark, Delaware), Fluka (Belgium), and Cerilliant (Round Rock, Texas). Appendix A, Table 1 shows the targeted compounds, category of each compound and CAS number.

Sample Collection

Samples of influent, effluent and sludge were collected from September 2016 to December 2017 monthly from two WWTPs that use Lake Winnebago as their effluent receiving water. Influent and effluent samples were collected in amber glass bottles, and biosolid samples were put in freezer bags and placed on ice for transport to the laboratory and frozen until extractions could be done.

Alternative Extraction Procedure

Aqueous Samples

Samples were thawed. Twenty-five milliliters (ml) of effluent from each WWTP and 10 ml of influent from each WWTP were aliquoted in triplicate into 50 ml falcon tubes. Spiked samples contained external standards and a mixed PPCP/Opioid standard. Each falcon tube top

was wrapped in parafilm, then 3 holes were poked into the parafilm and put in a -80 ºfreezer until fully frozen then placed on the lyophilizer. After lyophilization, samples were resuspended with 5 ml of 40:40:20 (methanol:acetone:5% glacial acetic acid) solute, vortexed and sonicated in a 50 ºC sonicator for ten minutes. Samples were centrifuged at maximum speed for 15 minutes and the supernatant was transferred to 20 ml scintillation vials. Five ml of solute was added after each centrifugation to the falcon tubes, centrifuged two more times ending with 15 ml of solute in each scintillation vial. Sample extracts were evaporated at 55 ºC. Dried samples were reconstituted with 1 ml of 50% MeOH, then vortexed, sonicated, and sample material was transferred to 1.5 ml centrifuge tubes and centrifuged for 15 minutes at maximum speed. Supernatant was transferred to liquid chromatography (LC) vials and analyzed by LC-MS/MS. Extraction procedure is in Appendix A, Table 2.

Biosolid Samples

Samples were thawed. One gram of biosolid material from each date and each WWTP was weighed and aliquoted in triplicate into 50 ml falcon tubes. Each falcon tube top was wrapped in parafilm, then 3 holes were poked into the parafilm and put in a -80 ºfreezer until fully frozen then placed on the lyophilizer. After lyophilization, samples were reconstituted with 5 ml of 80% methanol (MeOH) and 5 ml of 10% glacial acetic acid. One set of triplicate samples was spiked with external standards and a mixed PPCP/Opioid standard. Samples were vortexed and sonicated for 15 minutes then put on a shaker table overnight at room temperature. Samples were centrifuged at maximum speed for 15 minutes and supernatant was transferred to scintillation vials. After each transfer, 5 ml of 80% MeOH was added to falcon tubes, vortexed and sonicated, centrifuged again and supernatant transferred to the scintillation vials. This was done three times ending with a total of 15 ml of supernatant. Sample material was evaporated

then reconstituted with 1 ml of a combination of 100% acetonitrile (ACN), 1% ammonium formate and 0.1% formic acid. Samples were vortexed, sonicated and microcentrifuged for 15 minutes at maximum speed, then supernatant was diluted 1:5 and put in LC vials ready for HPLC/MS-MS analysis. Biosolids extraction procedure is in Appendix A, Table 3.

LC-MS/MS

Instrumental analysis was performed by liquid chromatography using a Shimadzu system equipped with an auto sampler and connected in series with a 4000 Q trap triple quadruple ion trap mass spectrometer operating with a Turbo Ion Spray Source in positive scheduled mode. Chromatographic separation was achieved with a Kinetex 1.6 µm C18 100A 50x3 mm column in a 40 º C column oven. Analytes were detected using a multiple reaction monitoring (MRM) mode. Mobile phase A consisted of distilled water, 0.1% formic acid and 5 ml ammonium formate and mobile phase B consisted of 100% CAN. Appendix A, Table 5 shows the elution gradient. The injection volume was 4 µL, flow rate was 3 ml/minute, rinsing volume was 1000 µL, needle stroke 54 mm, rinsing speed 35 µL/sec, sampling speed 15.0 µL/sec and purge time was 25.0 minutes. The total working time was 10 minutes.

Two parent-product transition mass-to-charge transitions (m/z's) were monitored for the analytes (Appendix A, Table 4). Calibration curves were used for quantification (minimum of 7 levels 0.1 to 50 µg/L). A continuous calibration verification sample was run every 12 samples within the batch of samples and calibration curves were run at the beginning and end of each total run. Analyte peak identification was performed based on retention time using Analyst software (Analyst® 1.6.3 by Sciex).

Statistical Analyses

Data were analyzed using R Statistical Environment (version 3.0.2 Rstudio Team (2020). Rstudio: Integrated Development for R. Rstudio, PBC, Boston, MA URL http://www.rstudio.com/). Actual concentrations were calculated for the detected compounds and percent recovery was calculated on the spiked samples. Kruskal-Wallis nonparametric tests were used to determine whether there is a statistically significant difference between two independent samples when there is a nonnormal distribution and a small sample size. A significant Kruskal-Wallis test indicates that at least one sample dominates another sample. The test does not identify where this dominance occurs. Differences between the overall concentration of influent between plants in each season, removal efficiency by plant and by analyte, and percent remaining by were tested using Kruskal-Wallis one-way analysis of variance statistical test for non-parametric data. The correlation coefficients (r^2) of each analyte were calculated.

Calculation of Mass Loads, Removal Efficiency and Percent Remaining

Daily mass load of each analyte from the two WWTPs was calculated by multiplying individual concentrations of each compound by the average daily flow rate using these equations:

$$M_{influent} = C_{influent} \text{ x average flow rate} \qquad \text{(Eq. 1)}$$

$$M_{effluent} = C_{effluent} \text{ x average flow rate} \qquad \text{(Eq. 2)}$$

$$S_{biosolids} = C_{biosolids} \text{ x average flow rate} \qquad \text{(Eq. 3)}$$

Where $M_{influent}$ is the daily mass load of the compound, $M_{effluent}$ is the daily mass load in the effluent and $S_{biosolids}$ is the daily mass load in the biosolids. $C_{influent}$, $C_{effluent}$, and $C_{biosolids}$ are the

concentrations of each compound in the influent, effluent and biosolids, respectively multiplied by the average daily flow rate of the WWTP.

Removal Efficiency of the compounds were determined by the percentage of reduction between the influent concentration of the compound in the aqueous phase and the concentration of the compound in the effluent aqueous phase by this equation:

$$\text{Removal rate (\%)} = \frac{(\text{Influent} - \text{Effluent})}{\text{Influent}} \text{ x } 100 \qquad \text{(Eq. 4)}$$

Percent Remaining is determined by the analyte concentration that is remaining in effluent after compounds pass through WWTP processes by this equation:

$$\text{\% Remaining} = \frac{\text{Effluent}}{\text{Influent}} \text{ x } 100 \qquad \text{(Eq. 5)}$$

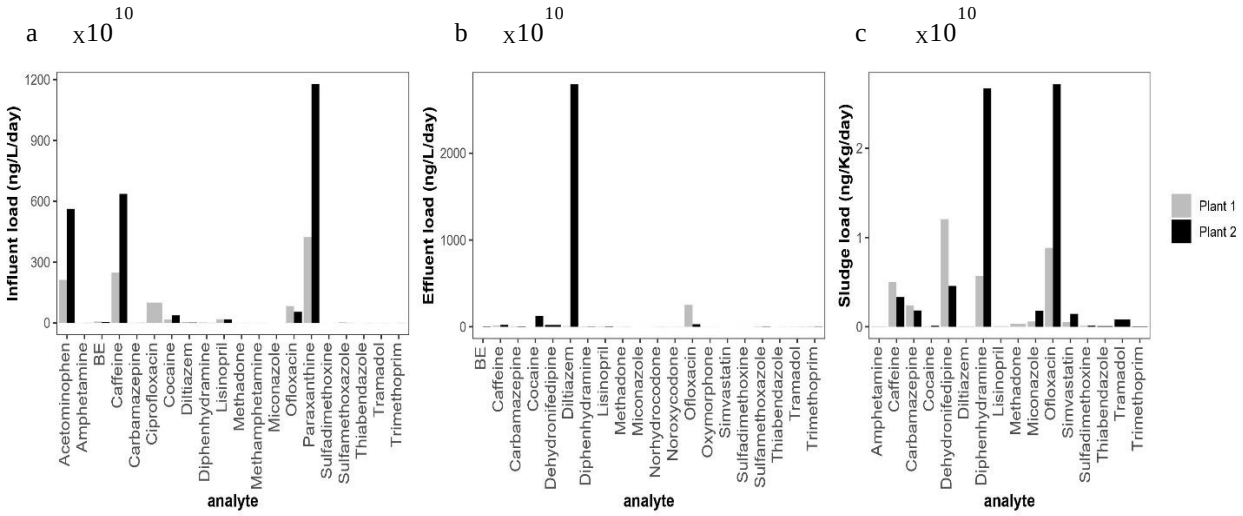
Results and Discussion

Mass balance examines the mass load of PPCPs and illicit drugs from influent, effluent and sludge. Figure 3.1a, b, and c shows the influent, effluent and sludge loads from the two WWTPs. The mass loads were calculated using Equations 1-3 based on concentrations using influent, effluent and sludge and daily WWTP flow rate. WWTP #1's daily flow rate was 12 million gallons per day (mgd) and WWTP #2's daily flow rate was 8 mgd. Seven compounds were detected in the influent load. Acetominophen and caffeine mass loads had slightly higher concentrations at 600 x 10^{10} ng/L/day with paraxanthine with a higher concentration at approximately 1200 x 10^{10} ng/L/day at WWTP #2. The same compounds were detected at WWTP #1 but with lower concentrations (Figure 3.1a). The effluent load (Figure 3.1b) showed diltiazem was still present at WWTP #2 in addition to a small concentration of cocaine. A study

by (Subedi and Kannan, 2015) compared mass loadings of effluents from a WWTP in India to a WWTP in New York, USA having the same size populations and found diltiazem in effluent from the New York study to be up to 13 times higher in mean concentrations compared to the India study. Another study (Lietz and Meyer, 2006) from the Miami-Dade South District Wastewater Treatment Plant found diltiazem at low µg/L concentrations in the effluent. The factors affecting any analyte's sorption onto sludge depend on pH, sludge type and the types of functional groups (Dubey et al., 2021). In the sludge samples 8 compounds were detected at WWTP #2 and 5 compounds were detected at WWTP #1(Figure 3.1c). At WWTP #2, diphenhydramine and ofloxacin had the greatest concentration with lower concentrations of caffeine, carbamazepine, dehydronifedipine, miconazole, simvastatin, and tramadol. At WWTP #1, Dehydronifedipine and ofloxacin were detected in addition to caffeine, carbamazepine, diphenhydramine at much lower concentrations. Ofloxacin belongs to the fluoroquinolone antibiotics. A study of antibiotics in Beijing, China (Gao et al., 2012a) found ofloxacin to have the highest concentration in all of their 18 sludge samples. A study by (Ajibola and Zwiener, 2022) detected ofloxacin in sewage sludge from two Nigerian Hospital WWTPs. Adsorption onto sludge may be the main pathway of elimination for fluoroquinolones that have high K_d and low K_{ow} values (ofloxacin K_{ow} value is negative 0.39) and may interact electrostatically during sorption to sludge (Dubey et al., 2021; Khadra et al., 2019; Radjenović et al., 2009). Diphenhydramine had low concentrations in influent and was not detected in effluent but was detected in the sludge load.

The octanol-water partitioning coefficient (K_{ow}) and hydrophobicity may determine the compounds affinity to sludge. For example, the anti-histamine diphenhydramine has a K_{ow} value of 3.27, but ofloxacin's K_{ow} value is -0.39. Some researchers (Carballa et al., 2008; Chiaia-

Hernandez et al., 2013; Ternes et al., 2004) suggest also using the solid-water distribution coefficient (K_d)value to determine sludge sorption. Compounds often contain polar functional groups (e.g. carboxylic moieties, aldehydes and amines) (Carballa et al., 2008) which may interact only with special parts of organic matter, thus using just K_{ow} values may yield different results.



Figures: 3.1a, 3.1b, 3.1c: Figure 3.1a is the mass load of influent from WWTPs #1 and #2, Figure 3.1b is the mass load of effluent from WWTPs #1 and #2, Figure 3.1c is the mass load of sludge from WWTPs #1 and #2.

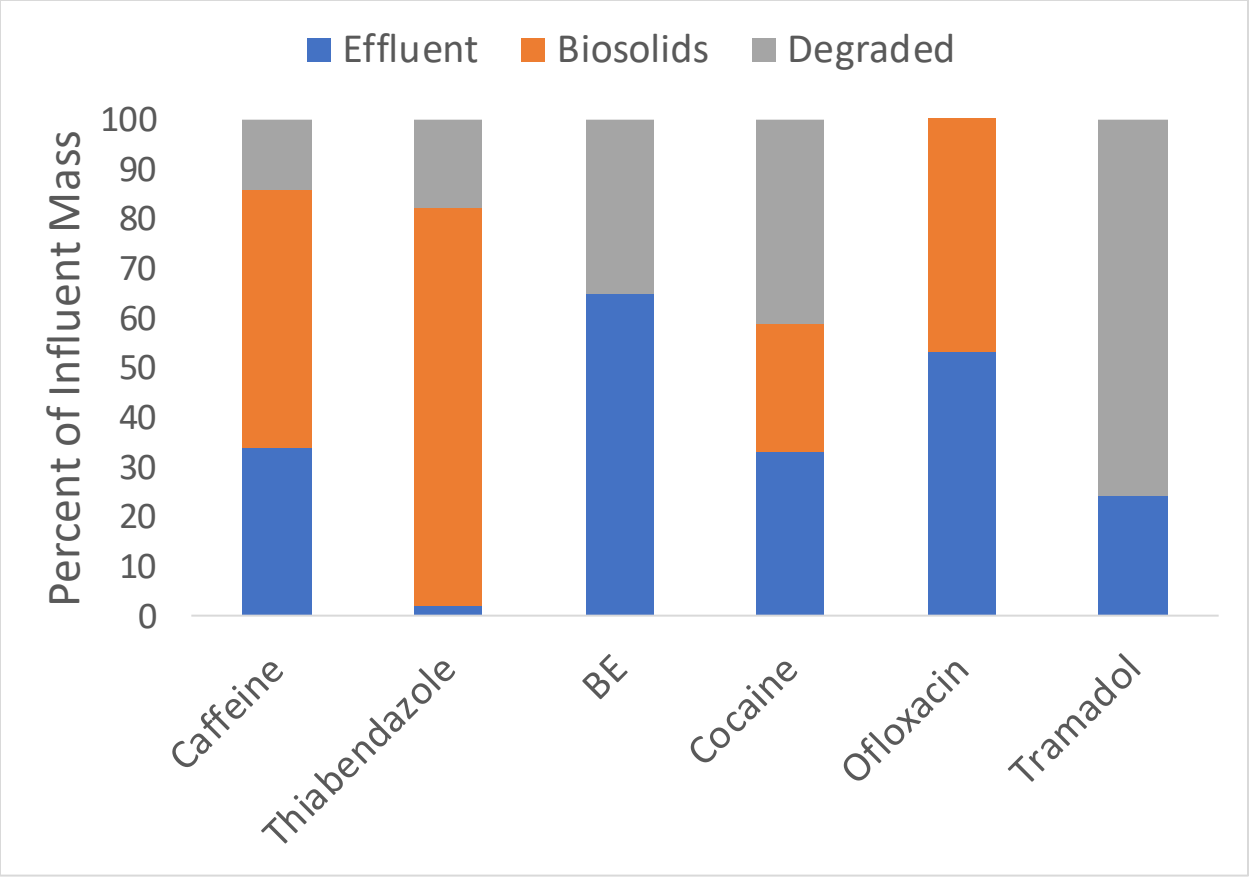


Figure 3.2: Bar graph showing percent of influent mass going to effluent, biosolids or being degraded from 6 selected compounds.

When influent enters a WWTP, where does the influent go? Figure 3.2 shows the breakdown of where the influent goes from 6 selected compounds. The majority of thiabendazole (80%) ends up in biosolids, approximately 50% of caffeine entering as influent ends up in biosolids along with 50% of ofloxacin and 30% of cocaine. BE, the metabolite of cocaine ends up in effluent (65%), with ofloxacin 50% in effluent, 30% for caffeine and cocaine, 20% for tramadol and a very small concentration of thiabendazole (1%) in effluent. The percent leftover from biosolids and effluent is the amount that is degraded. Ofloxacin is not degraded at all compared to

tramadol in which 80% is degraded, the other compounds (caffeine, thiabendazole, BE and cocaine) are < 40% degraded. Biological degradation of a chemical refers to the elimination of the pollutant by metabolic activity of living organism, usually microorganisms (Oller et al., 2011). A study by Ibrahim et al. (Ibrahim et al., 2014) found biodegradation by bacteria the most efficient technique in degrading caffeine. Some strains of bacteria that can degrade caffeine are *pseudomonas, aspergillus, penicillium, rhizopus* and *bacillus* sp. Another study by Topp et al. (Topp et al., 2006) observed caffeine in three agricultural soils and found biodegradation in all three soils was quite uniform upon the addition of caffeine-degrading bacteria or aerated biosolids. Perruchon et al. (Perruchon et al., 2017) studied thiabendazole which is a fungicide used in harvesting of fruits. The application of thiabendazole results in contaminated effluents and evaluated its degradation capacity under various conditions (range of pH, temperatures and thiabendazole concentration levels). Thiabendazole maintained its high degradation capacity in a wide range of pH (4.5-7.5) and temperatures (15-37 ℃). Liu et al. (Liu et al., 2023) looked at ofloxacin in a rural sewage treatment plant and built a wetlands and found that sludge has a strong adsorption effect on ofloxacin at the initial stage, but the adsorption capacity gradually decreases under the long-term. Also, the denitrifers *Microbacterium, Geobacter* and *Ignavibacterium*, were the main participants in ofloxacin biodegradation.

Removal Efficiency

Removal efficiency was calculated on the mean concentrations of influent and effluent using Equation 4. Figure 3.3 shows the mean removal efficiencies for WWTP #1 and #2. WWTP #1 removal efficiencies were less efficient (BE, lisinopril and ofloxacin) compared to WWTP #2. Both WWTPs were efficient in 100% removal of acetaminophen, amphetamine,

caffeine, methamphetamine, and simvastatin. Carbamazepine, an anti-epileptic, is considered a recalcitrant analyte with negative removal at both WWTPs. Previous studies (Al-Mashaqbeh et al., 2019; 2020; Gao et al., 2012b; Gros et al., 2013; Lajeunesse et al., 2012; Subedi et al., 2017) have shown carbamazepine to be persistent in effluent exceeding influent concentrations. This may be due to deconjugation during the treatment process or by transformation back into the parent compound. WWTP #1 was unable to efficiently remove the opioids compared to WWTP #2. The Kruskal-Wallis statistical test was significant for removal efficiency by plant (p=0.0003). Kamika et al. (Kamika et al., 2021) found that WWTPs perform poorly in removing some opioids such as methadone and hydrocodone. There was negative removal of cocaine from WWTP #1, whereas its metabolite BE was more efficiently removed at both WWTPs. A WWTP study in New Zealand (Kumar et al., 2019) found cocaine was detected at the highest concentration in the effluent.

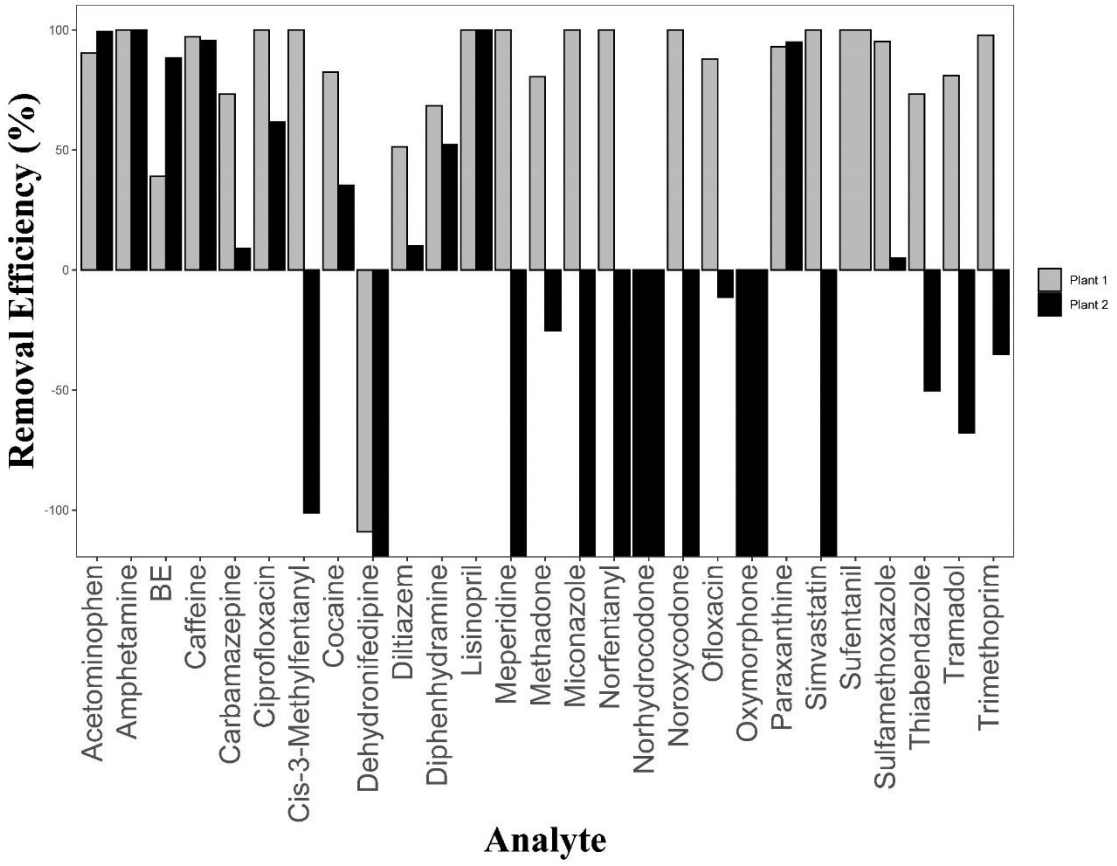


Figure 3.3: Removal efficiency in percent of detected analytes from WWTP #1 and #2. Lower portion of graph shows negative removal of detected analytes from WWTP #1 and #2.

Percent Remaining

Percent remaining (Eq. 3) are the analytes that entered the WWTPs as influent but remain in the effluent. Norhydrocodone and Noroxycodone (Figure 3.4) were detected in the effluent at over 100% for both WWTPs. Oxymorphone was detected in the effluent at over 100% at WWTP #2, and dehydronifedipine and simvastatin at WWTP #1. Benzoylecgonine, lisinopril and ofloxacin were detected in the effluent at less than 75% at WWTP #1, as well as lisinopril, miconazole, benzoylecgonine and caffeine at WWTP #2 (Figure 3.4). A possible explanation for compounds remaining in effluent is that metabolites and/or conjugate forms of PPCPs and illicit drugs are not detected in the influent and are deconjugated back in to the parent compounds through either biotic processes such as organisms, or abiotic processes such as sunlight, temperature, and precipitation occurring within the WWTP (Blair et al., 2015; Verlicchi et al., 2012). Other factors contributing to compounds remaining in effluent may be the physiochemical properties of the compounds, the WWTP retention time, and microbial activity within the plant at the time of sampling (Archer et al., 2017a). Archer et al. (Archer et al., 2017a) detected cocaine and benzoylecgonine higher in influent than effluent similar to the removal efficiency graph (Figure 3.3). The Kruskal-Wallis statistical test for percent remaining by plant was significant (p==0.0003).

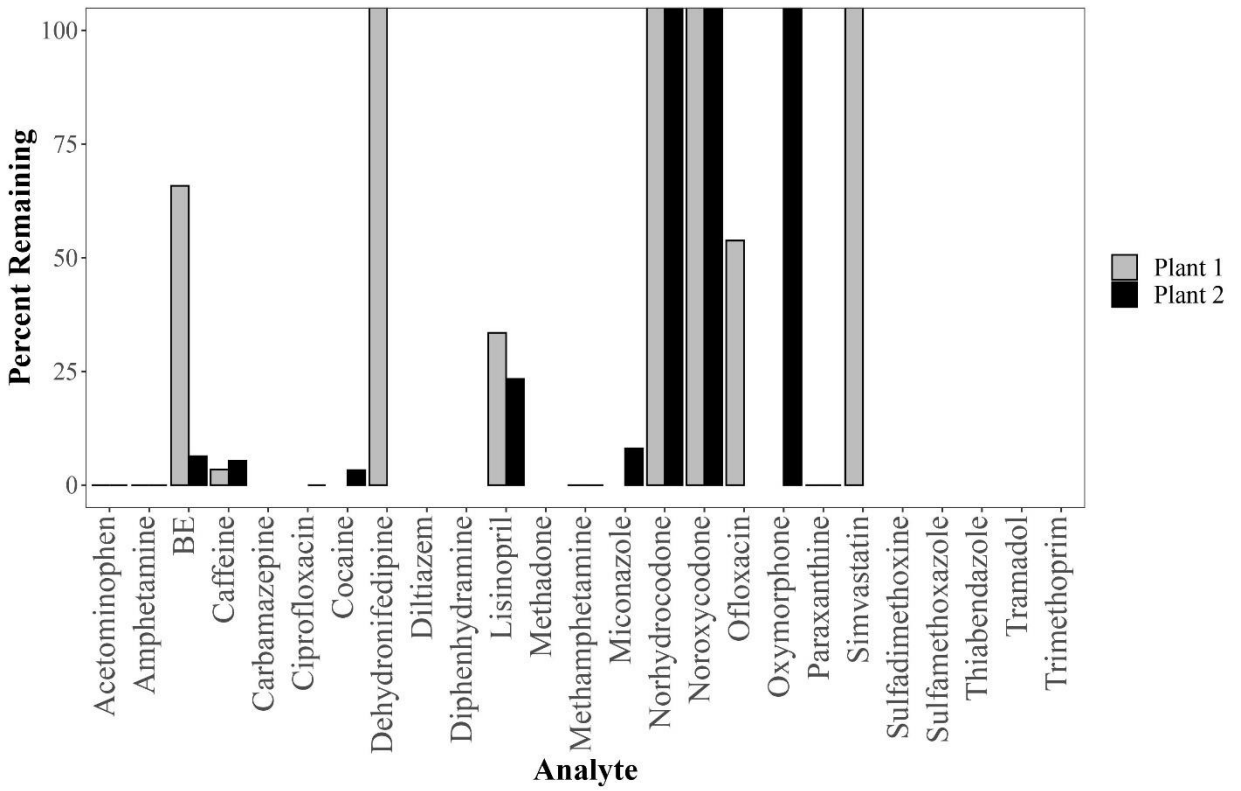


Figure 3.4: Percent remaining of detected analytes that entered WWTPs as influent but remain in effluent for both WWTPs #1 and #2.

Seasonality

Factors that may affect the presence of analytes in the environment could be the consumption of pharmaceuticals (i.e., using different antibiotics during the year), demographics, or wastewater treatment processes. Samples were collected in autumn (September 2016, October 2016 and November 2016), winter (December 2016, January 2017 and February 2017) spring (March 2017, April 2017 and May 2017) and summer (June 2017, July 2017 and August 2017) to examine seasonal trends. The concentration of analytes from two WWTPs were examined seasonally using Krusal-Wallis non-parametric tests. Figures 3.5 and 3.6 show the mean influent

concentrations of detected analytes at the two WWTPs by season. Figure 3.4 displays the detected analytes with influent concentrations greater than 125,000 ng/L and Figure 3.6 displays the detected analytes with influent concentrations less than 125,000 ng/L. The analytes that had mean influent concentrations <1000 ng/L are not included in these graphs. The winter season had higher mean influent concentrations for acetaminophen, caffeine, and its metabolite paraxanthine at WWTP #1 compared to WWTP #2 (Figure 3.5), and cocaine, and ofloxacin (Figure 3.6) had higher influent concentrations at WWTP #2 compared to WWTP #1 (Figure 3.6). There was significance between the two WWTPs for the winter season (p=0.0037). A study in Central Spain also found higher concentrations of analytes in winter compared to summer (Valcárcel et al., 2013). A seasonal study of WWTPs influent from Charleston, South Carolina (Hedgespeth et al., 2012) found acetaminophen with mean concentrations of 143,000 ng/L in the winter. A study of 5 WWTPs in southern California found acetaminophen influent concentrations to be the highest (81.5 ug/L) in the winter (Yu et al., 2013). Acetaminophen is a common pain reliever and fever reducer and consumption use because of winter ailments is higher in winter. In addition, out of 14 compounds studied, 10 compounds had higher influent concentrations in the winter. The central nervous system analyte caffeine was detected in the influent at high mean concentrations of 4968 ng/L in winter (Kosma et al., 2010). Caffeine is found in coffee and tea products, hot chocolate, chocolate, and soft drinks which are popular in the winter. Another researcher (Bahlmann et al., 2012) found caffeine concentrations peaked between November and April. The Kruskal-Wallis statistical test for differences for analytes and differences between plants was significant (p=0.0002).

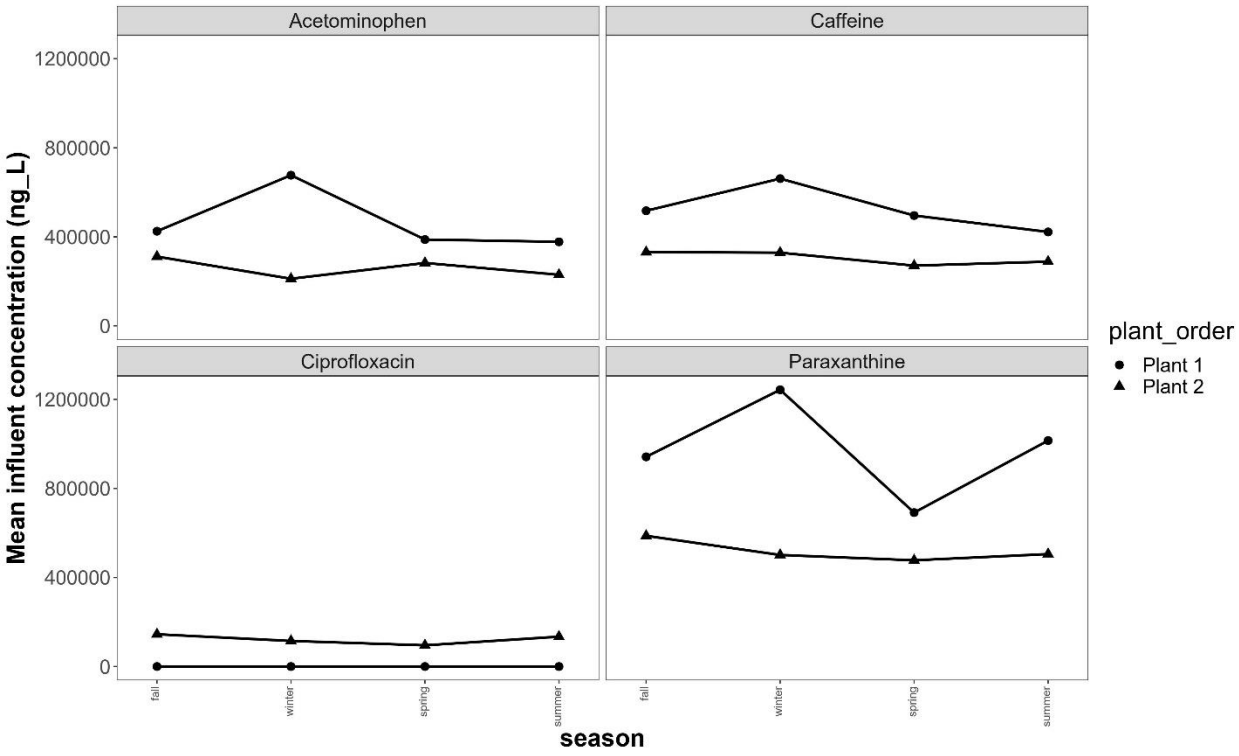


Figure 3.5: Mean influent concentration of detected analytes >125,000 ng/L for fall, winter, spring and summer for WWTPs #1 and #2.

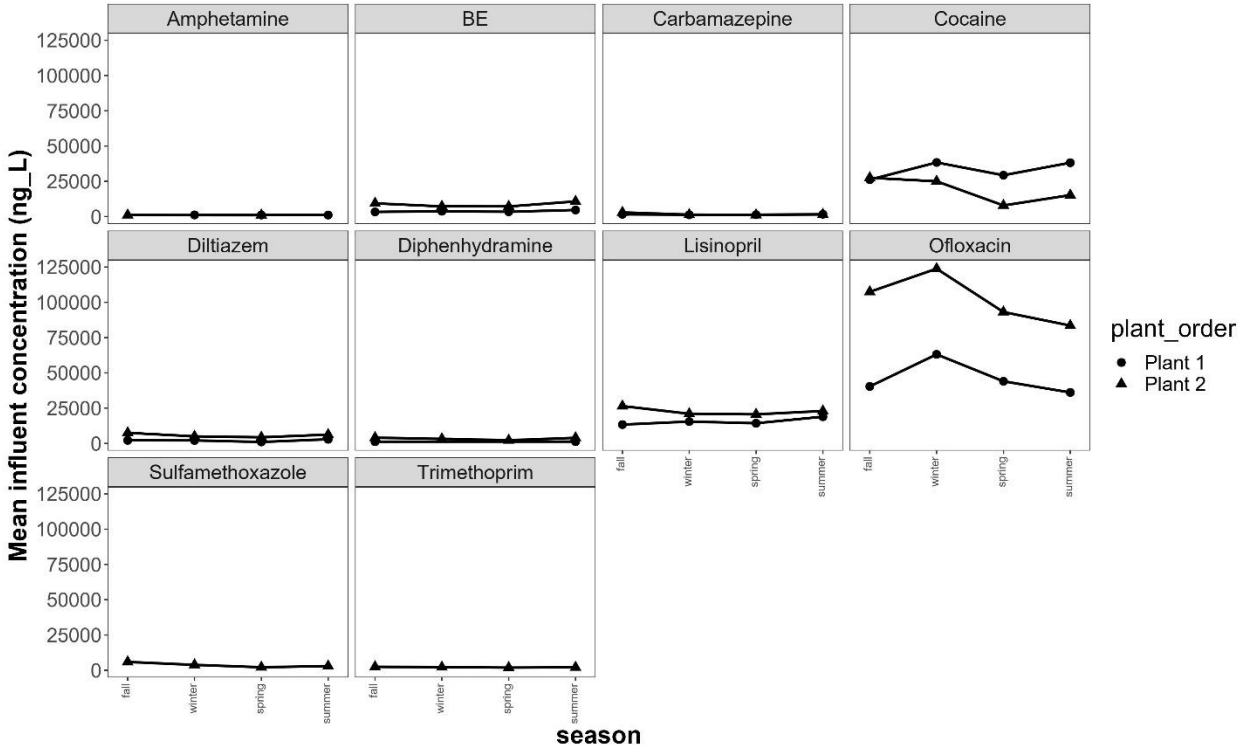


Figure 3.6: Mean influent concentration of detected analytes <125,000 ng/L for fall, winter, spring and summer for WWTPs #1 and #2. Analytes that were <1000 ng/L mean influent concentrations not included in this graph.

Removal Efficiency by Season

The graphs for seasonal removal efficiency (Figures 3.7, 3.8, 3.9, 3.10) include all detected analytes. Equation 4 was used for calculating removal efficiency at both WWTPs. Acetominophen, amphetamine, caffeine, methamphetamine and paraxanthine were all efficiently removed for all seasons and at both WWTPs. Carbamazepine continued to be a recalcitrant analyte at both WWTPs and for all seasons showing negative removal. Cocaine, dehydronifedipine, diltiazem, methadone norhydrocodone, noroxycodone, simvastatin, sulfadimethoxine, sulfamethoxazole, thiabendazole, tramadol and trimethoprim had negative

removal for all seasons at WWTP #1. In comparison, WWTP #2 was efficient at removing cocaine but had negative removal for 11 analytes for all seasons (Figures 3.7, 3.8, 3.9, 3.10,). Negative removal indicates that these PPCPs and illicit drugs are entering aquatic systems. These compounds may have the potential to interact and affect aquatic organisms, in addition to the illicit drugs having pseudo-persistence since they give continuous environmental input and maybe long-term exposure to aquatic organisms (Binelli et al., 2012; Horký et al., 2021; Maasz et al., 2021). Studies show 4 main drug groups that cause global concern: cannabinoids, synthetic drugs such as amphetamines, cocaine, and opioids (Adeleye et al., 2022; Petrie et al., 2015). These drugs can act synergistically and may cause toxic effects on the aquatic organisms even at low concentrations (Adeleye et al., 2022). The Kruskal-Wallis statistical test for between plants in each season was significant (fall p=0.0077, winter p=0.025, summer p=0.04). No significant difference was found for spring.

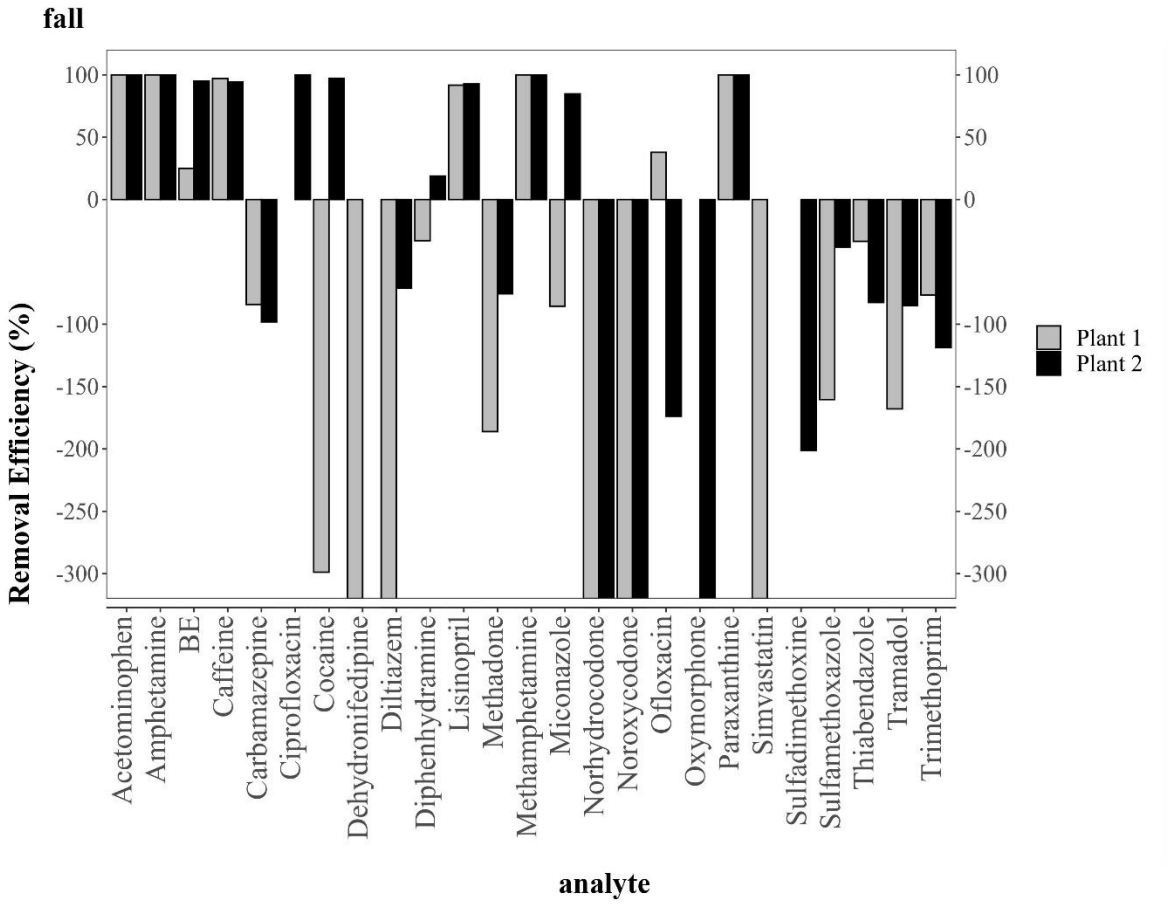


Figure: 3.7: Percent removal efficiency of detected analytes from WWTPs #1 and #2 for fall. Lower portion of graph represents negative removal of detected analytes remaining in effluent.

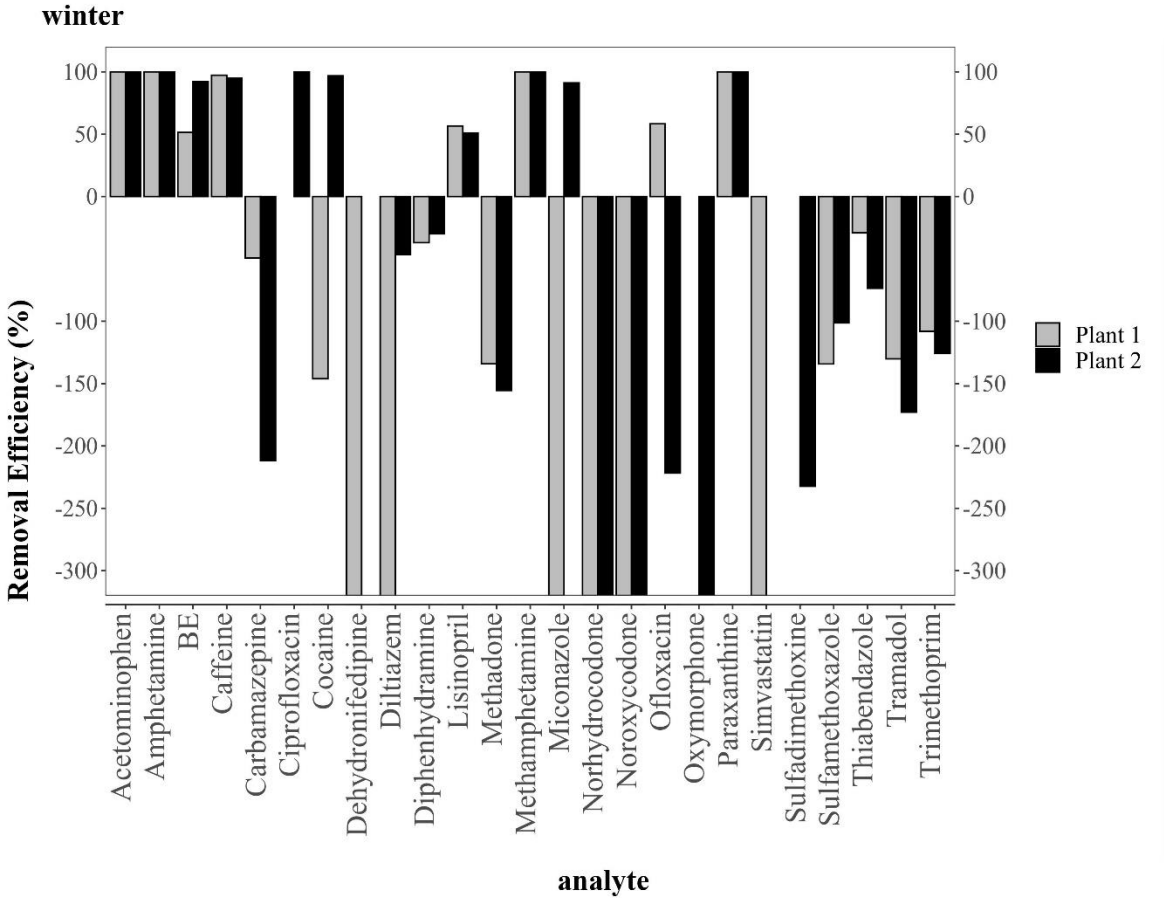


Figure 3.8: Percent removal efficiency of detected analytes from WWTPs #1 and #2 for winter. Lower portion of graph represents negative removal of detected analytes remaining in effluent.

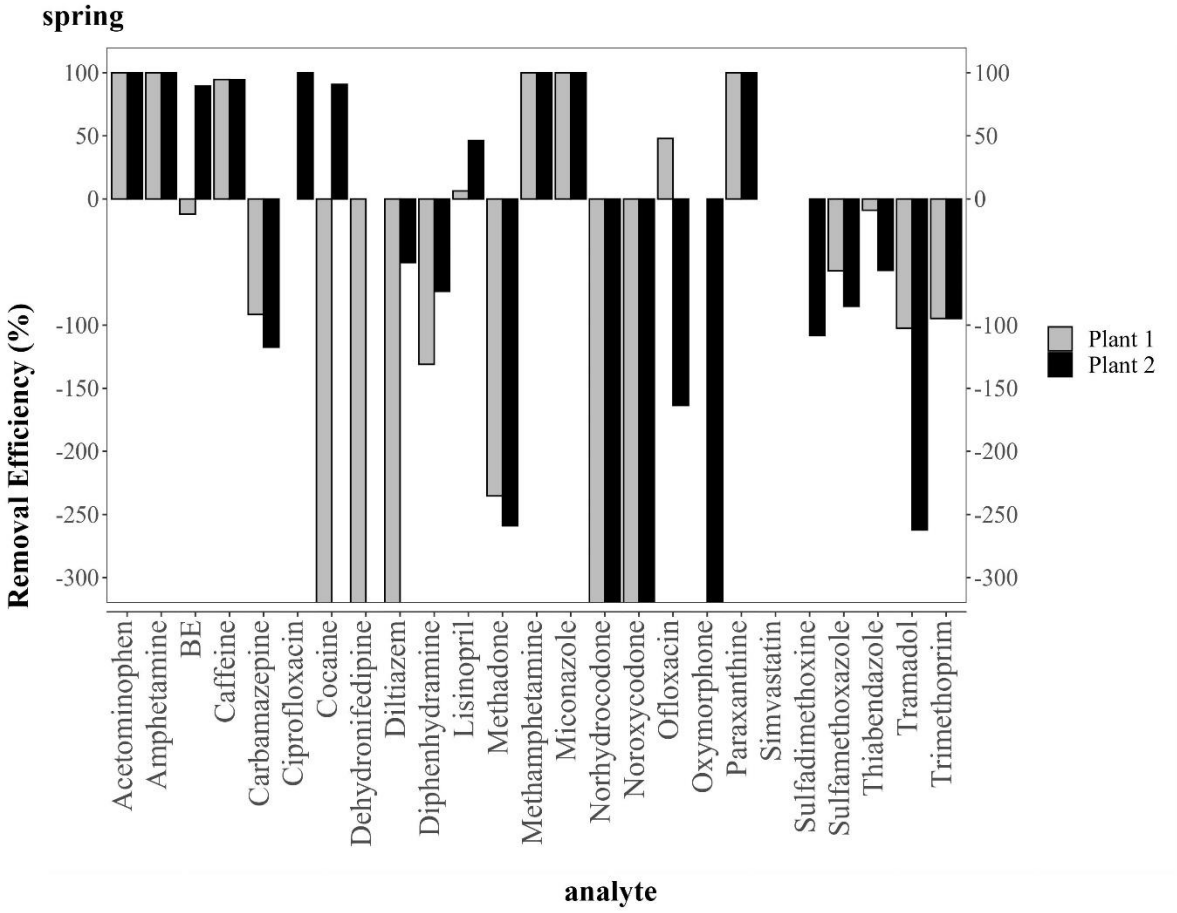


Figure 3.9: Percent removal efficiency of detected analytes from WWTPs #1 and #2 for spring. Lower portion of graph represents negative removal of detected analytes remaining in effluent.

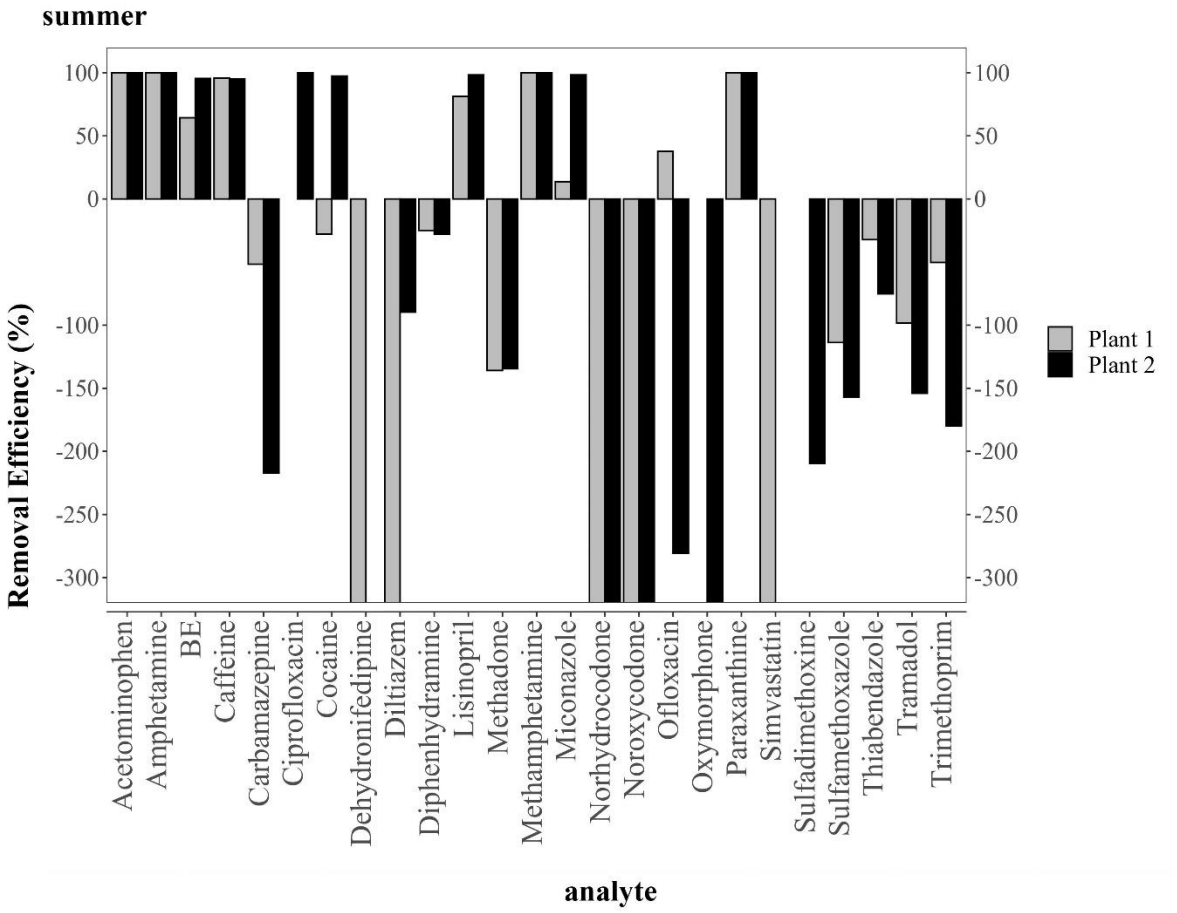


Figure 3.10: Percent removal efficiency of detected analytes from WWTPs #1 and #2 for summer. Lower portion of graph represents negative removal of detected analytes remaining in effluent.

Conclusions

This study had 5 objectives: to assess the distribution of 60 diverse compounds of PPCPs and illicit drugs, to use an alternative extraction procedure for aqueous and sludge samples, to examine removal efficiencies, and seasonality and lastly to calculate a mass balance to identify the distribution of the PPCPs and illicit drugs. Influent, effluent, and sludge samples were sampled from two conventional WWTPs and all objectives were met successfully. The seasonality studies found the winter months to have the highest concentrations of PPCPs and illicit drugs. This study was the first of its kind for this body of water and gives valuable information regarding PPCPs and illicit drugs that remain in effluent and potentially enters receiving waters. Little is known about the effects of these compounds in the aquatic environment and more research is needed in this area.

Chapter 4:

Survey of Pharmaceuticals, Personal Care Products (PPCPs) and Illicit Drugs from six urban river systems and seasonality differences.

Introduction

It remains a challenge to balance our land use with our water use to protect, restore, and enhance our natural resources. It is becoming an increasing problem to protect our urban rivers and streams from pollution because our land and water are forever linked together. Unfortunately, pharmaceuticals and personal care products (PPCPs) and illicit drugs are environmental contaminants detected in surface waters globally, as well as locally. These contaminants can enter aquatic ecosystems in multiple ways. They can enter through industry, agriculture, septic systems, or hospitals, but mainly wastewater treatment plants (Daughton and Ternes, 1999; Genthe et al., 2013). Major examples of PPCPs and illicit drugs entering aquatic systems are medicinal drugs, veterinary drugs, hormones, and antibiotics, in addition to illegal drugs such as cocaine, opioids, and amphetamine-type substances (Mohan et al., 2021; Nowal, 2018).

There are no requirements for monitoring or regulation of PPCPs and/or illicit drugs. Many researchers (Anand et al., 2022; Archer et al., 2017c) have found that the presence of PPCPs and illicit drugs in surface waters may have deleterious effects on the aquatic biota at concentrations of nanograms to micrograms per liter. These contaminants can have a negative impact on public health, ecosystems, and the economy (Meyer et al., 2019). There is an increasing trend in urbanization and lifestyle changes globally, with the consumption pattern of PPCPs and illicit drugs gradually changing alongside health ailments such as cardiovascular

disease, diabetes and changes in illicit drug use (Mohapatra et al., 2016). Global urbanization is growing rapidly. According to (Jensen and Wu, 2018) the urban population is expected to increase to 66% by 2050 from 54% in 2014. Rapid urbanization may increase the use of freshwater resources, but also increase contamination of water (Best, 2018; Flörke et al., 2018; van Vliet et al., 2017). World population is growing as well as global living conditions, promoting widespread use of PPCPs and illicit drugs and across all socio-economic levels of populations (Anand et al., 2022).

PPCPs and illicit drugs are found in surface waters all around the world. The concentrations of these drugs may vary depending on the region and consumption habits of the people (Li et al., 2016). Also how the PPCPs and illicit drugs are transferred to the water may be based on climate, rainfall and use of the soil (Lee et al., 2007). Van et al. (Van et al., 2021) investigated 56 compounds, 48 were found in river water in Hanoi and 33 compounds were found in Metro Manila. Concentrations of these compounds ranged from 7.5 to 20,789 ng/L in Hanoi and 118 to 3,394 ng/L in Manila. Ngo et al. (Ngo et al., 2021) examined PPCPs in the Cau River, Vietnam. They found 36 out of 56 PPCP samples were detected at concentrations from 8.21 to 529 ng/L. The PPCPs detected at > 70% concentrations were caffeine, sulfamethoxazole and lincomycin. The United States Geological Survey (USGS) conducted a survey and detected 84 PPCPs in 38 United States streams (Bradley et al., 2017). In 1999, the USGS conducted a national survey which sampled streams from 30 United States and found that organic contaminants were detected in 80% of streams sampled and also found multiple contaminants in a single water sample (Kolpin et al., 2002). The use of PPCPs and illicit drugs will likely increase throughout the world as more people will have access to these drugs and treatment which will have an impact on surface waters.

The objective of this research was to survey the occurrence and concentrations of 60 PPCPs/illicit drugs in six river systems that pass through urban and non-urban regions. An alternative extraction procedure compared to the commonly used solid phase extraction (SPE) was used to determine how efficient this extraction procedure was at detecting compounds in water samples. Samples were analyzed by liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). Data were further analyzed by seasonality.

Sample Sites

Sampling was conducted at 20 sites within five rivers and one creek in the Milwaukee Wisconsin River Basin (Figure 4.1). The Milwaukee River Basin is comprised of 882.3 square miles within the following counties: Milwaukee, Waukesha, Washington, Ozaukee, Fond du Lac, Sheboygan, and Dodge. The Basin is divided into the Milwaukee, Menomonee and Kinnickinnic River watersheds and the Fox River system. There are approximately 500 miles of perennial streams, more than 400 miles of intermittent streams, 35 miles of Lake Michigan shoreline, 57 named lakes, and over 1.3 million people. The southern quarter of the Basin is the most densely populated area in the state, holding 90% of the Basin's population. Wetlands encompass over 678,000 acres or 12% of the basin land area. The main land use is grasslands, which account for 56% of the Basin land cover. Throughout the Basin are habitat modifications such as channelization and dams which promote runoff and contaminated sediment (dnr.wisconsin.gov). Table 4.1 shows the sampling sites with the land use within the Milwaukee River Basin.

The Wilson Park Creek is a tributary to the Kinnickinnic River and included in the Kinnickinnic River watershed (Figure 4.1). The Wisconsin Department of Natural Resources describes this watershed as the most urban (90% of land cover) of the rivers studied in this paper. Thirty percent of the system is concrete lined and 30% are in an enclosed channel. It is a highly

stressed area and suffers from chronic aquatic toxicity from a variety of pollutants, agricultural runoff and discharges from storm sewer systems. The Fox River is heavily urbanized at the headwaters and mouth, then becomes agricultural in the middle drainage area then back to urban as the river flows southerly. The Menomonee River is mostly urban, and a portion of the river is heavily channelized and industrialized which is a major source of pollution for this river. The Root River is comprised of a mixture of urban and rural land uses. Stormwater runoff is a major source of pollution for the Root River watershed (dnr.wisconsin.gov).

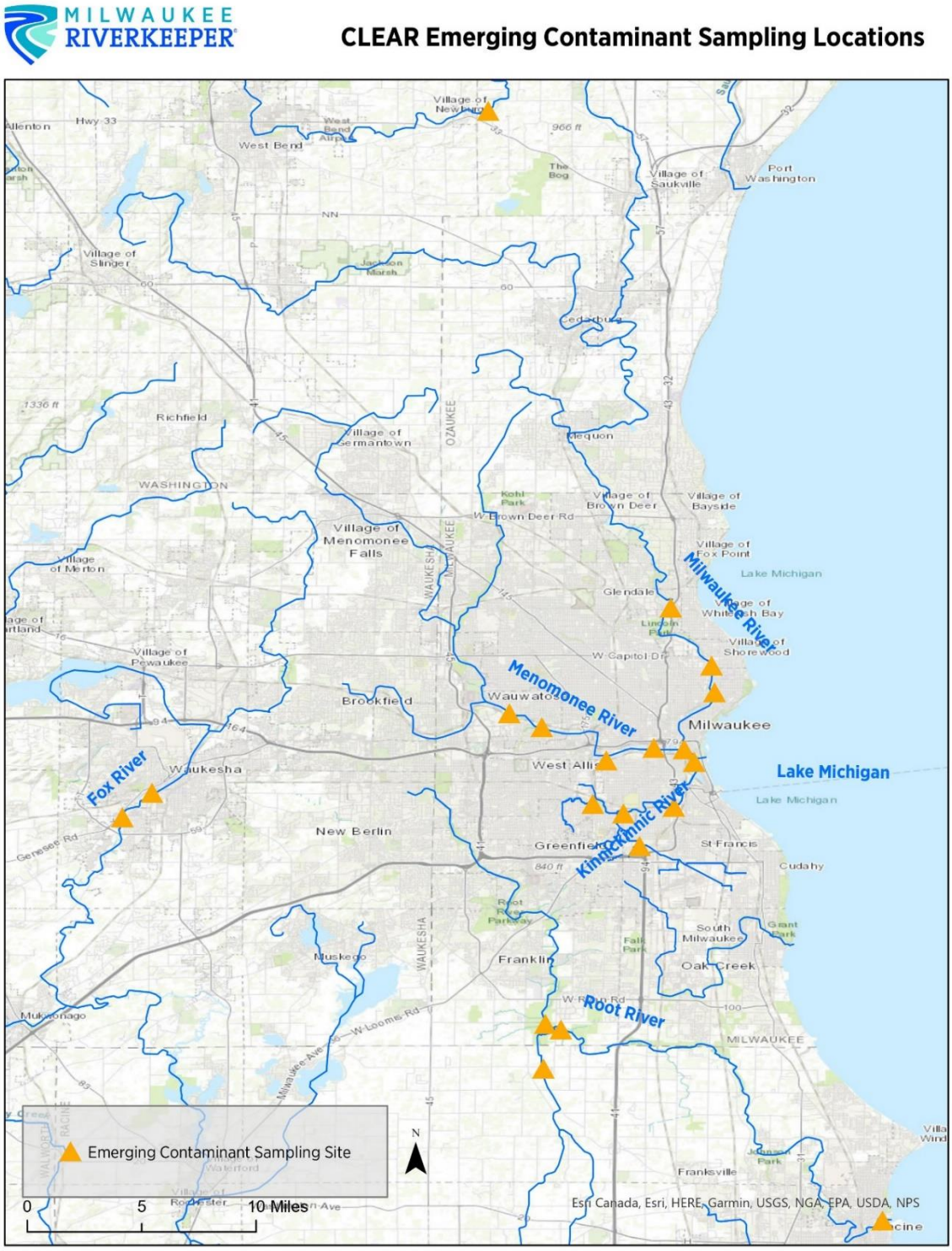


Figure 4.1: Sampling sites for 5 urban rivers are shown with gold triangles. Sampling sites at Wilson Park Creek are not shown on this map.

Chemicals

Sixty standards were analyzed in this research study, and all were of high purity grade (>90%). Chemicals were purchased via Sigma-Aldrich (St. Louis, Missouri), Cayman Chemical (Ann Arbor, Michigan), Acros (Newark, Delaware), Fluka (Belgium), and Cerilliant (Round Rock, Texas). Appendix A, Table 1 shows the targeted compounds, category of each compound and CAS number.

Sampling and Alternative Extraction Procedure

Volunteers were recruited and trained to collect samples by Milwaukee Riverkeeper®. Sampling occurred September, October and November for fall, March, April and May for spring, and June, July, and August for summer. Sampling consisted of a pole with polyvinyl chloride (PVC) pipe rubber-banded to a plastic 250 ml sample container. The sample container was rinsed 3 times prior to getting the sample to be extracted. One sample located downstream on Sunset Drive Bridge in Waukesha had to be lowered into the stream from the top of the bridge. Samples were collected on 8-4-18, 11-23-18, 3-30-19, 7-20-19, 11-19-19, 4-3-21, and 11-26-21 and kept frozen until they could be extracted. Ten milliliters (ml) of sample were aliquoted into 50 ml labelled falcon tubes. Each falcon tube top was wrapped in parafilm, then 3 holes were poked into the parafilm and put in a -80 ºfreezer until fully frozen. After freezing, the falcon tubes were put on the lyophilizer. After lyophilization, samples were resuspended with 5 ml of 40:40:20 (methanol:acetone:5% glacial acetic acid) solute, vortexed and sonicated in a 50 ºC sonicator for ten minutes. Samples were centrifuged at maximum speed for 15 minutes and the supernatant was transferred to 20 ml scintillation vials. Five ml of solute was added after each centrifugation to the falcon tubes, centrifuged two more times ending with 15 ml of solute in each labeled scintillation vial. Sample extracts were evaporated at 50 ºC. Dried samples were reconstituted

with 1 ml of 50% MeOH, then vortexed, sonicated, and sample material was transferred to 1.5 ml labeled centrifuge tubes and centrifuged for 15 minutes at maximum speed. The supernatant was transferred to 1 ml labeled liquid chromatography (LC) vials, then analyzed by liquid chromatography tandem mass (LC-MS/MS) spectrometry. External standards were made from 0 to 50 µg/L with 50% MeOH. Four fortified samples were made by adding 13^{c} phenylalanine to the 50 ml samples to be used in the field. See Appendix A, Table 2 for extraction procedure.

LC-MS/MS

Instrumental analysis was performed by LC-MS/MS using a Shimadzu system equipped with an auto sampler and connected in series with a ®4000 Q-Trap triple quadruple ion trap mass spectrometer operating with a Turbo Ion Spray Source in positive scheduled mode. Chromatographic separation was achieved with a Kinetex 1.6 µm C18 100A 50x3 mm column in a 40 º C column oven. Analytes were detected using a multiple reaction monitoring (MRM) mode. Two parent-product transition mass-to-charge transitions (m/z's) were monitored for most analytes (Appendix A, Table 4). Quantitation of peaks was by Analyst® 1.6.3 software by Sciex.

Mobile phase A consisted of distilled water, 0.1% formic acid and 5 ml ammonium formate and mobile phase B consisted of 100% acetonitrile. Appendix A, Table 5 shows the elution gradiant. The injection volume was 4 µL, flow rate was 3 ml/minute, rinsing volume was 1000 uL, needle stroke 54 mm, rinsing speed 35 uL/sec, sampling speed 15.0 uL/sec and purge time was 25.0 minutes. The total working time was 10 minutes.

Statistical Analyses

Data were analyzed using R Statistical Environment (version 3.0.2 RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL

http://www.rstudio.com/). to generate the box and whisker plots. Actual concentrations were calculated for the detected compounds. Concentrations on the seasonal graphs were converted to log base 10 concentrations. Kruskal-Wallis nonparametric tests were used to determine whether there is a statistically significant difference between two independent samples when there is a nonnormal distribution and a small sample size. A significant Kruskal-Wallis test indicates that at least one sample dominates another sample. The test does not identify where this dominance occurs. Calibration curves were used for quantification (minimum of 7 levels 0.1 to 50 µg/L). A continuous calibration verification sample was run every 12 samples within the batch of samples and calibration curves were run at the beginning and end of each total run. The correlation coefficients (R^2) of each analyte were over 0.99.

Results and Discussion

Sixty compounds were investigated in five urban rivers and one creek. Figures 4.2 through 4.7 box and whisker plots show caffeine was found in all rivers and the creek with the highest concentrations of all the detected compounds. Caffeine is a very widely used compound consumed daily for most people in addition to being found in chocolate, tea, and soda. In addition, caffeine is also used as an active ingredient in acetaminophen for example (Roveri et al., 2022). Other compounds detected at higher concentrations were metformin in the Kinnickinnic River, dehydronifedipine in the Root River and carbamazepine in Wilson Park Creek (Figures 4.3, 4.6, and 4.7). One study in the United States surveyed 37 streams and found caffeine as the second most detected compound in 77.5% of all samples (Bernot et al., 2016), while in another study of 38 streams in the United States they found caffeine in 74% of all samples (Bradley et al., 2017).

A study of streams that one can walk across in the Southeastern United States found caffeine in 49% of the samples and the diabetes medicine metformin in 89% of the samples and carbamazepine, an antiepileptic medicine in 28% of all the samples (Bradley et al., 2016). Another study of nonprescription pharmaceuticals in central Indiana streams found caffeine at trace concentrations (19 to 176 ng/L) at all 10 sampling sites (Bunch and Bernot, 2011), whereas this study found caffeine concentrations much higher at slightly under 2000 ng/L. Similar to this study, (Bunch and Bernot, 2011) found dehydronifedipine, diltiazem, diphenhydramine, thiabendazole and trimethoprim at concentrations slightly above detection limits. Interestingly, some of the urban rivers in this study lack WWTPs which are noted to be the primary source of pharmaceuticals to stream/river environments. A study on the assessment of pharmaceutical exposures on urban streams without WWTPs observed that pharmaceuticals were found in 75% of samples from streams without WWTPs. (Bradley et al., 2020). Some of the compounds detected were metformin in 68% of the sample sites, caffeine in 42% of the sample sites, in addition to carbamazepine at 41%. Some reasons for this are gray-water systems (Eriksson et al., 2003), green space and golf course wastewater reuse (Fatta-Kassinos et al., 2011a), animal waste runoff (Margalida et al., 2014) and private septic waste handling systems (Kibuye et al., 2019).

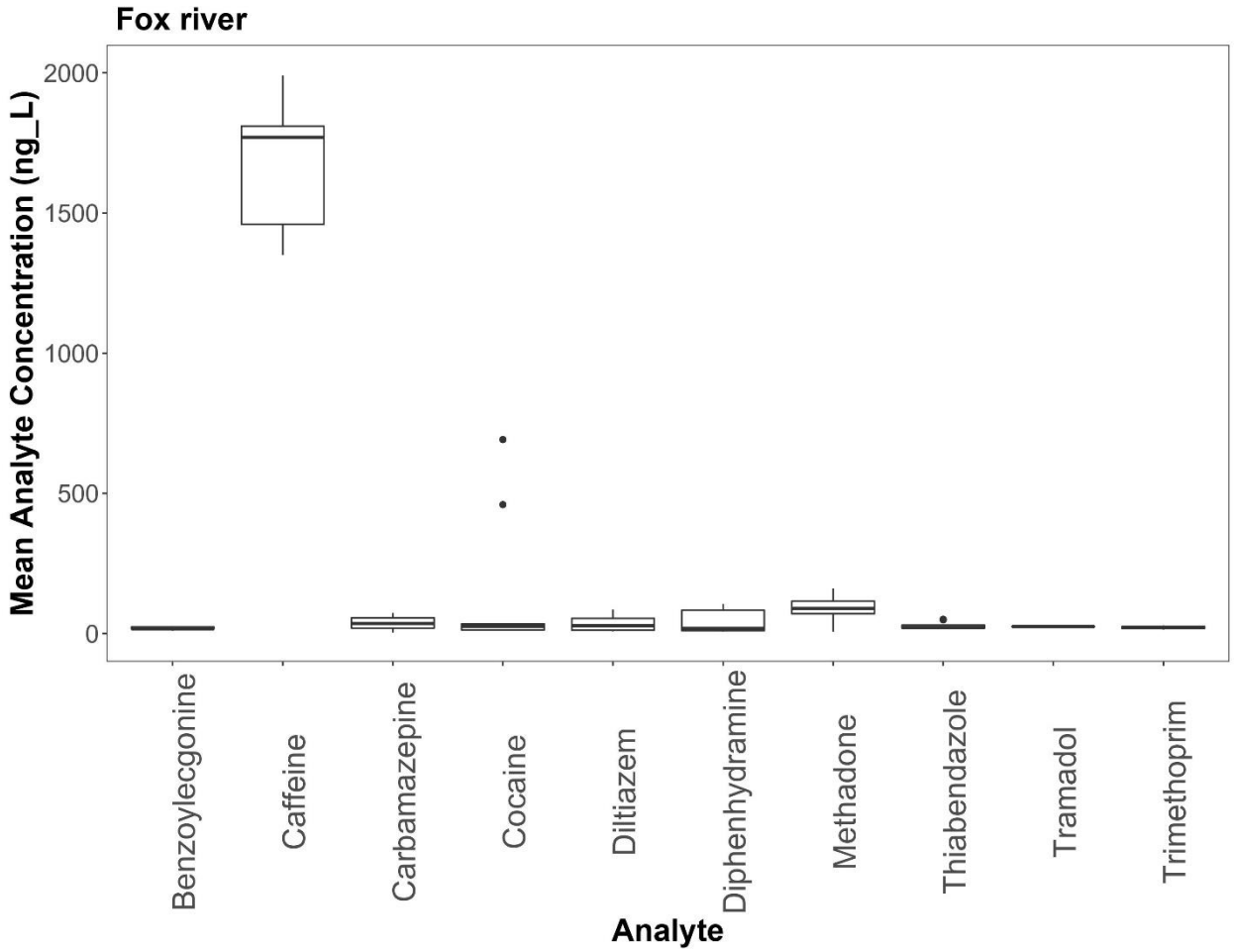


Figure 4.2: Box and whisker plots of mean analyte concentrations for detected analytes for the Fox River.

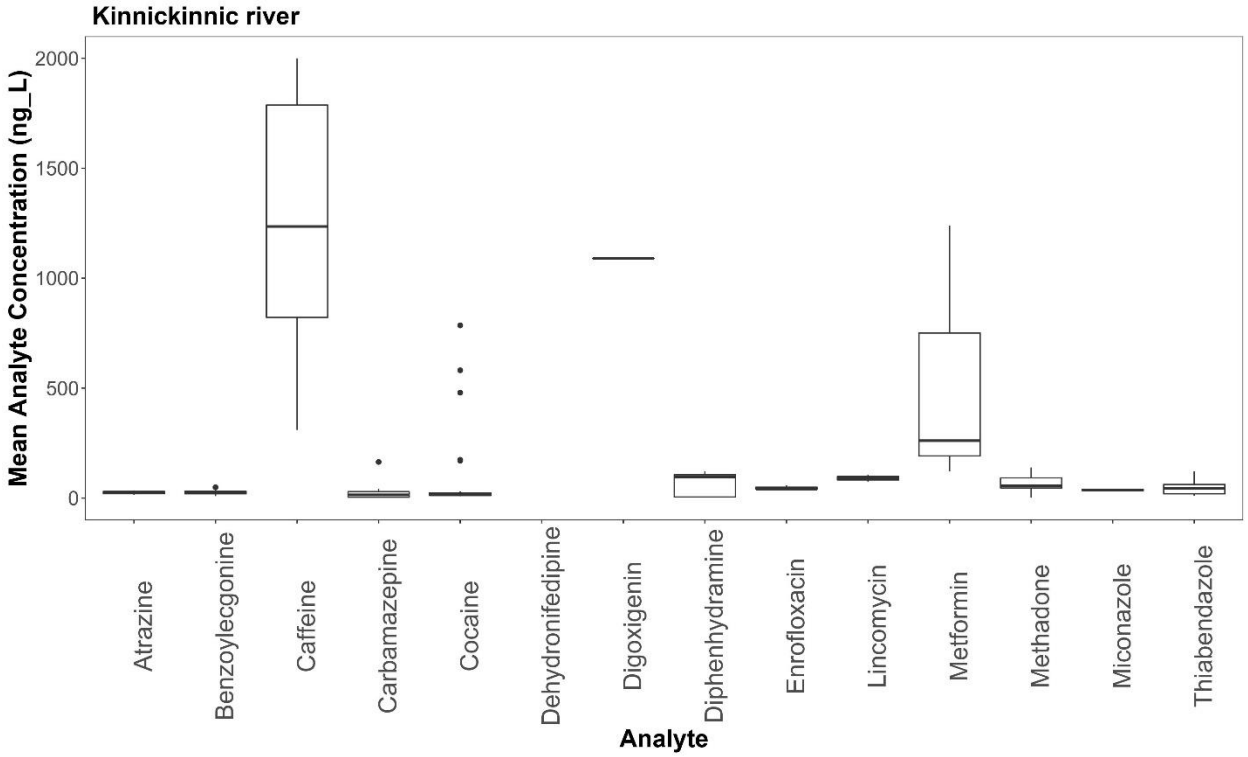


Figure 4.3: Box and whisker plots of mean analyte concentrations for detected analytes for the Kinnickinnic River.

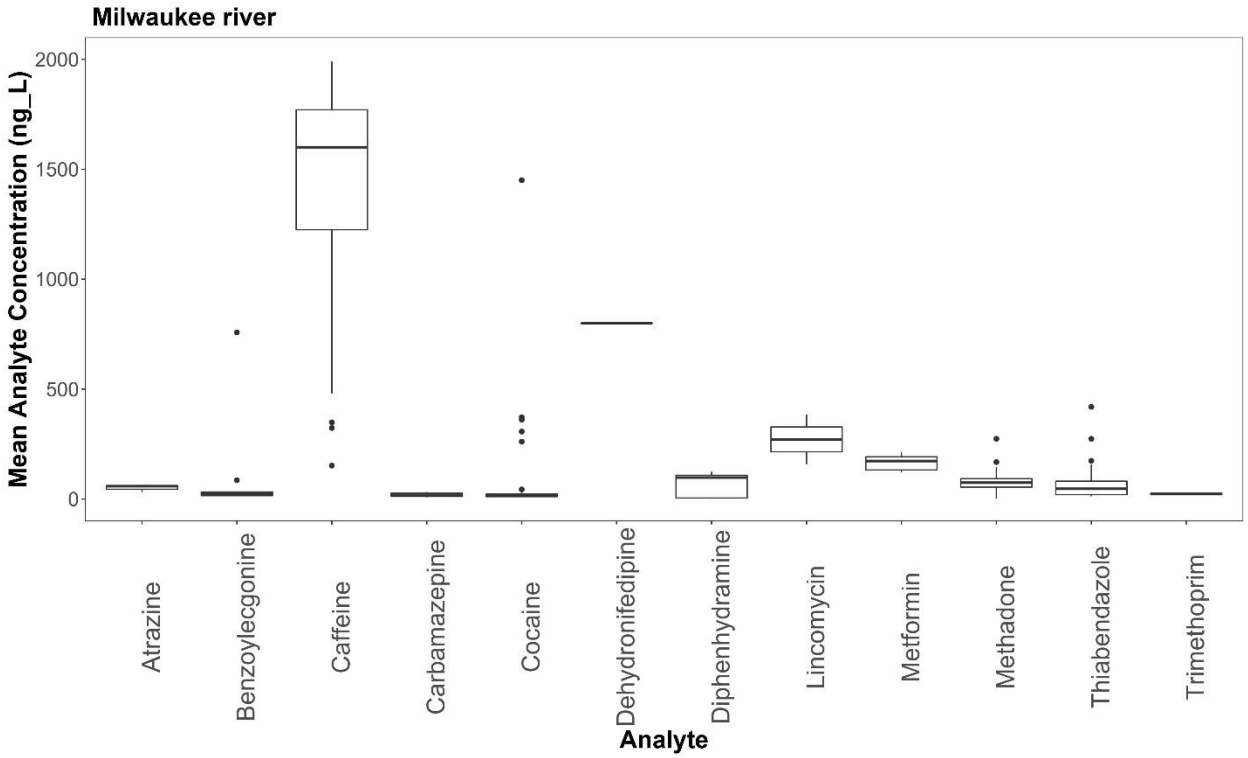


Figure 4.4: Box and whisker plots of mean analyte concentrations for detected analytes for the Milwaukee River.

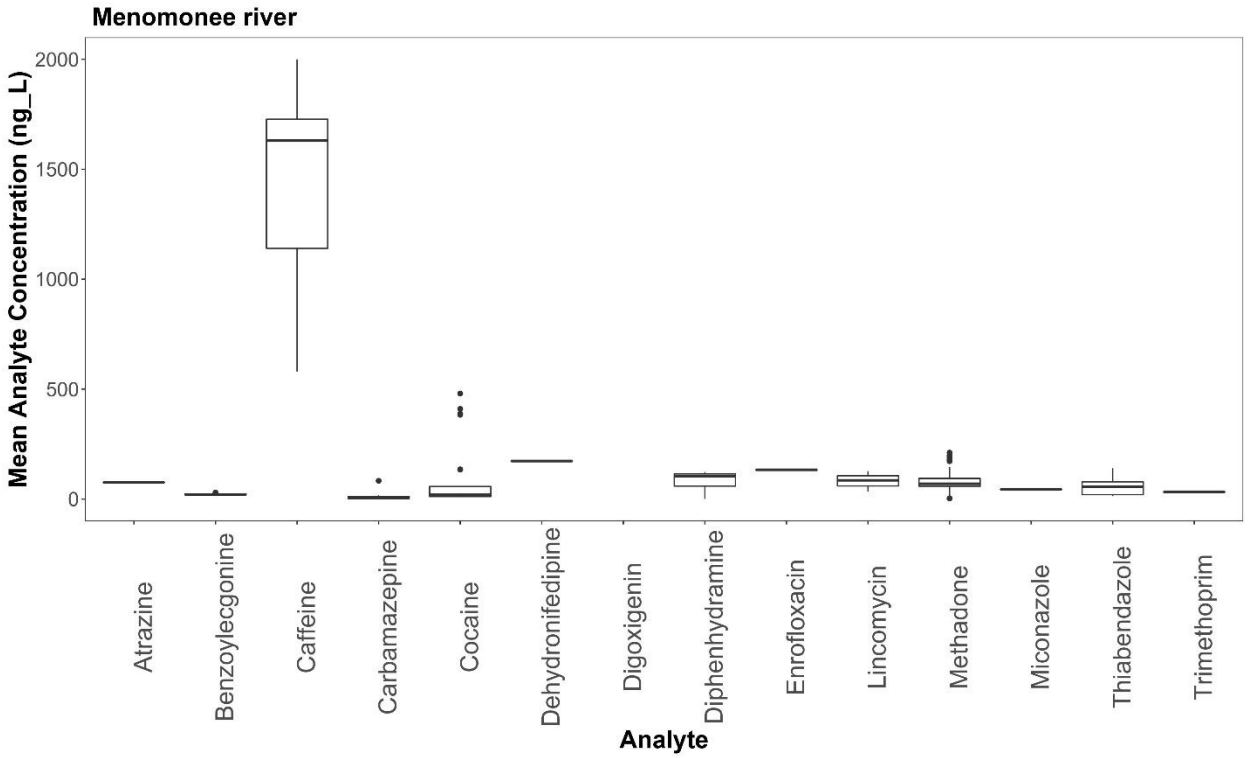


Figure 4.5: Box and whisker plots of mean analyte concentrations for detected analytes for the Menomonee River.

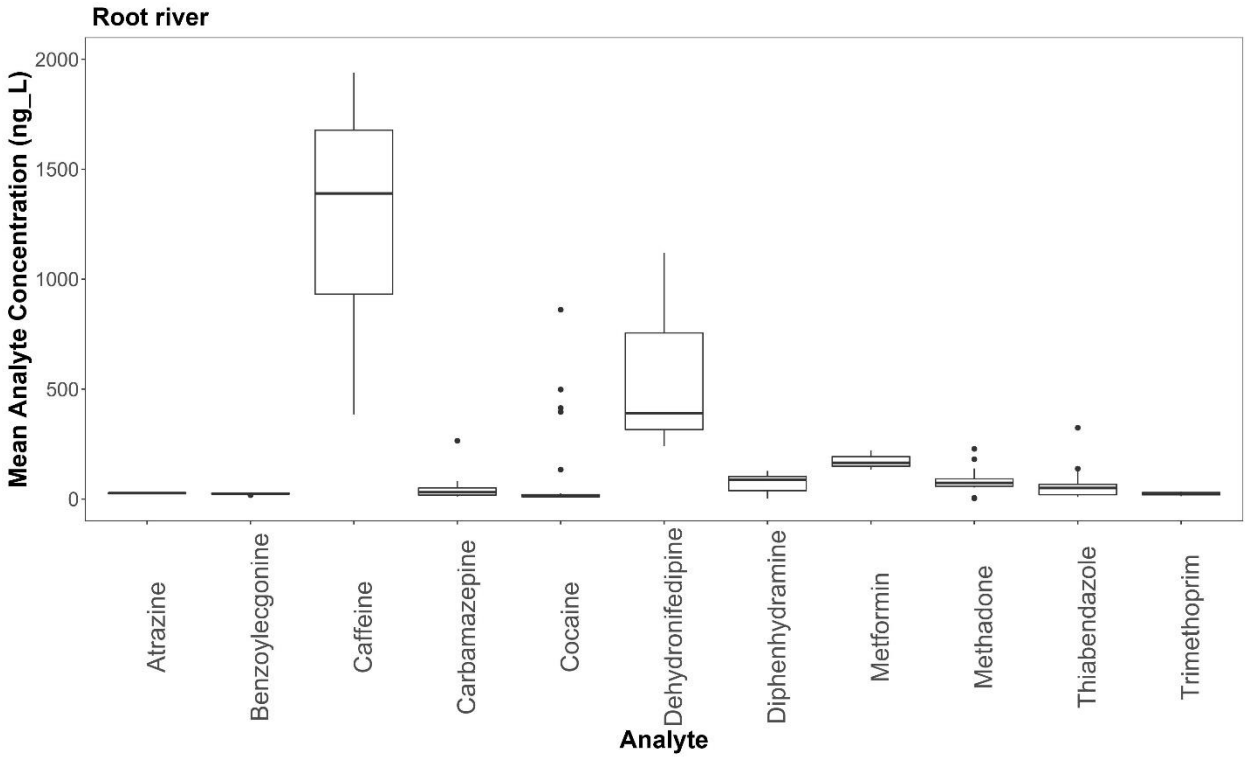


Figure 4.6: Box and whisker plots of mean analyte concentrations for detected analytes for the Root River.

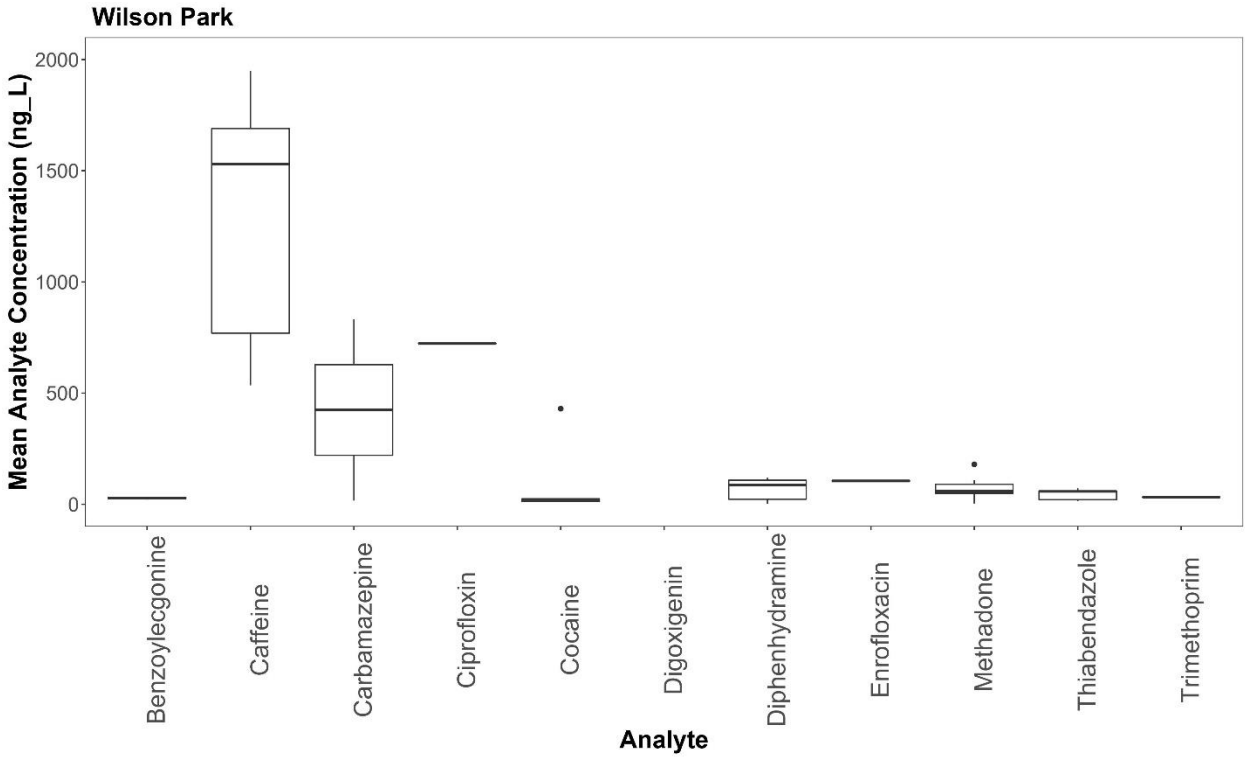


Figure 4.7: Box and whisker plots of mean analyte concentrations for detected analytes for the Wilson Park Creek.

Toxicity

PPCPs enter the aquatic environment through a variety of sources, such as externally applied PPCPs (e.g. lotions, sunscreens) from activities such as swimming and use of biosolids for fertilization. Aquatic organisms, such as fish and invertebrates that are near these sources of PPCPs are likely to be exposed to these chemicals throughout their entire life cycle. PPCPs are biologically active and can impact the metabolic activity of their cells. PPCPs are designed for use by humans or veterinary purposes and not these aquatic organisms. There is limited data on direct effects to aquatic organisms from exposure to these chemicals. Kovalakova et al. (Kovalakova et al., 2020) reviewed trimethoprim, sulfamethoxazole, ofloxacin and ciprofloxacin concentrations in surface waters (all detected in the rivers in this dissertation) and the ecotoxicity of these antibiotics towards different organisms. These antibiotics are often found in the environment as mixtures, such as sulfamethoxazole and trimethoprim are sometimes administered simultaneously. Duckweed (*Lemna* sp.) showed sensitivity to sulfamethoxazole. The EC_{50} values for ofloxacin and ciprofloxacin were between 0.1 mg/L and 0.7 mg/L for *Lemna minor* (Brain et al., 2014; Robinson et al., 2005*)*. These values are one order of magnitude lower than the lowest measured EC_{50} values for *P. subcapitata* which were 1.1 mg/L and 1.4 mg/L for ciprofloxacin and ofloxacin respectively (Isidori et al., 2005; Yang et al., 2008). Berninger et al. (Berninger et al., 2011) developed an aquatic toxicological method for the antihistamine diphenhydramine with aquatic plant and fish models. The plant model, *Lemna gibba*, was not adversely affected at exposures as high as 10 mg/L in comparison to the *Lemna* study by (Brain et al., 2014; Robinson et al., 2005) with the antibiotics ofloxacin and ciprofloxacin. In the fish model, *Pimephales promelas*, (Berninger et al., 2011) found pH affected the toxicity thresholds and feeding behavior (no-observed-effect concentration = 2.8 µg/L) than standardized survival or

growth endpoints. Lifelong exposure of antibiotics to aquatic organisms and aquatic plants may produce chronic health effects such as changes in behavior, reproduction, and growth.

Caffeine was detected in all river samples. According to traditional toxicity tests, caffeine alone does not appear to have toxic effects on aquatic organisms at the typical concentrations found thus far in the environment. However, environmentally relevant concentrations (e.g., 0.05 µg/L and 0.2 µg/L caffeine) have been shown to affect gill tissue of the California mussel (*Mytilus californianus*) at the molecular level, and little is known about effects of long-term exposure (del Rey et al., 2011). While caffeine most likely does not bioaccumulate and is not considered an acute threat, the detection of caffeine in water bodies often means the co-occurrence of organic wastewater compounds, including pharmaceuticals, pesticides, other emerging chemicals of concern (Moore et al., 2008; Quinn et al., 2009; Richards and Cole, 2006).

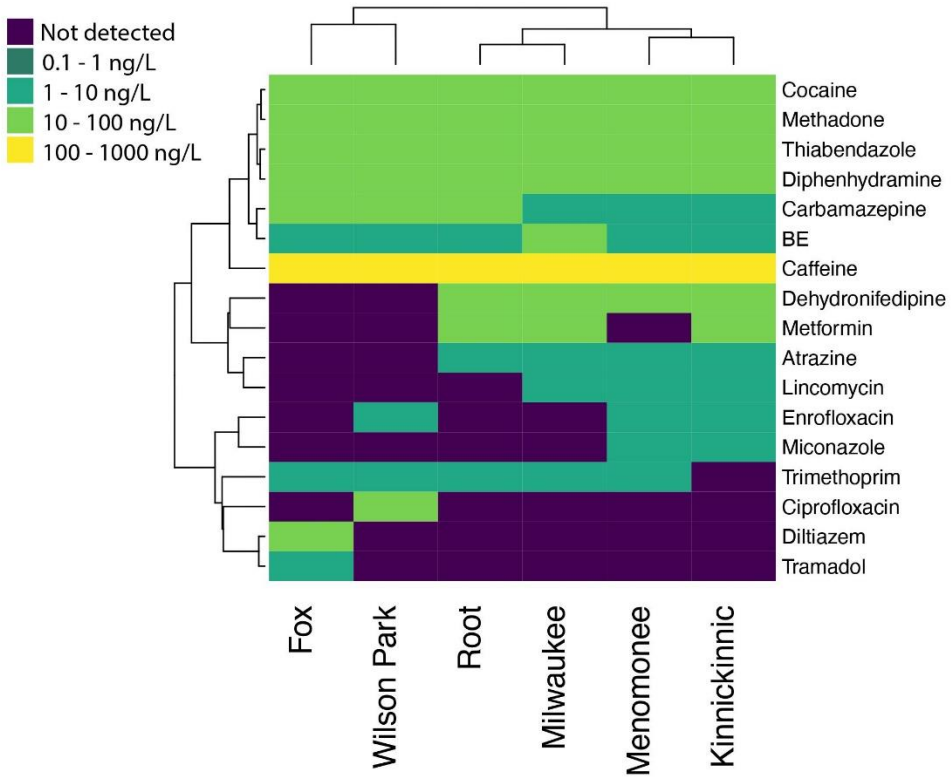


Figure 4.8: Heat map and cluster analysis of the 6 urban rivers, detected analytes and concentrations in ng/L.

The array of compounds detected in the Fox River and Wilson Park Creek are more like one another than to the other 4 rivers. The other rivers cluster together. Except for the Root River it makes sense geographically. The Root River sample location is most likely closer to the Milwaukee River than to the Fox River. The Wilson Park Creek is a pond, so it likely does not get the same kind of contaminants that the other rivers get. So, it makes sense that it is more like

the Fox River locations, which are further from the Milwaukee urban areas. Caffeine, (yellow color) was detected with highest concentration across all 6 rivers.

Seasonality

Samples from six urban rivers were taken in fall, spring, and summer, excluding any samples from winter. Caffeine was detected with the highest mean concentrations for all seasons in all 6 urban rivers (Figure 4.9 through 4.14). Peixoto et al. (Peixoto et al., 2022) created a model to indirectly determine caffeine concentrations at 20 sampling sites along the Atuba River , Brazil. The site located the furthest from the river's spring and also influenced by the discharge from the WWTP Atuba Sul had concentrations of 8.524 ug/L. They examined the demographic density and found a concentration rise of 3.081 ug/L possibly due to a higher density of irregular housing in this region. In addition, they explained the higher concentration of caffeine may be due to the concentration of caffeine might be higher in the dry seasons. Studies by (Ho et al., 2020; Peteffi et al., 2019) found caffeine concentrations on the Dnieper River in Ukraine at 192. µg/L, and on the Sinos River in Brazil at concentrations of 3.73 ug/L respectively. Fifteen PPCPs and caffeine were investigated in an urban stretch of the River Ganges for three seasons in two holy cities *Rishikesh* and *Haridwar* (India) (Singh and Suthar, 2021). caffeine showed the highest detection frequency (>90–100%) in the river and caffeine showed a higher load in summer, possibly due to their intense uses during this period. Another study (Silva-Filho, 2016) examined caffeine concentrations from samples collected in the Paquequer River, located at the city of Teresópolis, in the State of Rio de Janeiro, Brazil. Water samples were collected at seven points along the river, in summer, beginning of spring and end of winter in conservation areas, one tributary of the Paquequer River and four sites along the urban area of the city. Caffeine

concentrations were found in a range from 0.16 to 47.5 µg L-1 with the highest concentrations found in the urban areas. These studies show that caffeine is a global problem.

Cocaine was detected in all 6 rivers during all seasons (Figures 4.9 through 4.14) with Wilson Creek Park (Figure 4.14) having the lowest concentrations of cocaine. Summer months were found to be the initial start of drug use because of more idle time and increased social activities in the form of outdoor parties. Scientists from the National Institute on Drug Abuse (NIH) found the introduction of drugs was more likely to occur in the summer months and found marijuana use at 30% and cocaine use at 28%. BE, the metabolite of cocaine was detected in 4 of the 6 rivers (Figures 4.9, 4.10, 4.11, 4.14) for all seasons, and detected in spring and fall and summer in the Root River and in the Menomonee River, for fall and summer. Methadone, used for the treatment of morphine and heroin addiction was found in all 6 rivers and in all seasons. Dehydronifedipine was detected in 4 of the 6 rivers (Figures 4.10, 4.11, 4.12, 4.13) in the summer. Dehydronifedipine is a calcium channel blocker derived from nifedipine. The Root River samples contained detectable dehydronifedipine in the fall (Figure 4.13) with lower concentrations in the summer. The Fox River (Figure 4.9) and Wilson Park Creek (Figure 4.14) had no detectable dehydronifedipine. Thiabendazole, a compound used to treat fungal infections and used as an agricultural fungicide was detected in all 6 rivers and in all seasons (Figures 4.9, 4.10, 4.11, 4.12, 4.13, 4.14). Digoxigenin is a compound derived from digoxin which helps with atrial fibrillation. Digoxigenin was detected in the Kinnickinnic River, Menomonee River and Wilson Park Creek (Figures 4.10, 4.12 and 4.14) during the spring season. Tramadol, used as a pain reliever was only detected in the Fox River in the fall.

Atrazine, is used as an agricultural herbicide to control grasses and broadleaf weeds primarily with field and sweet corn, sorghum, and sugarcane and is approximately 100 times

more potent than glyphosate, the active ingredient found in Roundup® (de Albuquerque et al., 2020; Graymore et al., 2001). Atrazine was detected in the Kinnickinnic River, Milwaukee River and Menomonee River during the summer (Figures 4.10, 4.11, 4.12), and detected in the Root River in the fall (Figure 4.13) but not detected in the Fox River, or Wilson Park Creek. Bachetti et al. (Bachetti et al., 2021) collected samples on the Ctalamochita river basin for 5 years and found atrazine frequencies ranging from 67 to 100% in spring and 33% to 67% in fall. Atrazine concentrations increased during the warm-rainy season because of atrazine application to crops. Methadone and thiabendazole were detected in all 6 rivers for all seasons (Figures 4.9 through 4.14). Hu et al. (Hu et al., 2019) conducted a study of 11 abused drugs (including methadone) in the Beiyunhe River, an urban river flowing through North China, and found concentrations from 26.6 to 183.0 ng/L in water and determined the drugs originated from hospitals and sewage treatment plants.

Ciprofloxacin, and enrofloxacin, both antibiotics, were detected at Wilson Park Creek in the spring, and enrofloxacin, was also detected in the Menomonee and Kinnickinnic Rivers in the spring with no detection in the Fox, Milwaukee, and Root Rivers. Diltiazem, an antihypertensive drug was only detected in the Fox River during fall and spring.

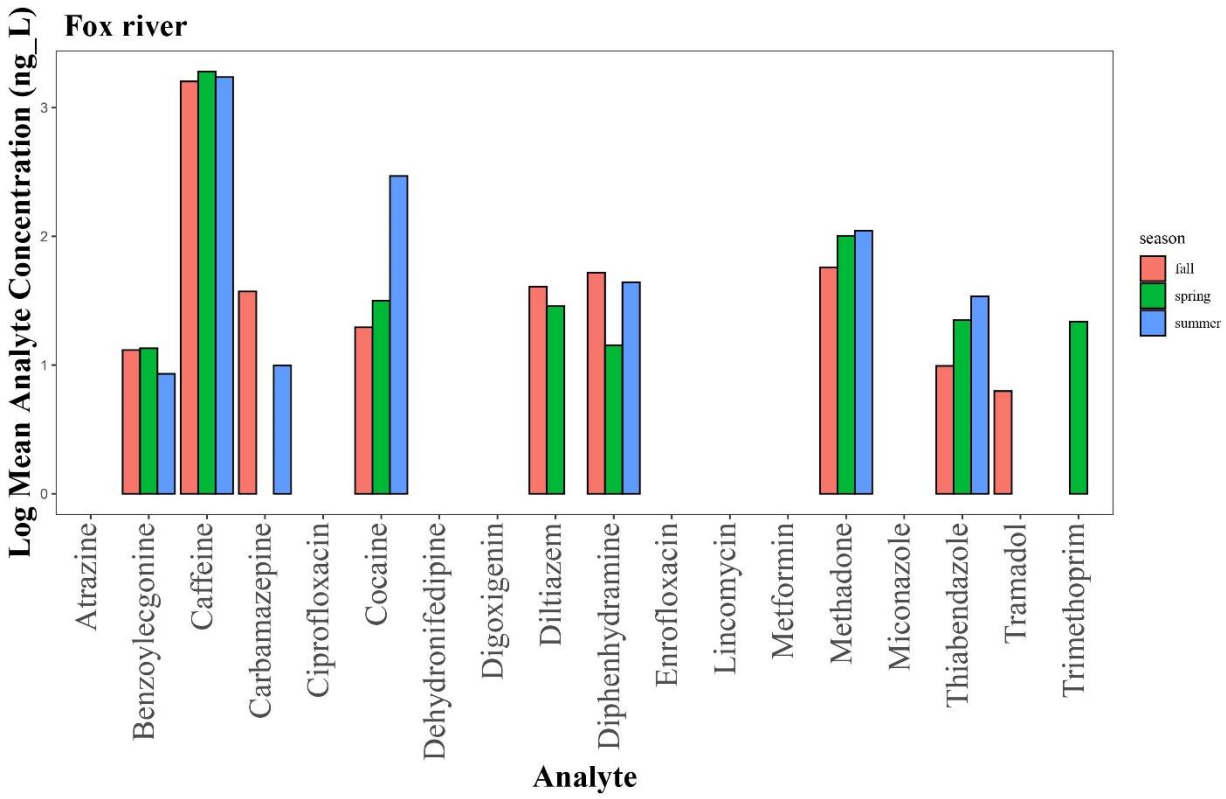


Figure 4.9: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Fox River. No sampling occurred in the winter months.

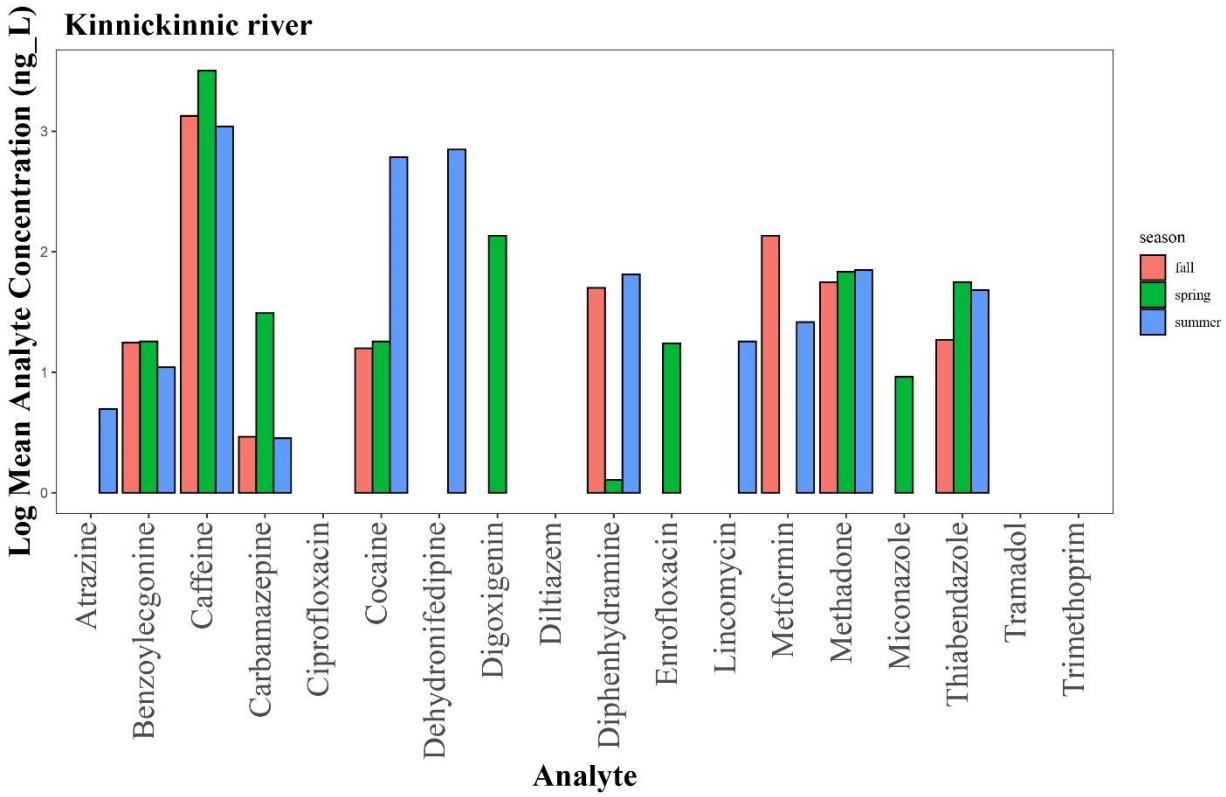


Figure 4.10: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Kinnickinnic River. No sampling occurred in the winter months.

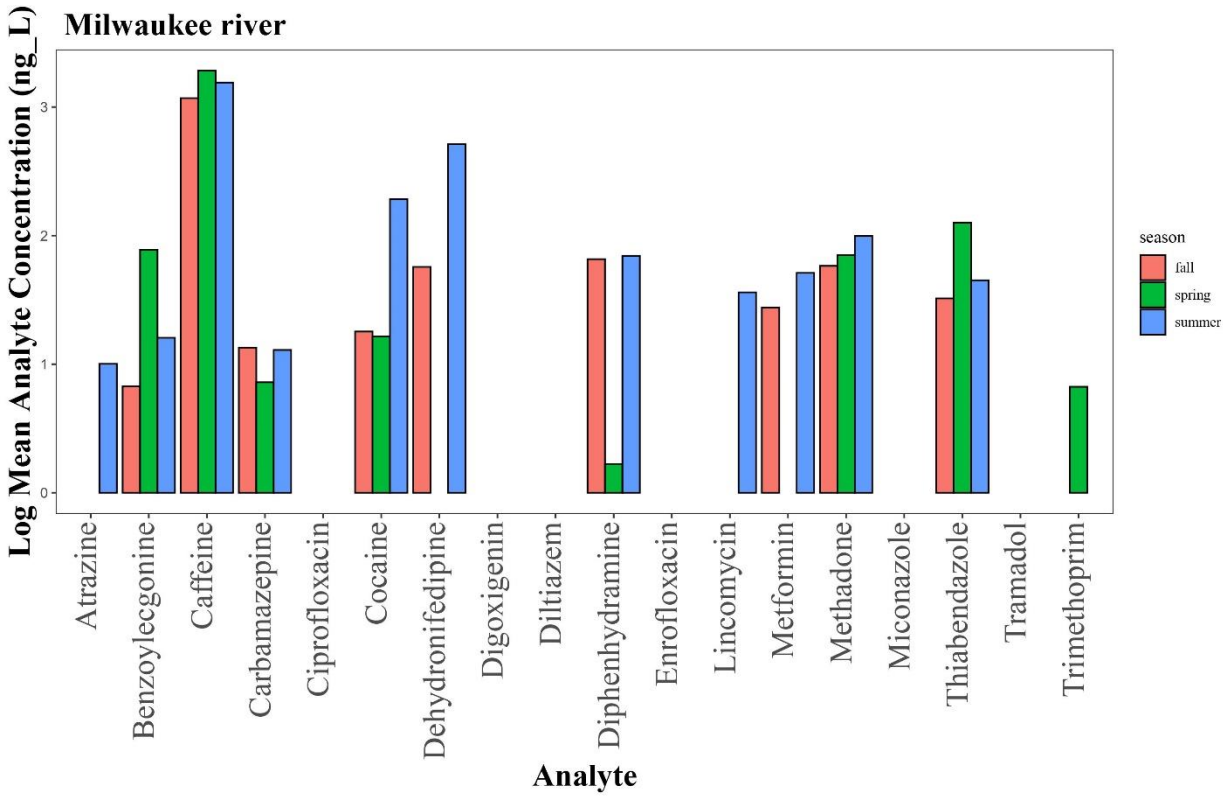


Figure 4.11: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Milwaukee River. No sampling occurred in the winter months.

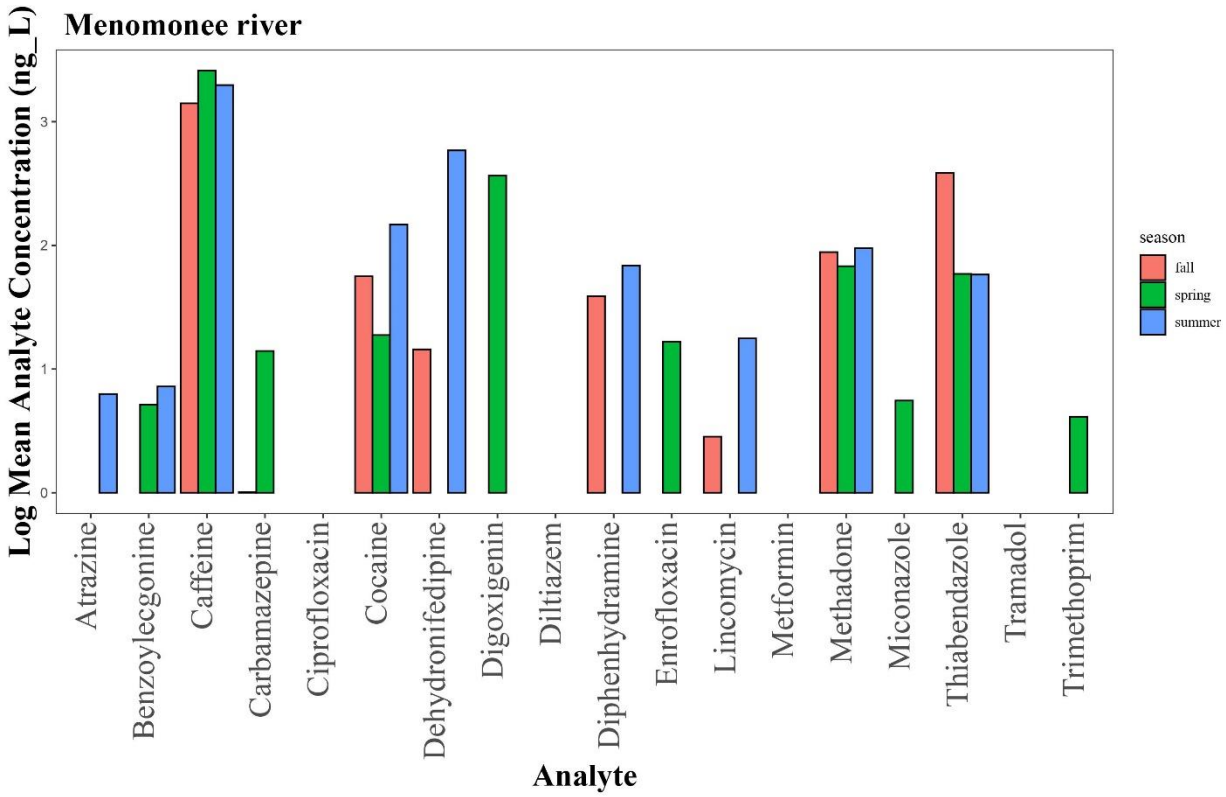


Figure 4.12: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Menomonee River. No sampling occurred in the winter months.

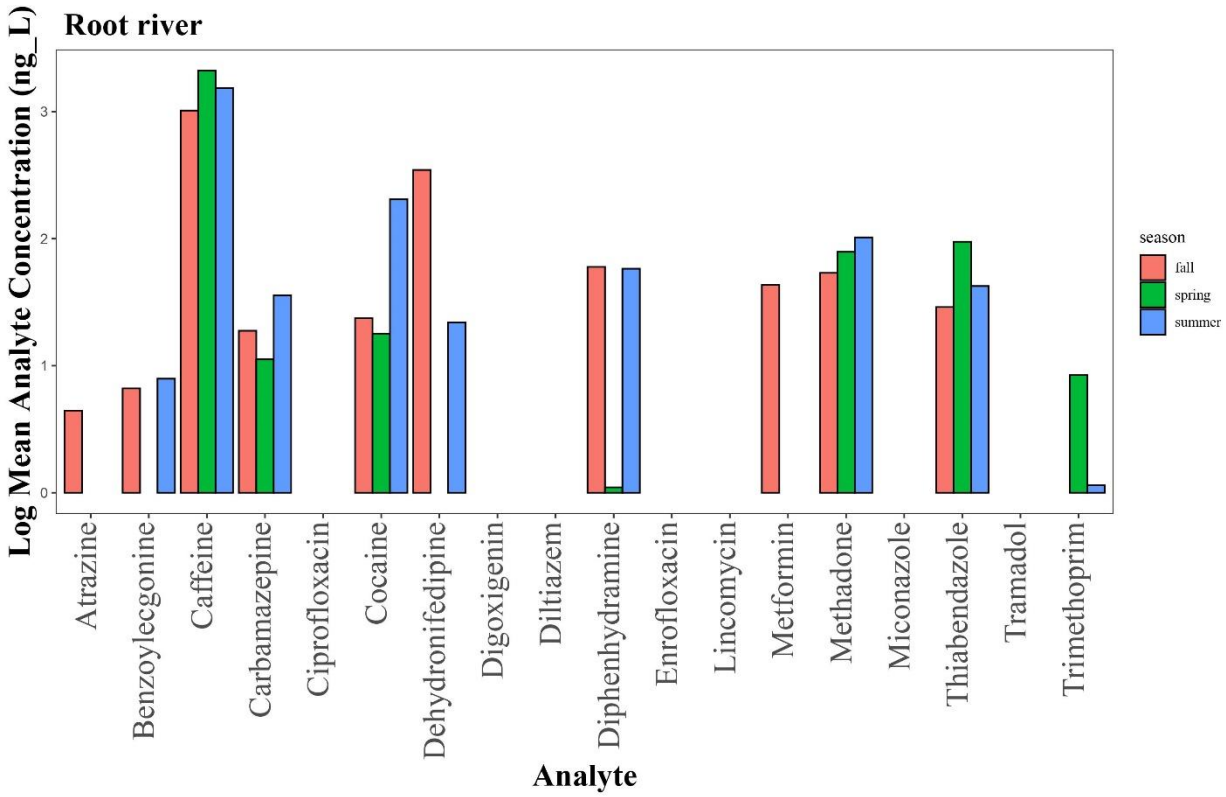


Figure 4.13: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Menomonee River. No sampling occurred in the winter months.

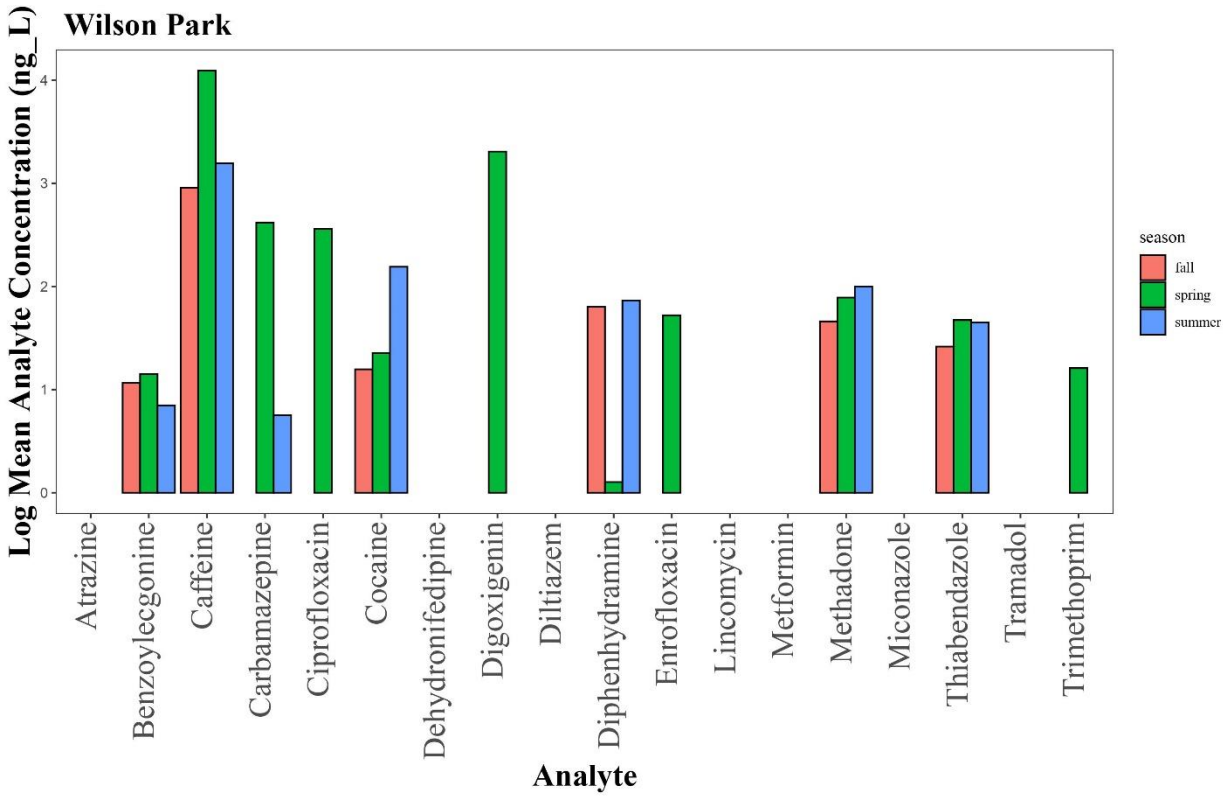


Figure 4.14: Log base 10 mean analyte concentration for detected analytes for fall, spring and summer in the Wilson Park Creek. No sampling occurred in the winter months.

Conclusions

The influence of land usage in urban rivers can affect what contaminants may be found in those rivers. Six urban rivers were examined for PPCPs and illicit drugs for actual concentrations and seasonality. The Milwaukee River basin contains the Menomonee River and the Kinnickinnic River in which the southern portion of the basin is the most densely populated with about 90% of the basin's population. Looking at just the Milwaukee River south watershed it is about 33% urban, 25% agriculture, 21% grassland, 12% forests, and 6% wetlands. The Menomonee River is mostly urban, heavily channeled and industrialized. Channelization can increase erosion, change the water quality, and lose some aquatic habitat. Chemicals and heavy metals may enter the river system where there is heavy industrialization. Examining the graphs for the Milwaukee River, Menomonee River and the Kinnickinnic River, many compounds were detected in these rivers, from atrazine, cocaine, methadone, and caffeine, primarily in the spring and summer. The Fox River is heavily urbanized at the headwaters, then becomes agricultural, and the Root River is a mixture of urban and rural land usage that is affected by stormwater runoff. Lastly, Wilson Park Creek is a tributary to the Kinnickinnic River, and is 30% concrete lined, and 30% enclosed channel which makes it a highly stressed system.

Sources of PPCPs and illicit drugs in urban rivers may vary under different seasons or weather conditions. For example, Mei et al. (Mei et al., 2018) studied 11 PPCPs along the Huangpu River in Shanghai, China during the dry season and wet season and found concentrations up to 1455 ng/L for carbamazepine, trimethoprim and caffeine similar to this study.

Chapter 5:

Conclusions and future directions for the study of pharmaceuticals and personal care products (PPCPs) and illicit drugs

This dissertation contributes information to the understanding of the diverse types of pharmaceuticals and personal care products and illicit drugs entering wastewater treatment plants as influent, being processed, and exiting as effluent to the receiving waters and/or as biosolids and being landfilled. An alternative method for extracting compounds in water samples was used instead of the standard solid phase extraction protocol used in most literature (Appendix A: Tables 2 and 3). This alternative method was able to detect various types of compounds in influent, effluent, and sludge. Chapter 2's box and whisker plots (Figures 2.1) showed compounds detected with the alternative method in the 4 categories, though some compounds at very low concentrations. Surface water box and whisker plots showed this alternative method was able to detect many compounds (Figure 2.2). Chapter 3 (Figure 3.3 a,b,c,d) shows the detected analytes from influent, effluent, but also from sludge using this alternative method. This research showed that many compounds are not being removed from the effluent and are entering the surface waters in concentrations ranging from ng L^{-1} to µg L^{-1} (Quesada et al., 2019) and are discharged into the environment continuously. These compounds in the surface waters may have an impact on aquatic and terrestrial life, even at low concentrations and more research needs to be done on long-term effects. These pharmaceuticals found in surface waters may affect the aquatic invertebrate communities. Many species emerge in the spring for reproduction and become food for other organisms. One study found the invertebrate emergence occurred sooner and at a greater rate when exposed to certain pharmaceuticals (Richmond et al., 2017). More research needs to be done since many of these pharmaceutical effects are unknown.

Pharmaceuticals and personal care products (PPCPs) are commonly used for cosmetics, hygiene, medical care, etc. Pharmaceutical compounds, such as antibiotics, have a market value of 1462 billion dollars in 2021 (González Peña et al., 2021; Ziylan-Yavas et al., 2022). The personal care products, such as sunscreen, deodorants, etc. are the second more common group (Ziylan-Yavas et al., 2022). North America has the leading role in the U.S. pharmaceutical industry with China having the highest growth rates worldwide (González Peña et al., 2021). Many factors such as reduced taxes and lowered drug prices in the U.S, sedentary lifestyles and ageing drive the growth of market sales in the U.S. (González Peña et al., 2021). As the pharmaceutical market grows and consumption increases, pharmaceuticals entering the environment increases due to poor management, and disposal mechanisms to name a few. Wastewater effluent is the major source of these compounds in surface waters (Figure 2.2). Chapters 2 and 3 in this dissertation confirmed the inefficiency of wastewater treatment plants in removing these compounds as shown by negative removal (Figures 2.3, 2.4, 3.5 through 3.8).

Chapter 4 presented research on pharmaceuticals and illicit drugs from 6 urban rivers. This study does not involve wastewater treatment plants. Most of the compounds detected were potentially from private sewer systems, gray water and/or agricultural runoff. A diverse group of compounds were detected with caffeine having the highest concentrations (Figures 4.2 – 4.7).

Antibiotic resistance is a serious and growing occurrence that has emerged as one of the most important public health concerns of the 21st century. An increasing number of pathogenic bacteria have developed resistance to commonly used antibiotics. The Center for Disease Control (CDC) (CFDC, 2019) reports that in the United States approximately 2 million people are infected with bacteria resistant to antibiotics and ultimately 23,000 people will die because of these infections. There is concern that the release of antibiotics into the environment might be

contributing to the increase of antibiotic resistance. A major contributor to antibiotics in the environment is effluent released from WWTPs or from veterinary practices. There is a global need to produce new antibiotics. Infectious diseases cause 1/5th of all deaths globally each year and are the leading killer of children under 5 years of age (Martens and Demain, 2017). Research should address antibiotic resistance and its potential to transfer to human and animal pathogens that may affect health.

The current opioid crisis is officially the deadliest drug crisis in American history. Drug overdoses are now the leading cause of death for Americans under the age of 50 years (Salmond and Allread, 2019). In 2016, synthetic opioids have surpassed prescription opioids as the leading cause of drug over dose deaths (Salmond and Allread, 2019). The National Institute on Drug Abuse (NIDA) (NIDA, 2020) reported in 2017 that more than 72,000 drug overdose deaths occurred in the United States (Salmond and Allread, 2019). Overdoses killed more Americans in 2017 than guns (37,400), car accidents (38,000), or breast cancer (40,000) (Salmond and Allread, 2019). The states with the highest age-adjusted drug overdose death rates were West Virginia (52.0%), Ohio (39.1%), New Hampshire (39.0%), Pennsylvania (37.9%) and the District of Columbia (38.8%) (Salmond and Allread, 2019). Twenty-six states experienced drug overdose increases from 2015 to 2016 and all were located in the Northeast, Midwest and South (Hedegaard, 2020). Demographics of the opioid crisis show that rates are higher for males than females, though women aged 40-64 years are the fastest growing group for rates of death (Salmond and Allread, 2019). Ethnicity showed Caucasian accounted for 80.7% of opioid overdose deaths, followed by Blacks and Hispanics at 8.25 and 7.3%, respectively (Altekruse SF, 2020). American Indian, Alaskan Natives, Asians and Pacific Islanders accounted for 1% of opioid overdose deaths (Altekruse SF, 2020). Socioeconomic

status played an important role in the risk factor for opioid overdose (Altekruse SF, 2020). People with less than a four-year college degree had higher risk factors for opioid overdose than people with graduate degrees (Altekruse SF, 2020). This may be due to not having a stable employment opportunity (Altekruse SF, 2020). Providers are more cautious in prescribing opioids to non-Whites due to the fact that the premise is that non-Whites are more likely to abuse or sell the drugs (Hansen, 2016).

Opioid use varies from country to country. In the United States, opioid use is the highest of the twenty most populous countries, more than 50% higher than Germany (Figure 5.1). In poorer countries, such as India, Nigeria and the Congo, opioids are not prescribed as often for pain relief because there is a taboo regarding these types of drugs (Dan, 2018). The use of opioids has had an effect on life expectancy in the United States as a result of overdose deaths and suicides (Boté, 2019). The average life expectancy has decreased from 78.9 years in 2014 to 78.6 years in 2017 (Boté, 2019). Between 2014 and 2015, there was a 19% increase in overdose deaths among teenagers and infections from drug injections are continuing to be a problem (Boté, 2019). Illicit drug use may impact the health and safety of children and adolescents, such as accidental ingestion of prescription drugs, misuse of drugs during pregnancy, poor prenatal care and low birth weight, economic losses due to purchase of drugs, and more children entering foster care, due to parent's incarceration, drug treatment or death (Boté, 2019). A study by Khoury et al. (Khoury et al., 2010) found a strong association between childhood traumatization, substance use disorders and post-traumatic stress disorder. Another public health issue is the safety of first responders when they come into contact with the potent and fast-acting fentanyl and its analogs during emergency responses (Boté, 2019). There is an economic cost to train first responders to recognize respiratory distress, disorientation, and

cardiac arrest, in addition to training on using personal protective equipment and how to administer naloxone for opioid overdoses (Boté, 2019). Recently, public vending machines were implemented in Nevada for naloxone dispensation (Narcan) and research has found a reduction in opioid overdose fatalities (Allen et al., 2022).

Drug use is a worldwide problem and typically begins during adolescence. Some of the factors identified with adolescent drug use are high impulsivity, rebelliousness, emotional impairment, lack of religion, pain, total screen times to name a few (Nawi et al., 2021). Familial risk factors were maternal smoking, low parental education, negligence, poor supervision etc. and having peers using drugs (Nawi et al., 2021). Some preventive measures were desire to maintain good health, school connectedness, strong religious beliefs and paternal awareness of drug abuse (Nawi et al., 2021).

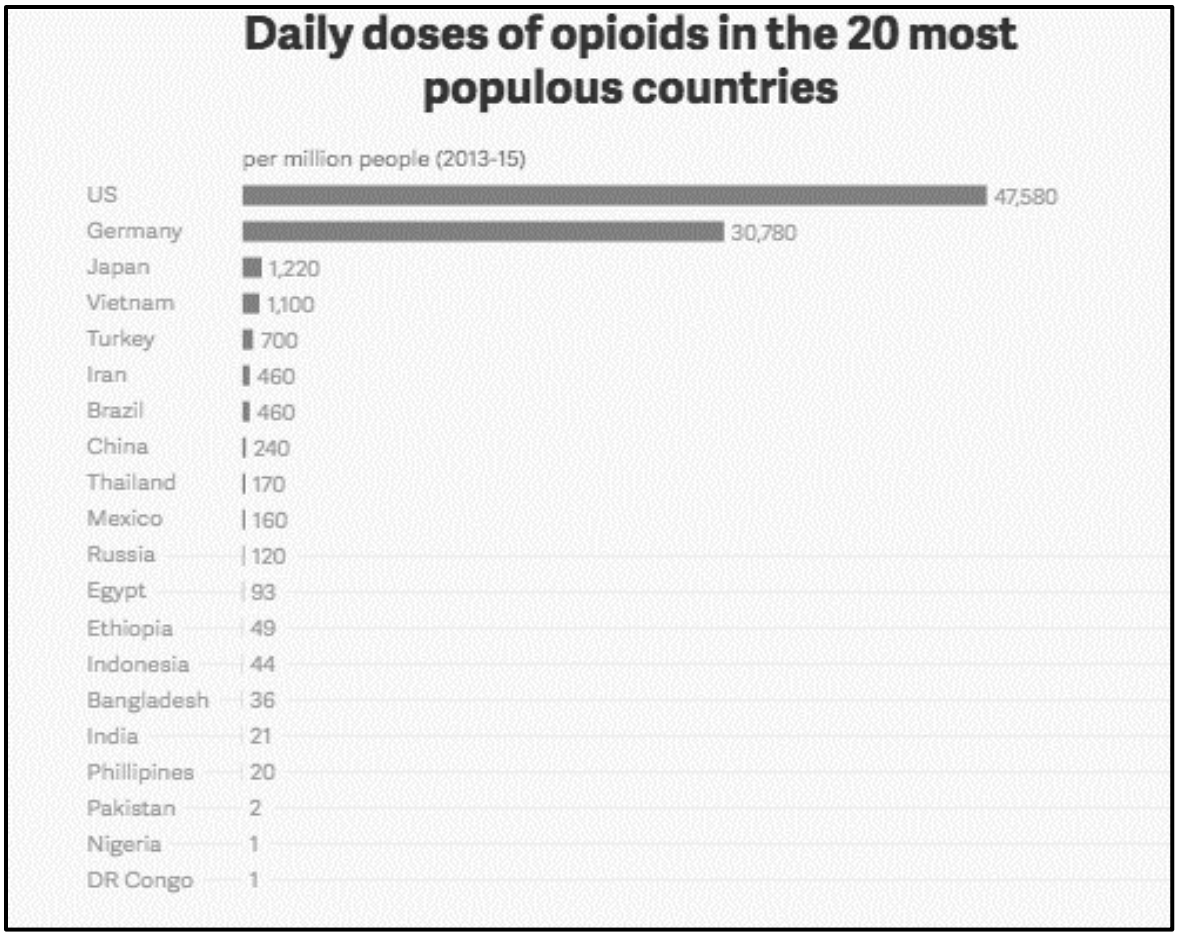


Figure 5.1: Data showing opioid use in 20 most populous countries from International Narcotics Control Board 2017 (Dan, 2018).

Drug use is a public health emergency. First and foremost, it must be recognized as a public health emergency. Doctors and therapists need to understand their role since currently the focus is more on treatment. The primary factors identified in the opioid epidemic are the aggressive marketing programs that pharmaceutical companies generate, a failure to treat mental health problems and lack of proper training to manage pain (Smith, 2020). Clinicians should become more proactive in public health whether in the clinic, the hospital or the community (Smith, 2020).

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Appendix A: Chapters 2 and 3 Supplementary Materials

Table A1: Targeted compounds, category of each compound and CAS number (n=60)

Classification	Compound	Category	CAS #	Company
General	Atrazine	Herbicide	1912-24-9	Sigma-Aldrich
	Acetominophen	Analgesic	103-90-2	Cayman
	Albuterol	Anti-asthmatic	18559-94-9	Sigma-Aldrich
	Carbamazepine	Anti-epileptic	298-46-4	Sigma-Aldrich
	Cotinine	Nicotine Metabolite	486-56-6	Sigma-Aldrich
	Diltiazem	Calcium Channel	33286-22-54	Sigma-Aldrich
	Dehydronifedipine	Blockers	21829-25-4	Sigma-Aldrich
	Diphenhydramine	Anti-histamine	21829-25-4	Acros
	Thiabendazole	Fungicide	1872-46-4	Fluka
	Miconazale		229-16-478	Alfa Aesa

	Lisinopril	Ace Inhibitor	83915-82-7	Sigma-Aldrich
	Cimetidine	Anti-acid Reflux	51481-61-9	Sigma-Aldrich
	Ranitidine		66357-35-5	Sigma-Aldrich
	Metformin	Anti-diabetic	1115-70-4	Sigma-Aldrich
	Simvastatin	Anti-lipidemics	79907-63-9	Sigma-Aldrich
Antibiotics	Carbadox		6804-07-5	Sigma-Aldrich
	Ciprofloxacin		85721-33-1	Acros
	Enrofloxacin		93106-60-6	Sigma-Aldrich
	Lincomycin		7179-49D	Sigma-Aldrich
	Lomefloxacin		98079-51-7	Sigma-Aldrich
	Ofloxacin		82419-36-1	Sigma-Aldrich
	Oxacillin		1173-88-2	Sigma-Aldrich
	Penicillin G		69-57-8	Sigma-Aldrich
	Penicillin V		87-08-1	Sigma-Aldrich
	Roxithromycin		91296-87-6	Fluka
	Sulfachloropyridazine		80-32-0	Sigma-Aldrich
	Sulfadiazine		68-35-9	Sigma-Aldrich
	Sulfadimethoxine		122-11-2	Sigma-Aldrich
	Sulfamerazine		127-58-2	Sigma-Aldrich

	Sulfamethazine		57-68-1	Sigma-Aldrich
	Sulfamethizole		144-82-1	Sigma-Aldrich
	Sulfamethoxazole		723-46-6	Sigma-Aldrich
	Sulfathiazole		21409-26-7	Sigma-Aldrich
Opioids	4-ANPP		61086-44-0	Cerilliant
	Carfentanyl		78995-18-3	Cerilliant
	Cis-3-methylfentanyl		76-57-3	Cerilliant
	Codeine		437-38-7	Supelco
	Fentanyl		125-29-1	Cerilliant
	Hydrocodone		466-99-9	Cerilliant
	Hydromorphone		76-99-3	Supelco
	Methadone		57-42-1	Cerilliant
	Meperidine		465-65-6	Cerilliant
	Naloxone		639-46-3	Supelco
	Morphine		16590-41-3	Cerilliant
	Naltrexone		1211527-24-0	Cerilliant
	Norfentanyl		71968-04-2	Cerilliant
	Norhydrocodone		54426-25-0	Cerilliant
	Noroxycodone		76-42-6	Cerilliant
	Oxycodone		76-41-5	Cerilliant
	Oxymorphone		60651-17-3	Cerilliant
	Sufentanil		36282-47-0	Supelco

	Tramadol		33741	Cerilliant
Recreational	6-monoacetylmorphine		2784-73-8	Cerilliant
	Amphetamine		300-62-9	Cerilliant
	Caffeine		58-36-2	Cerilliant
	Cocaine		50-36-2	Supelco
	Methamphetamine		7632-10-2	Cerilliant
	Paraxanthine		611-59-6	Cerilliant

Classification indicates how the analytes are grouped to match the graphs.

Table A2: PPCP/Opioid Extraction Procedure from Lyophilized Water Samples

1. Prior to lyophilization
    a. Measure out 25 ml OR 10 ml of sample water of each type in triplicate (3 unspiked/3 spiked)
    b. Spike each sample with the PPCP mixed standard. (I have been using 10,000 ug/L concentration)
    c. DO NOT SPIKE OR DO TRIPLICATES FOR RIVERKEEPER SAMPLES.
    d. Vortex samples
    e. Parafilm each sample, poke holes in parafilm; put in -80 degree C freezer until frozen
    f. When each sample is completely frozen, put on lyophilizer. It takes approx. 2 days for lyophilization.
2. After lyophilization
    a. Remove samples from lyophilizer
    b. Remove parafilm and drop the center of the parafilm into the falcon tube containing the sample

c. Resuspend dried material with 40:20:20 methanol:acetone:water with 5% acetic acid to equal a total volume of 5 ml for each sample)
d. Vortex samples
e. Sonicate samples in 50 degree C sonicator for 10 minutes
f. Centrifuge samples in falcon tubes for 15 minutes
g. Remove supernatant from falcon tubes and put into 20 ml scintillation vials
h. Do this 3 times.
i. Put samples in scintillation vials on evaporator at 50 degrees C under N2 gas.

3. After Evaporation
    a. Resuspend samples in scintillation vials with with 1000 uL of 50% MeOH
    b. Vortex samples
    c. Sonicate samples for 10 minutes
    d. Transfer sample material to 1.5 uL centrifuge tubes and centrifuge for 15 minutes at 15,000 RPM's.
    e. Put supernatant into LC vials
    f. Make standards (0 to 100 ug/L in 50% MeOH)

Table A3: Extraction Procedure for Wastewater Sludge

Weigh out 1 gram of sludge per sample and put in labeled 50 ml falcon tubes. (Do this in duplicate or triplicate).

a. Parafilm the top of each sample; use a small pipet tip to poke holes in the tops of each parafilmed falcon tube.
b. Put parafilmed falcon tubes in a -80-degree freezer until frozen (usually around 2 hours). When each sample is frozen, put frozen samples into freeze-flasks and attach the flask to the lyophilizer. If using several flasks, wait until lyophilizer vacuum pressure is down to 0.040 psi (-50 C) before adding another flask. It usually takes 2 to 3 days for complete lyophilization to occur.
c. Remove samples from lyophilizer
d. Remove parafilm with razor blade (clean with MeOH each time) and drop the center of the parafilm into the falcon tube containing the sample.

Reconstitute dried material with 10 ml combined (5 ml 80%MeOH) and (5 ml 10% glacial acetic acid)

Spike or not spike with Mixed PPCP/Opioid standard

(Using the 1000 µg/L mixed standard spike with 200 µL)

Vortex samples

Sonicate samples in a 50 degree waterbath for 15 minutes

Put on shaker table overnight at room temperature

Centrifuge samples in falcon tubes at maximum speed for 15 minutes

Remove supernatant from falcon tubes and put into 20 ml labeled scintillation vials

After transferring supernatant, add 5 ml of 80% MeOH to each sample

Do the centrifuge and removal of supernatant into scintillation vials a total of 3 times. Should end up with 15 ml of supernatant in the scintillation vials.

Vortex and sonicate between centrifuges

Evaporate scintillation vials at 50 degrees C under N_2 gas. This may take overnight to dry down.

After evaporation: Reconstitute with 1 ml of the following combination (50% of the 100% ACN Buffer B, plus 5 ml of 1% ammonium formate and 1 ml of 0.1% formic acid Buffer A to make 1L).

Vortex and sonicate

Transfer sample material to 1.5 µL labeled centrifuge tubes and centrifuge at maximum speed for 15 minutes.

Dilute samples 1:5 (for 1 ml of sample: 200 uL of sample: 800 uL of buffer B/A)

Put in labeled LC vials.

Table A4: Tandem mass spectrometry parameters using the 4000 Q-Trap triple quadruple ion trap mass spectrometer in positive scheduled mode.

Analyte	**Parent Mass Charge (m/z)**	**Daughter Mass Charge (m/z)**	**Molecular Formula**	**MW (g/mol)**	**Retention Time (minutes)**	**DP (volts)**	**CE (volts)**
6- MAM 1 6- MAM 2	328.170 328.170	165.200 210.900	$C_{19}H_{21}NO_4$	327.000	4.98	71 71	53 35
Acetaminophen 1 Acetaminophen 2	152.189 152.189	110.140 164.843	$C_8H_9NO_2$	151.163	4.15	56 56	23 45
Amphetamine 1 Amphetamine 2	136.100 136.100	119.30 91.200	$C_9H_{13}N$	135.210	4.70	41 41	13 27
Albuterol 1 Albuterol 2	240.898 240.898	223.00 149.30	$C_{13}H_{21}NO_3$	239.311	4.27	71 71	15 25
Ampicillin 1 Ampicillin 2	350.102 350.102	106.200 160.200	$C_{16}H_{18}N_3NaO4S$	349.406	4.98	46 46	23 19
Atrazine 1 Atrazine 2	216.179 216.179	173.90 67.900	$C_8H_{14}ClN_5$	215.680	6.82	11 11	25 53
Benzoylecgonine 1 Benzoylecgonine 2	290.172 290.172	168.30 105.20	$C_{16}H_{10}NO_4$	289.331	5.40	76 76	27 41
Caffeine 1 Caffeine 2	195.077 195.077	138.194 110.210	$C_8H_{10}N_4O_2$	194.19	5.10	61 61	29 33
Carbadox 1 Carbadox 2	263.173 263.173	230.808 89.9330	$C_{11}H_{10}N_4O_4$	262.22	5.40	46 46	19 43
Carfentanyl 1 Carfentanyl 2	395.085 395.085	363.20 335.20	$C_{24}H_{30}N_2O_3$	394.512	6.54	181 181	5 5
Cimetidine 1 Cimetidine 2	254.022 254.022	160.20 95.100	$C_{10}H_{16}N_6S$	252.34	4.41	66 66	21 37

Carbamazepine 1 Carbamazepine 2	237.129 237.129	194.166 178.947	$C_{15}H_{12}N_2O$	236.269	6.68	51 51	29 47
Ciprofloxacin 1 Ciprofloxacin 2	332.200 332.200	230.830 287.987	$C_{17}H_{18}FN_3O_3$	331.346	5.60	66 66	55 27
Cis-3-methylfentanyl 1 Cis-3-methylfentanyl 2	351.263 351.263	202.10 105.20	$C_{23}H_{30}N_2O$	350.50	6.54	81 81	33 53
Cocaine 1 Cocaine 2	304.210 304.210	182.10 77.100	$C_{17}H_{21}NO_4$	303.353	5.97	66 66	27 81
Codeine 1 Codeine 2	300.186 300.186	199.40 128.20	$C_{18}H_{21}NO_3$	299.364	4.69	56 56	41 81
Cotinine 1 Cotinine 2	176.750 176.750	80.00 98.20	$C_{10}H_{12}N_2O$	176.22	2.48	76 76	35 29
Diltiazem 1 Diltiazem 2	415.143 415.143	177.964 149.871	$C_{22}H_{26}N_2O_4S$	414.52	6.54	66 66	39 67
Dehydronifedipine 1 Dehydronifedipine 2	345.116 345.116	284.00 152.20	$C_{17}H_{16}N_2O_6$	344.32	6.82	76 76	37 87
Diphenhydramine 1 Diphenhydramine 2	256.203 256.203	167.266 152.011	$C_{17}N_{21}NO$	255.361	6.54	36 36	21 53
Enrofloxacin 1 Enrofloxacin 2	360.231 360.231	316.180 245.416	$C_{19}H_{23}ClFN_3O_3$	395.9	5.79	71 71	29 39
Fentanyl 1 Fentanyl 2	336.834 336.834	188.20 132.10	$C_{22}H_{28}N_2O$	336.471	6.41	81 81	31 45
Hydrocodone 1 Hydrocodone 2	300.196 300.196	199.00 128.30	$C_{18}H_{21}NO_3$	299.368	4.69	91 91	41 81
Hydromorphine 1 Hydromorphine 2	286.990 286.990	158.200 157.200	$C_{17}H_{19}NO_3$	285.243	4.12	56 56	43 61
Lisinopril 1 Lisinopril 2	406.296 406.296	84.000 246.10	$C_{21}H_{31}N_3O_5$	405.495	4.85	66 66	53 33
Lincomycin 1 Lincomycin 2	407.229 407.229	126.040 359.230	$C_{18}H_{34}N_2O_6S$	406.538	4.96	66 66	39 27
Lomefloxacin 1 Lomefloxacin 2	352.188 352.188	307.989 265.130	$C_{17}H_{19}F_2N_3O_3$	351.408	5.67	51 51	25 33
Methamphetamine 1 Methamphetamine 2	150.450 150.450	91.200 119.20	$C_{10}H_{15}N$	149.237	4.98	46 46	23 15
Meperidine 1 Meperidine 2	248.162 248.162	220.10 174.40	$C_{15}H21NO2$	247.33	5.97	66 66	29 29
Metformin 1 Metformin 2	130.168 130.168	59.00 71.00	$C_4H_{11}N_5$	129.164	1.14	36 36	19 29
Methadone 1 Methadone 2	310.271 310.271	265.10 105.20	$C_{21}H_{27}NO$	309.445	6.68	51 51	21 41
Miconazole 1 Miconazole 2	416.937 416.937	159.104 68.8470	$C_{18}H_{14}Cl_4N_2O$	416.123	7.11	66 66	43 43
Morphine 1 Morphine 2	286.114 286.114	185.30 165.30	$C_{17}H_{19}NO_3$	285.34	4.12	86 86	49 51
Naloxone 1 Naloxone 2	328.322 328.322	310.00 212.10	$C_{19}H_{21}NO_4$	327.27	4.68	61 61	27 53
Naltrexone 1 Naltrexone 2	343.131 343.131	325.20 271.10	$C_{20}H_{23}NO_4$	341.401	4.84	71 71	31 39

Norfentanyl 1 Norfentanyl 2	233.182 233.182	84.20 54.90	$C_{14}H_{20}N_2O$	232.32	5.40	31 31	27 59
Norhydrocodone 1 Norhydrocodone 2	286.10 286.10	199.00 241.10	$C_{17}H_{19}NO_3$	285.34	4.97	70 70	39 32
Noroxycodone 1 Noroxycodone 2	302.10 302.10	187.10 227.10	$C_{17}H_{19}NO_4$	301.34	4.83	40 40	33 40
Oxycodone 1 Oxycodone 2	316.189 316.189	240.80 298.00	$C_{18}H_{21}NO_4$	315.364	4.83	61 61	51 9
Oxymorphone 1 Oxymorphone 2	302.011 302.011	284.00 227.00	$C_{17}H_{19}NO_4$	301.337	4.83	11 11	27 39
Ofloxacin 1 Ofloxacin 2	362.214 362.214	318.246 261.124	$C_{18}H_{20}FN_3O_4$	361.373	5.43	46 46	31 39
Oxacillin 1 Oxacillin 2	402.047 402.047	144.40 186.20	$C_{19}H_{19}N_3O_5S$	401.436	6.68	116 116	33 23
Paraxanthine 1 Paraxanthine 2	181.125 181.125	123.797 68.7780	$C_7H_8N_4O_2$	180.16	4.55	61 61	29 45
Penicillin G 1 Penicillin G 2	367.172 367.172	160.181 217.278	$C_{16}H_{18}N_2O_4S$	334.40	6.54	46 46	21 31
Penicillin V 1 Penicillin V 2	383.135 383.135	160.161 114.131	$C_{16}H_{18}N_2O_5S$	350.38	6.68	46 46	23 51
Ranitidine 1 Ranitidine 2	315.191 315.191	176.00 130.20	$C_{13}H_{22}N_4O_3S$	314.404	4.55	46 46	25 35
Roxithromycin 1 Roxithromycin 2	837.529 837.529	679.489 158.278	$C_{41}H_{76}N_2O_{15}$	837.047	6.68	86 86	31 45
Simvastatin 1 Simvastatin 2	419.077 419.077	161.20 163.10	$C_{25}H_{38}O_5$	418.574	7.10	61 61	41 41
Sufentanil 1 Sufentanil 2	387.186 387.186	111.20 77.100	$C_{22}H_{30}N_2O_2S$	386.552	6.54	61 61	51 97
Sulfachloropyridazine 1 Sulfachloropyridazine 2	284.991 284.991	155.979 91.9530	$C_{10}H_9ClN_4O_2S$	284.72	5.97	46 46	23 41
Sulfadiazine 1 Sulfadiazine 2	251.101 251.101	155.998 91.9680	$C_{10}H_{10}N_4O_2S$	250.278	4.69	51 51	23 41
Sulfadimethoxine 1 Sulfadimethoxine 2	311.072 311.072	156.092 91.9580	$C_{12}H_{14}N_4O_4S$	310.33	6.54	61 61	29 45
Sulfamerazine 1 Sulfamerazine 2	265.065 265.065	156.013 91.9710	$C_{11}H_{12}N_4O_2S$	264.305	5.14	51 51	23 41

Sulfamethazine 1 Sulfamethazine 2	279.113 279.113	186.181 156.046	$C_{12}H_{14}N_4O_2S$	278.33	5.54	71 71	27 27
Sulfamethizole 1 Sulfamethizole 2	270.675 270.675	156.001 91.9670	$C_9H_{10}N_4O_2S_2$	270.333	5.54	36 36	23 43
Sulfamethoxazole 1 Sulfamethoxazole 2	254.056 254.056	156.011 91.9800	$C_{10}H_{11}N_3O_3S$	253.279	6.11	46 46	23 39
Sulfathiazole 1 Sulfathiazole 2	256.042 256.042	156.089 91.9790	$C_9H_9N_3O_2S_2$	255.319	4.96	46 46	23 39
Thiabendazole1 Thiabendazole 2	201.983 201.983	174.837 131.001	$C_{10}H_7N_3S$	201.249	5.26	61 61	35 49
Tramadol 1 Tramadol 2	265.136 265.136	105.10 77.100	$C_{16}H_{25}NO_2$	263.38	6.55	96 96	29 75
Trimethoprim 1 Trimethoprim 2	291.155 291.155	229.913 260.975	$C_{14}H_{18}N_4O_3$	290.323	5.21	71 71	33 37

"Analyte" refers to each individual compound; the 1 and 2 after each compound represent notation two unique ion transitions used per analyte. Abbreviations: DP = declustering potential, CE = collision energy

Table A5: Elution Gradiant

Time	Module	Event	Percent B
1	Pumps	Pump B Conc	2
4	Pumps	Pump B Conc	30
5	Pumps	Pump B Conc	95
6	Pumps	Pump B Conc	95
7	Pumps	Pump B Conc	95
8	Pumps	Pump B Conc	95
8.01	Pumps	Pump B Conc	2
10	Controller	Stop	

Table A6: Average Percent Recovery from all analyte samples from WWTP #1 using Equation 2.

Analyte	WWTP#1 Percent Recovery
4-ANPP	0
6-MAM	0
Acetominophen	0
Albuterol	0
Amphetamine	17.9
Benzoylecgonine	57.7
Caffeine	81.9
Carbadox	1.07
Carbamazepine	18.8
Ciprofloxacin	198.5
Cis-3-methylfentanyl	29.5
Cocaine	2706
Dehydronipedipine	161
Diltiazem	436
Diphenhydramine	25.8
Enrofloxacin	170.7
Fentanyl	5.3
Lincomycin	0
Lisinopril	10
Lomefloxacin	66.5
Meperidine	69.6
Methadone	57.8
Methamphetamine	13.5
Miconazole	7.8
Morphine	0
Naloxone	0
Naltrexone	0
Norfentanyl	34.1
Norhydrocodone	6.1
Noroxycodone	4.7
Ofloxacin	191.7
Oxycodone	5.9
Oxymorphone	8.2
Paraxanthine	22.6
Simvastatin	7.52

Sufentanil	46.1
Sulfachloropyridine	1.7
Sulfadiazine	0.6
Sulfadimethazine	1.3
Sulfamerazine	0.4
Sulfamethazine	0
Sufamethizole	1.24
Sulfamethoxazole	2.3
Sulfathiazine	0
Thiabendazole	55.5
Tramadol	58.8
Trimethoprim	16.5

Table A7: Average Percent Recovery from all analyte samples from WWTP #2 using Equation 2.

Analyte	WWTP#2 Percent Recovery
4-ANPP	7.05
6-MAM	271.3
Acetominophen	129.3
Albuterol	51.6
Amphetamine	93.2
Benzoylecgonine	79.5
Caffeine	138.8
Carbadox	0
Carbamazepine	54.4
Ciprofloxacin	753.0
Cis-3-methylfentanyl	38.3
Cocaine	2953.6
Dehydronipedipine	215.4
Diltiazem	684.6
Diphenhydramine	43.8
Enrofloxacin	191.0
Fentanyl	7.88
Lincomycin	80.2
Lisinopril	145.0
Lomefloxacin	298.0
Meperidine	82.8
Methadone	81.4
Methamphetamine	59.7
Miconazole	13.6
Morphine	93.7
Naloxone	89.5
Naltrexone	43.8
Norfentanyl	91.1
Norhydrocodone	73.6
Noroxycodone	88.4
Ofloxacin	146.0
Oxycodone	78.2
Oxymorphone	97.5
Paraxanthine	158.6
Simvastatin	12.9

Sufentanil	78.8
Sulfachloropyridine	29.7
Sulfadiazine	24.5
Sulfadimethazine	38.1
Sulfamerazine	24.5
Sulfamethazine	7.96
Sufamethizole	18.0
Sulfamethoxazole	50.1
Sulfathiazine	15.8
Thiabendazole	66.5
Tramadol	75.6
Trimethoprim	61.6

Table A8: Average Percent Recovery from all analyte samples from surface water using Equation 2.

Analyte	Surface Water Percent Recovery
4-ANPP	0
6-MAM	144.6
Acetominophen	80.2
Albuterol	51.3
Amphetamine	67.0
Benzoylecgonine	65.4
Caffeine	67.7
Carbadox	9.64
Carbamazepine	69.7
Ciprofloxacin	489.3
Cis-3-methylfentanyl	34.4
Cocaine	2155.4
Dehydronipedipine	782.6
Diltiazem	722.0
Diphenhydramine	29.0
Enrofloxacin	91.2
Fentanyl	6.66
Lincomycin	49.5
Lisinopril	89.6
Lomefloxacin	211.6
Meperidine	60.6
Methadone	64.7
Methamphetamine	46.2
Miconazole	12.6
Morphine	29.5
Naloxone	17.5
Naltrexone	16.6
Norfentanyl	65.6
Norhydrocodone	49.0
Noroxycodone	72.0
Ofloxacin	134.6
Oxycodone	63.7
Oxymorphone	77.2
Paraxanthine	114.7
Simvastatin	12.1

Sufentanil	59.3
Sulfachloropyridine	13.6
Sulfadiazine	19.0
Sulfadimethazine	30.5
Sulfamerazine	14.3
Sulfamethazine	0.02
Sufamethizole	9.31
Sulfamethoxazole	28.8
Sulfathiazine	7.84
Thiabendazole	50.0
Tramadol	70.8
Trimethoprim	49.2

Appendix B: Chapter 4 Supplemental Data

Table B1: Sampling site for each water body with latitude and longitude

Water Body	**Sampling Site**	**Lat/Long**
Kinnickinnic River	Jackson Park	42.9971/ -87.9678
Kinnickinnic River	St. Lukes Medical Hospital	42.9915/ -87.9481
Kinnickinnic River	KK River Trail	42.9963/ -87.9189
Kinnickinnic River	Marcus Amphitheater	43.023611/ -87.9036111
Milwaukee River	Riveredge Nature Center	43.43488/ -88.0338
Milwaukee River	Hubbard Park	43.08446/ -87.89267
Milwaukee River	Riverside Park	43.0677/ -87.890778
Milwaukee River	Third Ward	43.031667/ -87.91
Milwaukee River	BayShore	43.12314/ -87.918
Menomonee River	Hoyt Park	43.0544/ -88.0225
Menomonee River	Jacobus Park	43.0456/ -87.9999
Menomonee River	Canal Street	43.04261/ -87.9592
Menomonee River	Emmber Lane	43.0325/ -87.9292
Wilson Park Creek	Howard Avenue	42.97054/ -87.93816
Root River	Oakwood Road	42.858153/ -87.9976746
Root River	West 7 Mile Road	42.82993/ -87.9988977
Root River	Downstream of 60th Street	42.854646/ -87.987839
Root River	River Mouth	42.734004/ -87.7846966
Fox River	Sunset Drive Bridge (Waukesha)	42.98865/ -88.264929
Fox River	Fox River Sanctuary	43.004154/ -88.246193

Appendix C: Detailed Protocols

Steps for Optimizing Compounds Using Miller LC-MS/MS Compound Optimization by Infusion

1. Put compound into solvent potentially to be used for LC-MS/MS at a concentration of 10-20 ug/L. Draw this up in the 1.0 ml glass syringe and set this up to pump at 20-40 µL/minute on the infusion pump.
2. Open Analyst on the computer. Go to the Hardware Configuration section in the left-hand panel and activate the "4000 Qtrap Only" option.
3. Go to Manual Tuning and choose Q1 MS (Q1) for the Scan Type. Enter in appropriate start and stop values for the scan (bottom hundred and top hundred for compound, i.e., if looking for compound with m/z=459, start = 400 and stop = 500). Enter in 1 sec. for Time. Hit start and look for the parent ion m/z value. Change to positive or negative mode depending on compound.
4. Do another type of scan called a Product Ion (MS2) scan. Enter in the parent mass in the Product of: box. Choose appropriate Start and Stop m/z values for expected daughter ions and 1 sec, for Time. Turning on MCA might be helpful here to determine the most prevalent compound.
5. Staying in Manual Tuning mode (note purple "T" button indented in top icon menu) and click on Compound Optimization under "Tune and Calibrate" on the left-hand panel. Select infusion and click "Next" twice. For the final window, input the compound name, the molecular weight in Daltons (note: most literature identified the value searched for with the H+ added, so subtract 1 from the m/z value), and the number of charges. Click finish. This will give the most prevalent ion transitions and parameters such as Collision Energy (CE), Declustering Potential (DP), etc. (factors that affect fragmenting the ions).

FIA Optimization – Gases

6. Go back to Hardware Configuration and activate the option called "MS + LC". Create an MRM Acquistion Method by going to "Build Acquisition Method" on the left under Acquire. Enter in the top three highest transition ions as (Compound) q, (Compound) c1, and (Compound) c2, example: MCLRq, MCLRc1 and MCLRc2. Also enter in DP, CE and CXP per ion transition. Make the method run for two minutes at 50% B (organic). Save the Acquisition Method in the format: date_name of compound.
7. Check the column oven to see if there is a column inserted. If there is, remove the solvent lines from the buffers and put both A and B into the appropriate storage solvent. Run the pumps for five minutes. Stop the pumps and remove the column from the oven, making sure to put the caps on top and bottom and placing it in the correct storage box.
8. Replace the column with a small metal tubular insert.
9. Click again on Compound Optimization in the left-hand panel. This time select FIA. You must select the appropriate method (the one that you just created). Continue clicking next and finish. All the gases that will be optimized should already be selected. FIA infusion will run for at least an hour and produce a final method optimized for all transition ions.

Sequence of steps for optimizing compounds on AB Sciex 4000 QTrap

1. Q1 scan in manual tuning
2. Product ion scan in manual tuning
3. Compound optimization (syringe infusion)
4. Further compound optimization (optional: multiple injections without column using shortened optimized method)
5. Full acquisition method with column and adjusting retention time separation.

Column Care: Changing Columns:

1. The LC should be turned on and neither of the pumps are flowing

   Take both pump lines (A & B) and put them in the appropriate storage solvent for the column currently in the column oven:

   a. HILIC: 85% Acetonitrile (ACN)
   b. C18: 100% ACN or 100% methanol (MeOH)
   c. C8: 100% ACN or 100% MeOH
2. Remove the red line from column and put beaker underneath column to collect fluid; if guard column is separate (ex.: HILIC) from normal column, pump solvent through each individually.

   Pump storage solvent through the lines for at least 5 minutes; press pump buttons on two top doors of LC; press pump buttons again to stop.

3. Remove column from oven; replace the appropriate caps (red for C18 and
4. Change the mobile phase buffers to the appropriate ones for the method; purge both the pump valves and storage solution in the autosampler. To turn both knobs on the pumps at least one full rotation to the left.

   Purge for 5 minutes. Press the purge button only on the bottom of the LC when finished. Make sure knobs are turned back and locked into position.

5. After purging: press the pump buttons and insert the new column into the oven when the mobile phase solutions (buffers) are flowing through the lines and dripping into the top of the column. This will decrease the probability of leaks.
6. Make sure the needle rinse solvent is 80% MeOH.

Cleaning Columns:

1. Turn the column upside down and run the appropriate solvent through into a waste beaker for approximately 30 minutes.
2. If there is a guard column, remove the regular column and put it right-side up. Run solvents through the attached guard column/column. Let the waste drip into a beaker.

LC General Care:

1. Purge waste should be emptied into the waste carboy into the hood. Solvents to include on the chemical list are methanol, acetonitrile, formic acid, water, ammonium formate, ammonium acetate (no abbreviations).

Procedure for Quantitative Analysis of LC-MS/MS Sample Runs in Analyst

1. Concentration units are in ng/ml (=µg/L)
2. Open Analyst (make sure correct Project Folder is selected in upper middle drop-down menu).

Building Quantitation Method

1. Under Quantitation on left hand side, select Build Acquisition Method (this step selects the method).
2. Find Data file in "Select Sample" box. Should be labeled "year, date, name", example: 20221229_PPCPOpioids_Pos_Scheduled. Click on the Batch Name and this will bring up the list of samples pertaining to that date in the next box
3. Select the highest standard (usually 100 or 50 µg/L) and click "okay".
4. A new window will appear and click on the Integration tab at the top.
    a. This process will create a quantitation method giving the program a template based on the analytes detected in the standard.
    b. In the Analyte box – select an analyte from the drop-down menu. A peak will appear in a lower window for that analyte. For example: Acetominophen 1 (the quantitative ion), next analyte will be acetaminophen 2 (the confirmatory ion). These analytes should be present at the same retention time (ex.: 5.19 minutes).
    c. If an analyte is at a different Retention Time and the peak is not highlighted:
        i. Check that the other ion transitions are present at the same time
        ii. Highlight the peak at the given retention time
        iii. Select peak icon (on right side at top)
        iv. If there is a peak at a different retention time from normal, highlight peak at the consistent retention time (maybe it will not be the highest peak for all analytes). Analyst will automatically select the highest peak, but some analytes may have same/similar enough ion transitions that one peak will

be the same, but the confirmatory ions will have slightly different retention times.

d. Some runs may say "no peak" – this could be an issue with the standard (i.e., compound not included in the standard mix), method (if a scheduled method, retention time window incorrect/not large enough to include retention tine shifts for the analyte), and/or mobile phases (if made incorrectly, can affect retention time of compounds from column.

5. Select File on top toolbar in Analyst
6. Select "Save As" and save the quantitation method using the same name as the batch/data file name.

Creating a Results File

7. Double-click on "Quantitation Wizard" in the left-hand panel.
8. Scroll through the left-hand window and single-click on the name of the data-file you are analyzing samples from.
    a. Select all samples for quantitation by single-clicking and highlighting them. Do not select the first high standard that was used to create the template. This standard should be the first one listed at the top above the full list of standards that make up the standard curve.
    b. Once all samples are highlighted (except the one from "a" above), select the > arrow; the names of the sample runs will go into the furthest right-hand box under "Available Samples" ("Selected Samples")
9. Hit "Next" button twice
10. Create Quantitative set – choose the existing quantitation method that you just created (it will be a .gif file).
11. Click "Finish". The Analyst software should open up a spreadsheet with each analyte for either the first standard or the blank standard run in your selected data.

Quantitating The Data

12. Save the Results File immediately by clicking on File – Save As in top left corner of Analyst, using the same datafile name.
13. Right-click in the tan area above the spreadsheet.
14. The column that is labeled "Sample Type", fill-in with the following: Standard (i.e. have known concentrations of chemical reference materials; typically listed as 0.1 to 50 or 100), Blank – with internal standards", "Double blank- with internal standards" or "Unknown" which are the samples. Do this for the top set of standards as well as the bottom set of standards. If there are blanks in the run, change those blanks to say "unknown".
15. Leave the column that is labeled "Analyte Concentration" blank
16. From the dropdown menu that appears, select "Analyte" and choose the first analyte you want to analyze.

17. Look at the Q1 analyte first
18. Scroll down to an unknown sample and look for a peak; scroll quickly through all the unknown samples
    a. If no peak, uncheck the standard boxes in the "Use Record" column and do not fill-in any concentrations under the column labeled (Analyte Concentrations) and uncheck the box under "Use Record". This is done so that the standard where there is no peak height is not included as a standard. This will improve the accuracy of your standard curve.
    b. If there is a peak: enter the concentrations of the standards in the run (top and bottom) in the column that is labeled "Analyte Concentrations". Then quantitate for that compound (ex.: acetaminophen 1) for each sample. No need to view analyte c2. When identifying your peaks, visually set the minimum peak height to be twice the baseline height for a signal to noise ratio of 2:1. You can accept peak height at 3:1 and higher. This prevents Analyst from calling background noise a peak in blanks or samples.
    c. Blanks should not have any peaks.
        i. If Blanks have peaks: highlight a blank area and hit the second button from the left on the row of icons in the spreadsheet, then hit "apply".
        ii. OR set the minimum height to 10,000, then hit apply and it should say "no peak".
    d. For the analytes with peaks, go to calibration icon at top and look at the standard curve. If there is no contamination, click on "linear through zero", and this will give a R^2 value, then accept the curve.
19. When the first chromatogram appears, four separate chromatograms may show up in the window underneath the spreadsheet. If this happens, right-click in one of the four sub-windows, and click on "options". A new window will come up. Under number of rows and number of columns change to "1". Select Zoom Y-axis to 100.00 percent of largest peak.
20. In the tan area above the chromatogram, click on the button with the counter-clockwise arrow to show options for smoothing, manual integration, etc. Note that these are options for adjusting your peak areas to get a better quantitative measurement from your sample – you may not need to smooth or manually integrate each sample.
    a. In the tan area above the chromatogram, click on the 3rd icon (from the left, excluding arrow buttons) which is the "Manual Integration" – this allows you to draw a line across the bottom of the peak manually.
    b. In the tan area above the chromatogram, you can also adjust the smoothing width from a dropdown menu – this will average the lines across a specific number of points across the peak you are selecting. The peak must be highlighted to smooth it. The lowest number you use is best (example: 3) to get a good peak. Click "apply" after changing the smoothing width.
    c. To view the next analyte, right-click in the tan area above the spreadsheet – click on "Analyte" and select the next one and repeat the above.

21. Save after viewing each analyte. To do this, make sure you click on the upper half-window with the spreadsheet so that a blue box appears around it. Save by either clicking on the floppy disk icon in upper left or by going to File – Save/Save As.

Transferring Data to Excel

1. To make an Excel spreadsheet
    a. Open file "Quantitation Results"
    b. Right-click on chromatogram spreadsheet and select "summary"
    c. A window appears – scroll down and select "calculated concentration"
    d. A new spreadsheet will list all the analytes and their calculated concentrations, no peaks, etc.
    e. Place cursor in white box next to "sample name" in the spreadsheet and right-click and spreadsheet will get highlighted
    f. Go to edit – then copy – then paste into a new Excel spreadsheet
    g. Save As
2. In new saved spreadsheet
    a. Do a find and replace for all <0 and no peaks and replace with zeros
    b. Delete first column that lists numbers (1,2,3,4,etc.)
    c. Delete columns with confirmatory ions, delete all the standards, delete internal standard columns, delete any blanks, and delete the number one's after all the Q1 analysts.
3. To find actual concentration in each sample
    a. Copy the analyte names and paste them vertically in the first column and horizontally below the first set of concentrations. You will end up with analyte names on the vertical axis and analyte names on the horizontal axis.
    b. Underneath each analyte name, you will calculate the actual concentration by putting in the column and row information and divide that by the volume used (i.e., 10 ml or 25 ml). For example: if the analyte name is in column B and row 2 and you used 10 ml for the volume, then you would type in =B2/10 in the copied spreadsheet below the first spreadsheet. This will give you the actual concentration for that sample.
    c. Do that for every analyte, then copy down to the end of the list of analytes.
    d. Units will be in µg/L.
4. To change units to ng/L
    a. Copy the analyte names and paste them vertically in the first column and horizontally below the first set of concentrations. You will end up with analyte names on the vertical axis and analyte names on the horizontal axis.
    b. Take the actual concentration calculated in the second copied spreadsheet.
    c. Take the value listed for each analyte and multiply by 1000. Example: If analyte is listed in column B, row 30 as 0.0900, then type in =0.0900*1000.
    d. Do that for every analyte, then copy down to the end of the list of analytes.
    e. Units will be in ng/L.

f. Save As

Protocol for putting samples on the LC-MS/MS

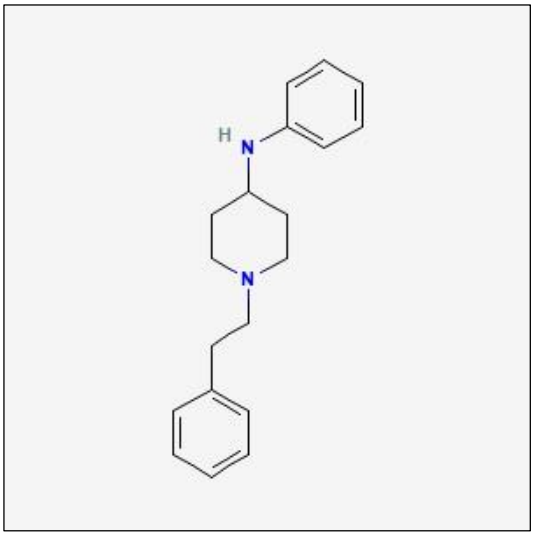
1. Under Acquire in left-hand column
2. Select "Build Acquisition Batch". This step selects the method.
3. Check to see if in the correct folder
4. Under Set – Name the batch (example: 20220901_PPCP/Opioids_Pos_Scheduled
5. Select Add Set
6. Select Add Samples, another new box will come up.
7. Delete prefix "data"
8. Number of new samples is equal to the number of vials plus standards; return
9. Another new box will come up
    a. Sample Name (to identify sample again, use name that is on LC vials)
    b. Vial position
        i. Start with two 50 µg/L or 100 µg/L standards
        ii. Blank
        iii. Standards
        iv. Samples (about every 12 samples add a blank)
        v. Standards
    c. Under Method Editor: choose your method from scroll down menu
    d. Submit at top
    e. Submit in the box
10. If LC-MS/MS has been turned off: go to Hardware Configuration on left hand side and select 4000 LC & MS, then enter samples
11. Go to View
    a. Sample Que must be open
    b. Select Acquire at top of menu
    c. Select Equilibrate from pull down menu (this turns on the pumps to get up to pressure and changes the temperature the method selected).
        i. If pumps are activated – green light will be on the LC
        ii. If pumps are not activated – press pump again
    d. Select and Equilibration time (usually one minute) for each sample
    e. When icons in right-hand corner at bottom of computer are green – equilibration is finished.
12. Select Start Sample at top under Acquire.

To View Sample Runs

1. Open Data File on left hand side
2. Find name of sample (batch name). This will bring up the chromatogram.
3. To see individual ions on chromatogram, select “extract ions”
4. Hit okay
5. To go back to the screen showing the samples in the que – select Acquire on left hand side.

Appendix D: Chromatography spectra and molecular structures for 60 compounds

4-ANPP



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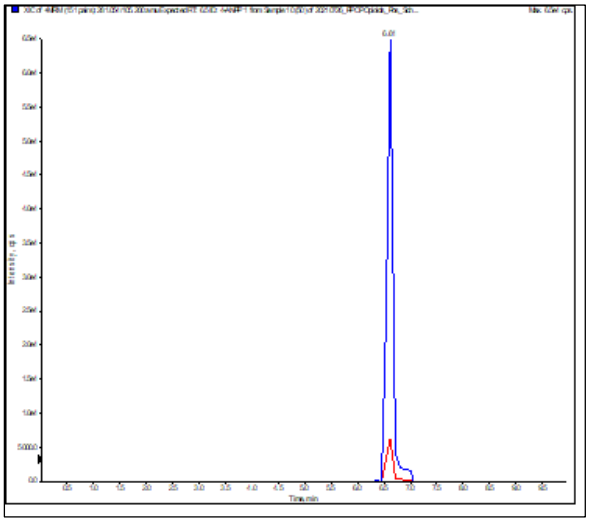
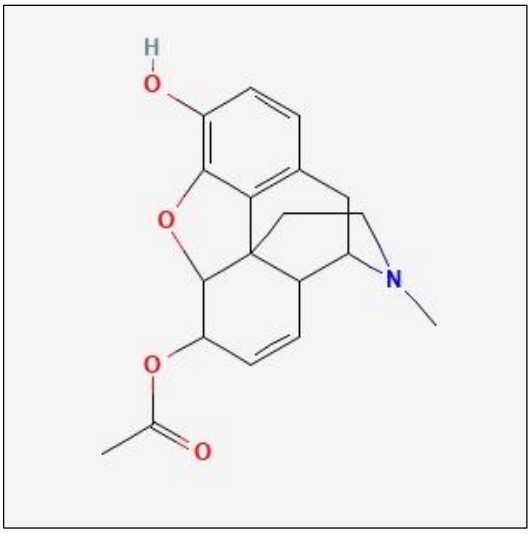


Chromatogram of 4-ANPP showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

6-Monoacetylmorphine (6-MAM)



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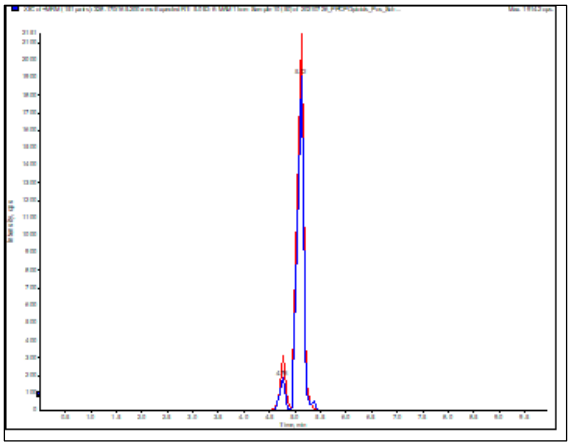
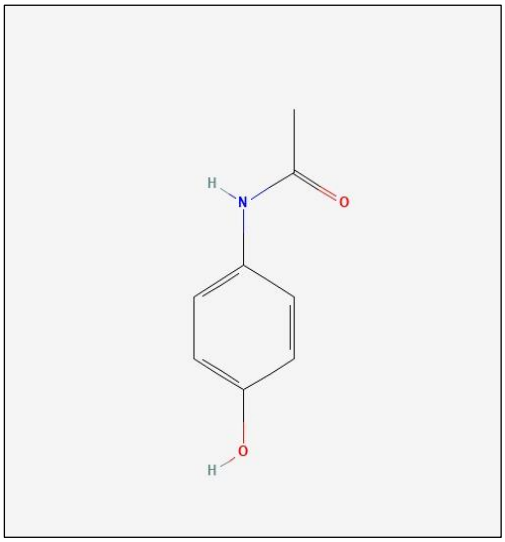


Chromatogram of 6-MAM showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Acetominophen



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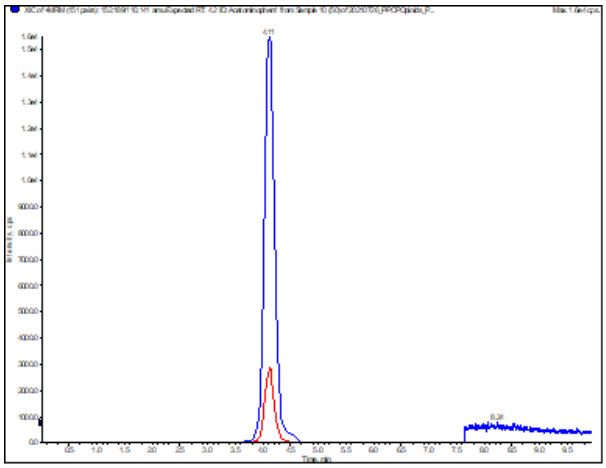
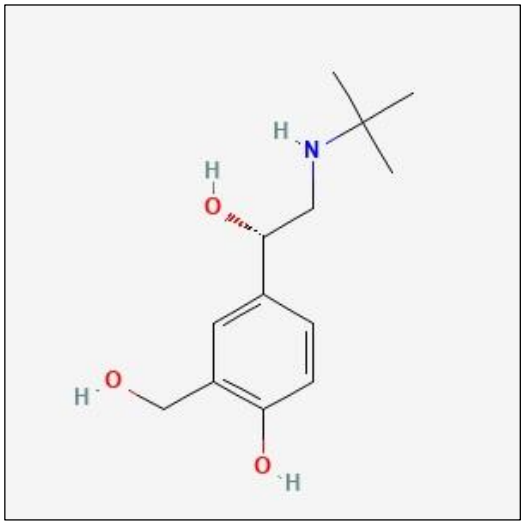


Chromatogram of acetaminophen showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Albuterol



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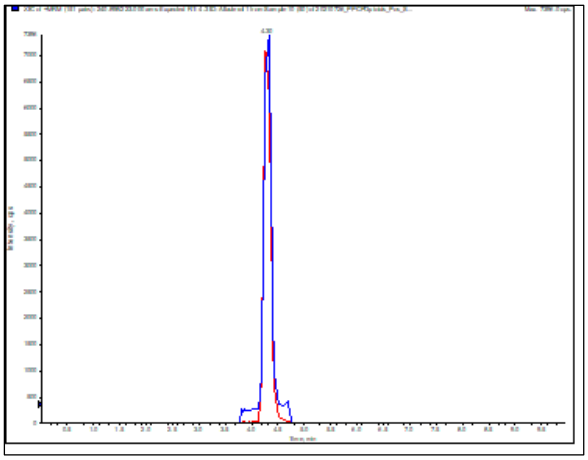
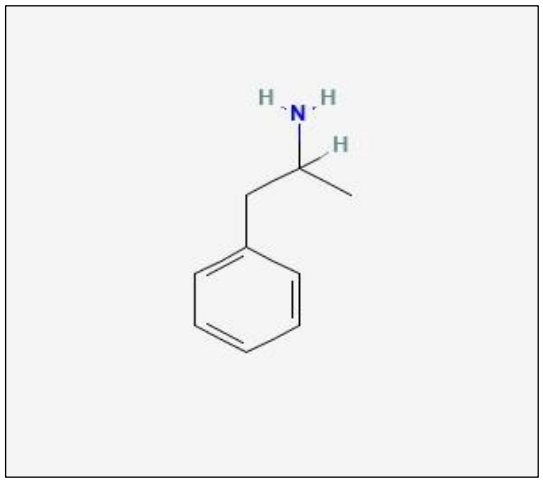


Chromatogram of albuterol showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Amphetamine



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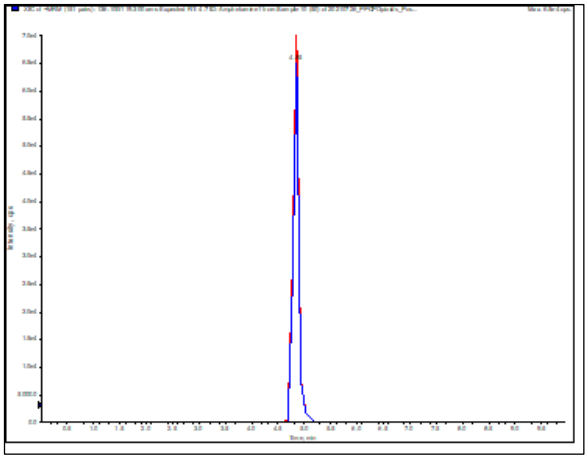
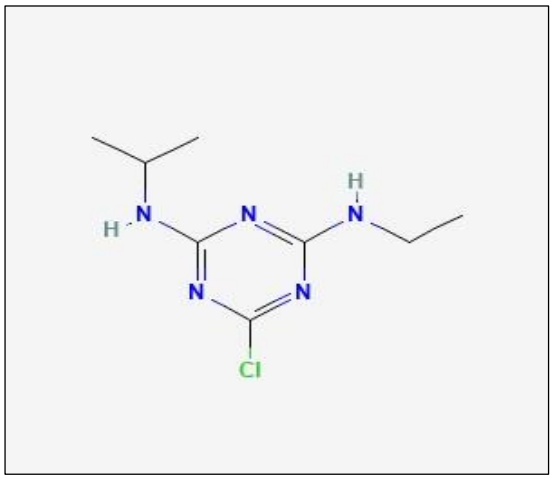


Chromatogram of amphetamine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Atrazine



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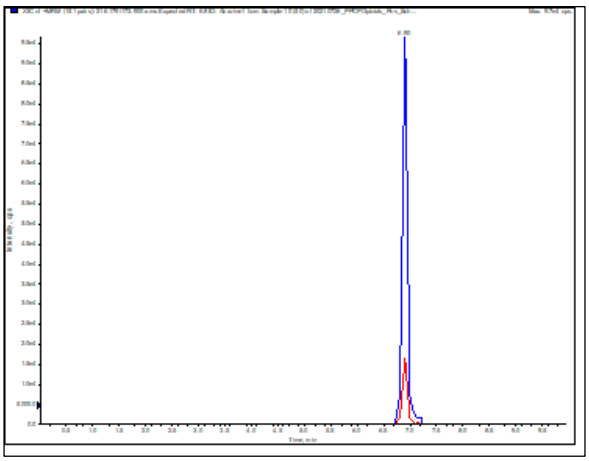
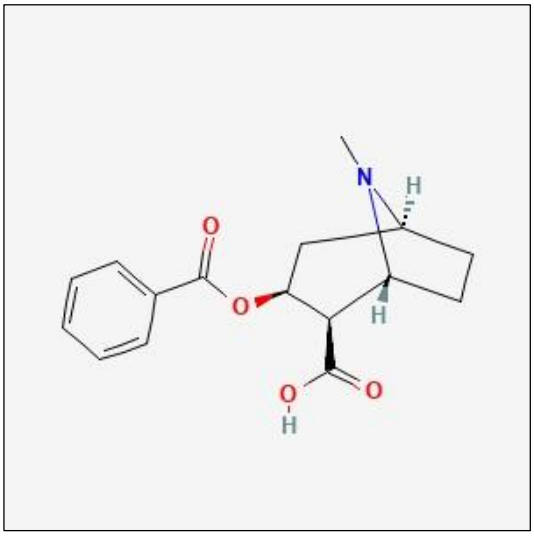


Chromatogram of atrazine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Benzoylecgonine



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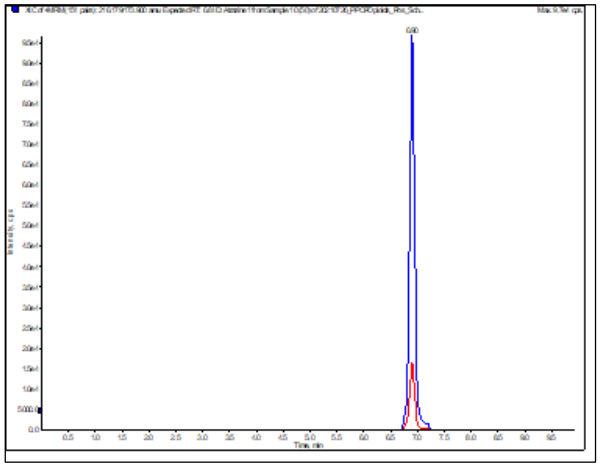
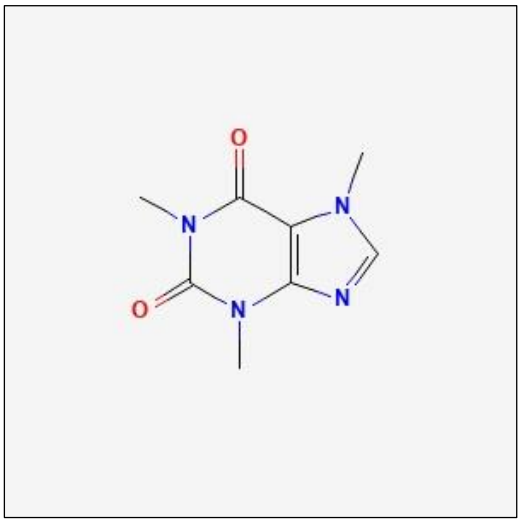


Chromatogram of benzoylecgonine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Caffeine



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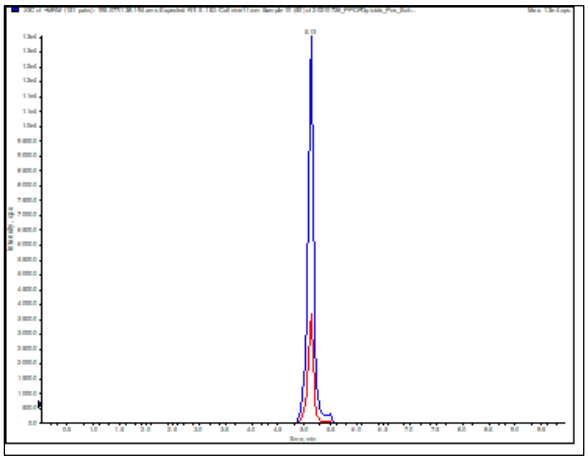
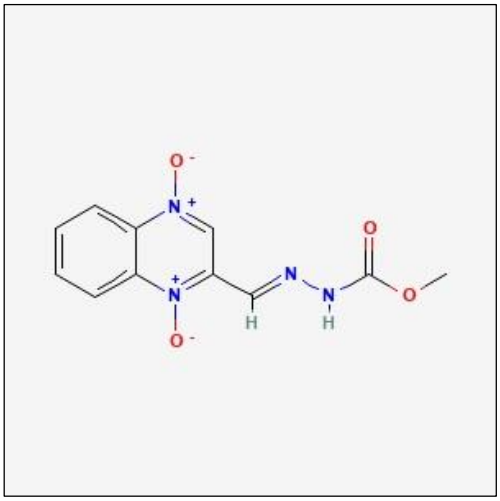


Chromatogram of caffeine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Carbadox



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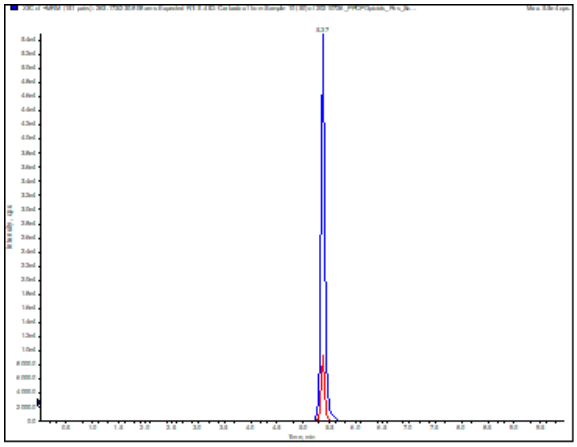
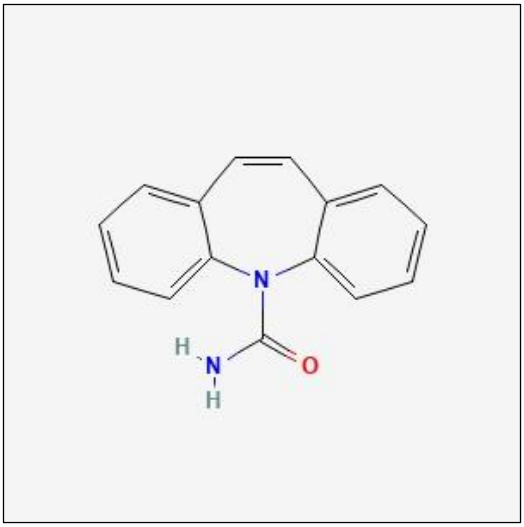


Chromatogram of carbadox showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Carbamazepine



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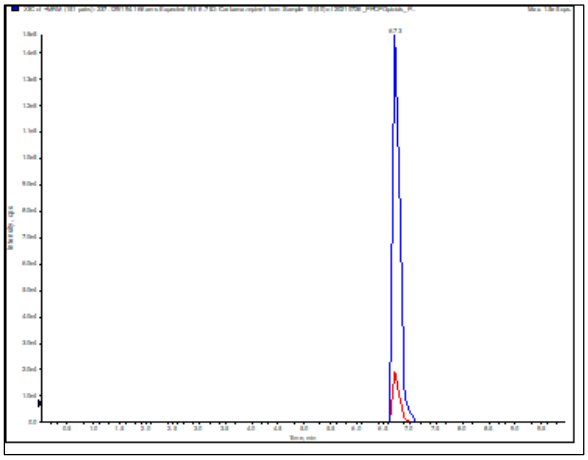
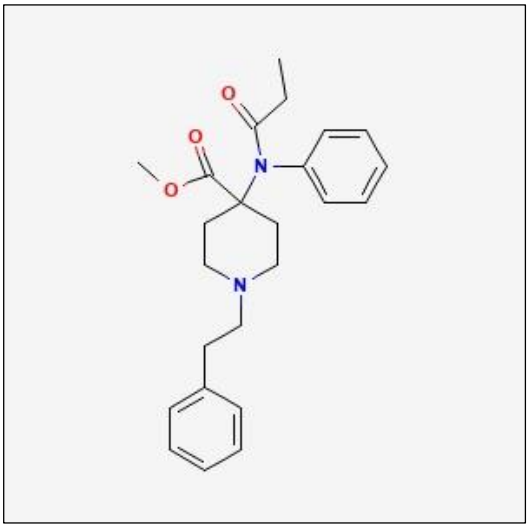


Chromatogram of carbamazepine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Carfentanil



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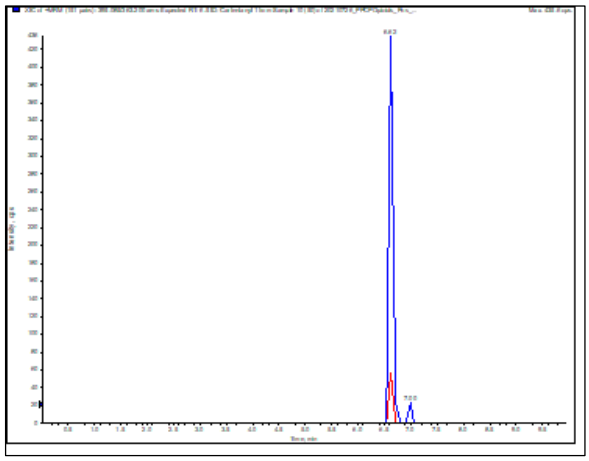
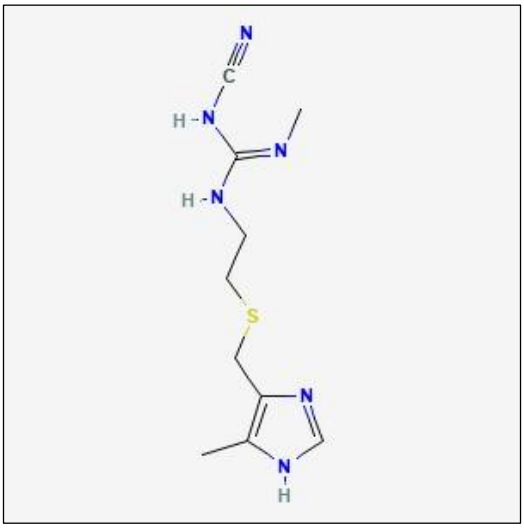


Chromatogram of carfentanil showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Cimetidine



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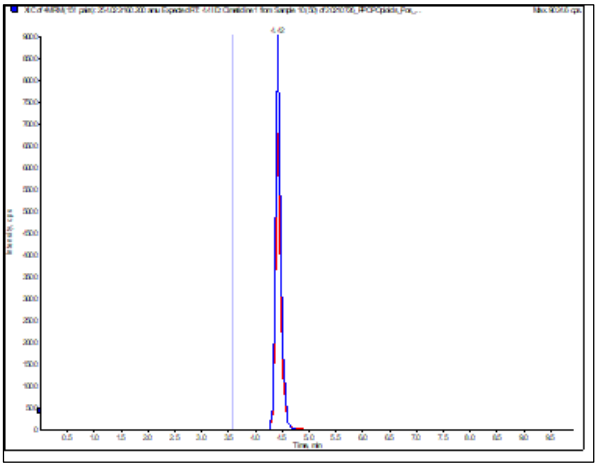
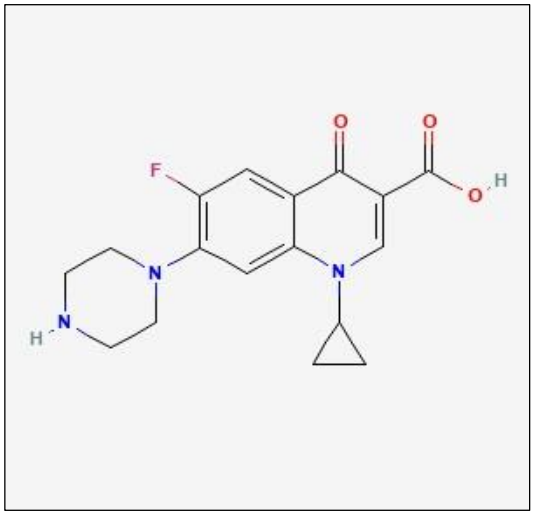


Chromatogram of cimetidine showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Ciprofloxacin



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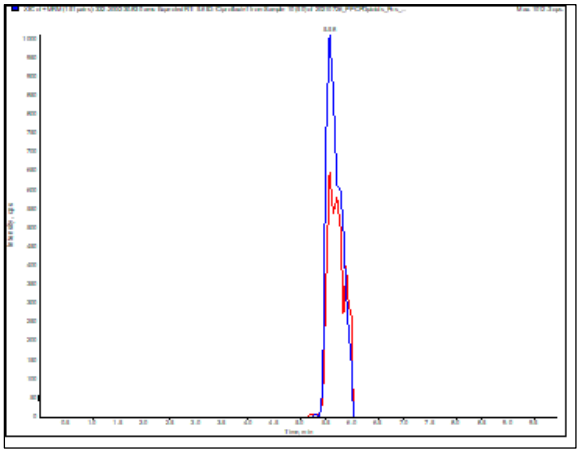
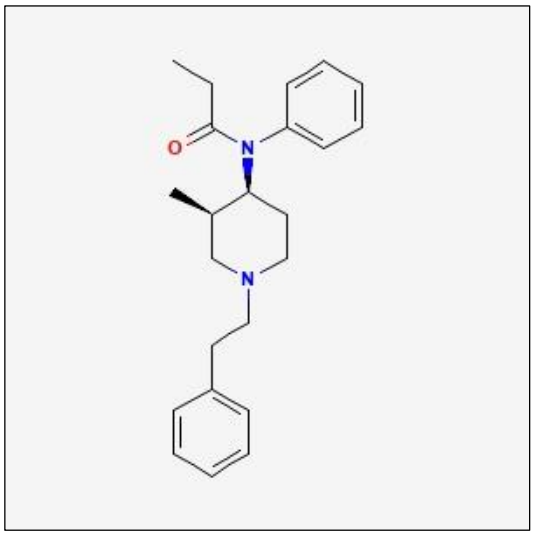


Chromatogram of ciprofloxacin showing Q1 and Q2 peaks generated from LC - MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Cis-3-methylfentanyl



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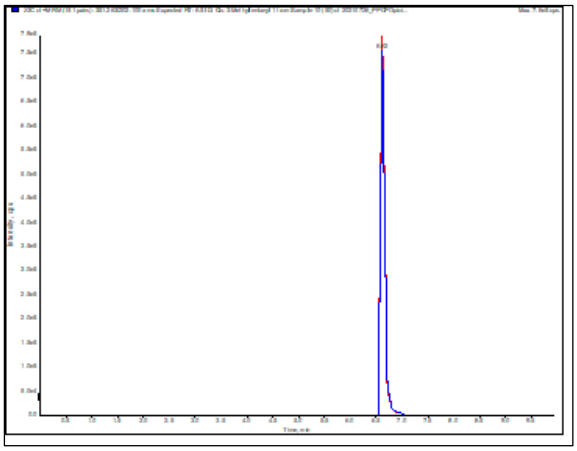
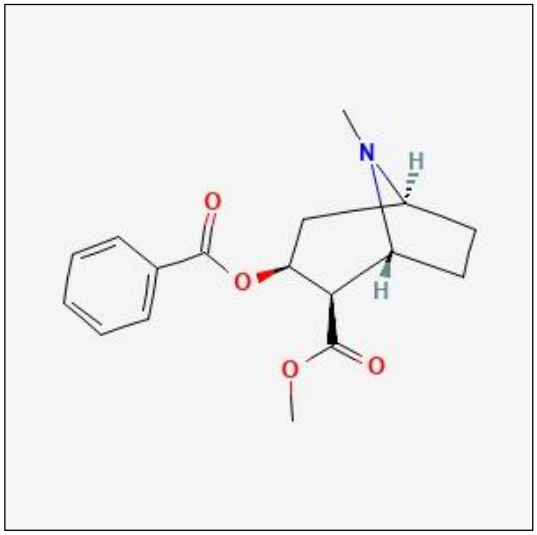


Chromatogram of cis-3-methylfentanyl showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Cocaine



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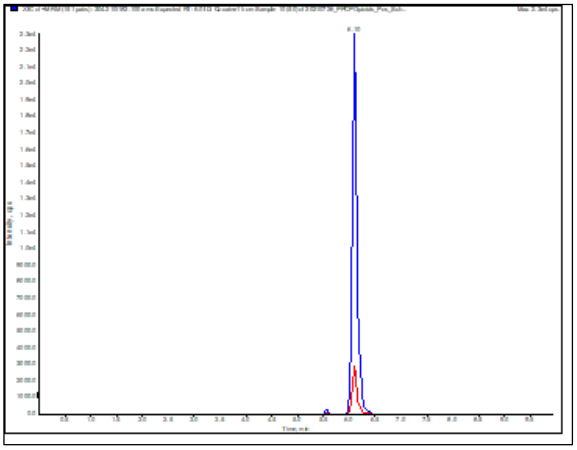
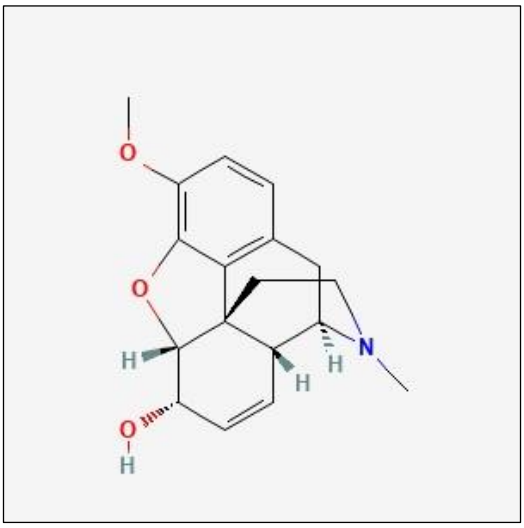


Chromatogram of cocaine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Codeine



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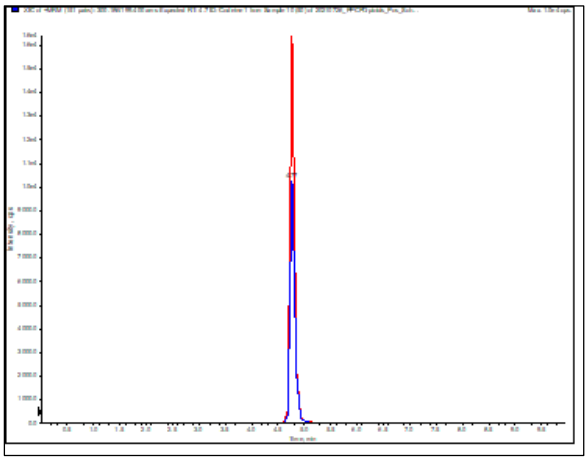
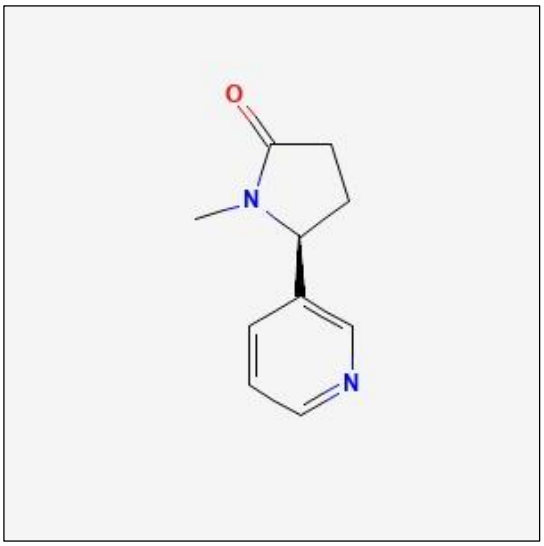
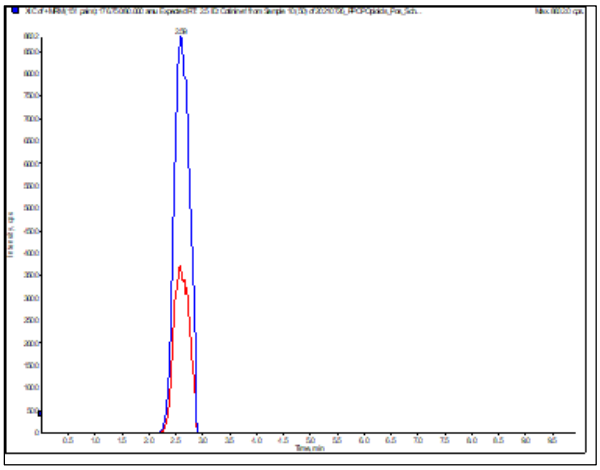


Chromatogram of codeine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Cotinine

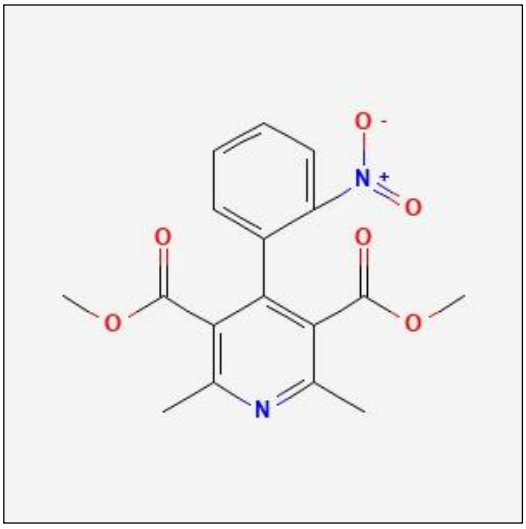


pubchem.ncbi.nim.nih.gov

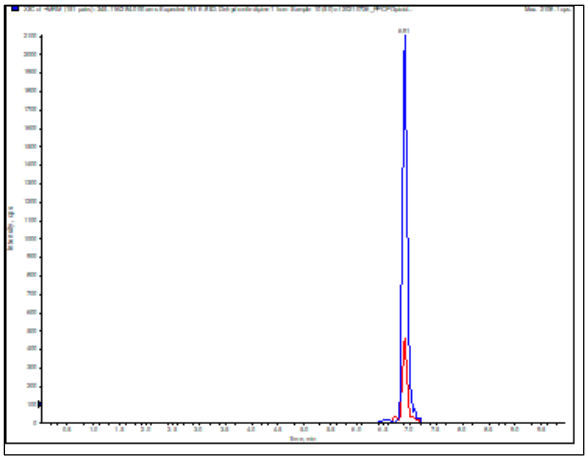


Chromatogram of cotinine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Dehydronifedipine

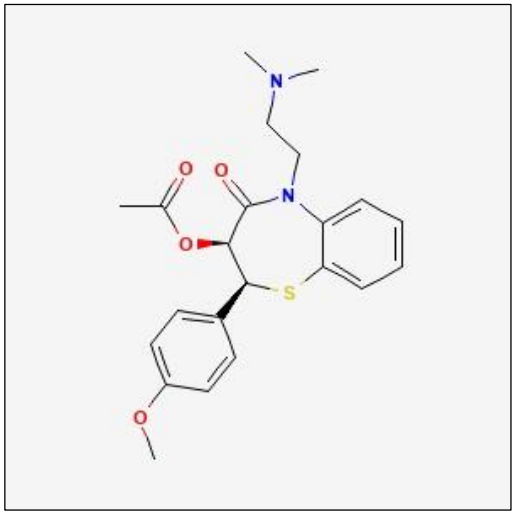


pubchem.ncbi.nim.nih.gov

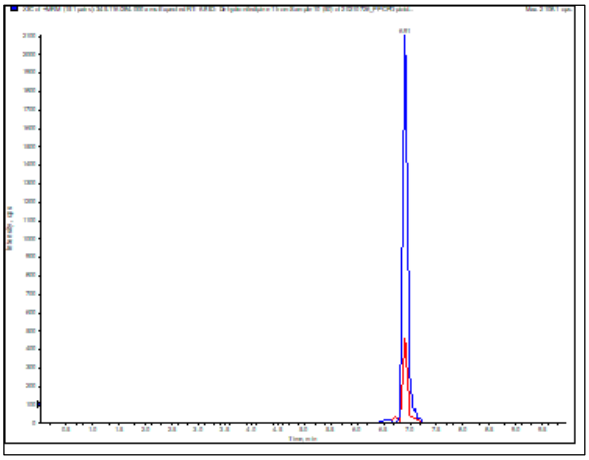


Chromatogram of dehydronifedipine showing Q1 and Q2 peaks generated from LC - MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Diltiazem

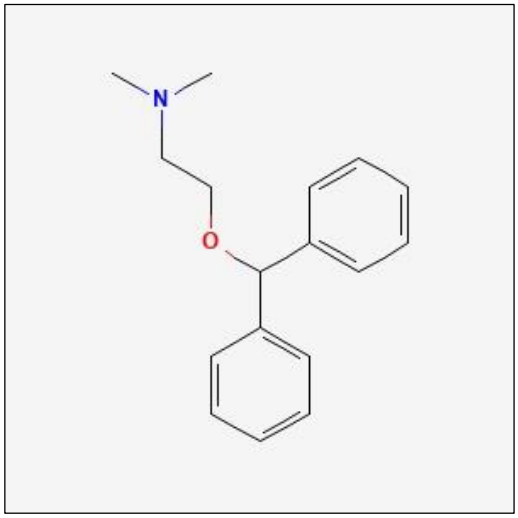


pubchem.ncbi.nim.nih.gov

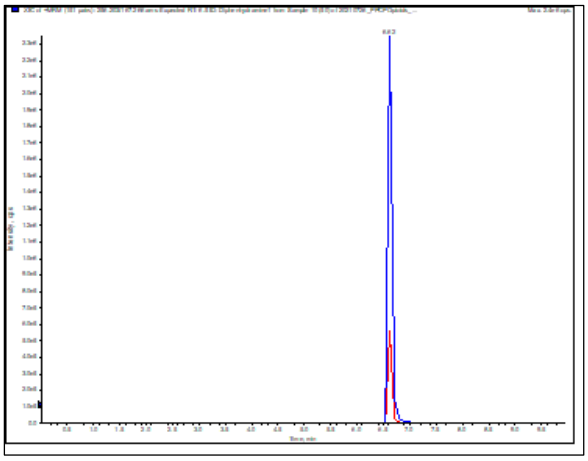


Chromatogram of diltiazem showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Diphenhydramine

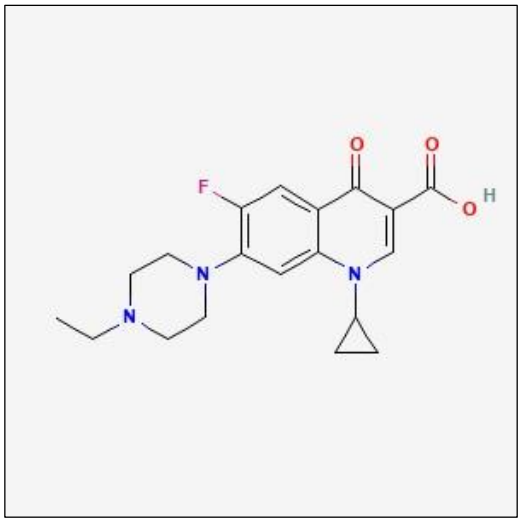


pubchem.ncbi.nim.nih.gov



Chromatogram of diphenhydramine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Enrofloxacin



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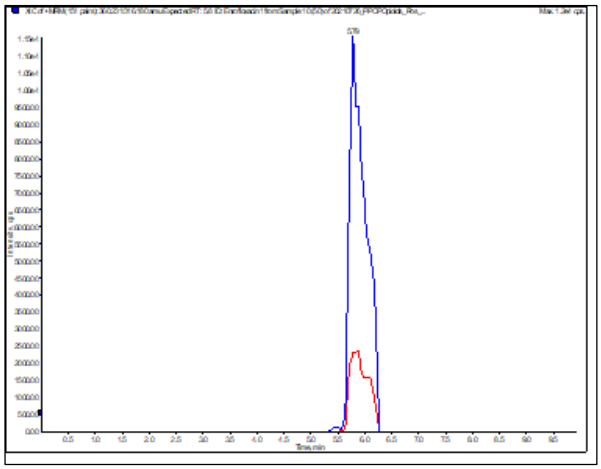
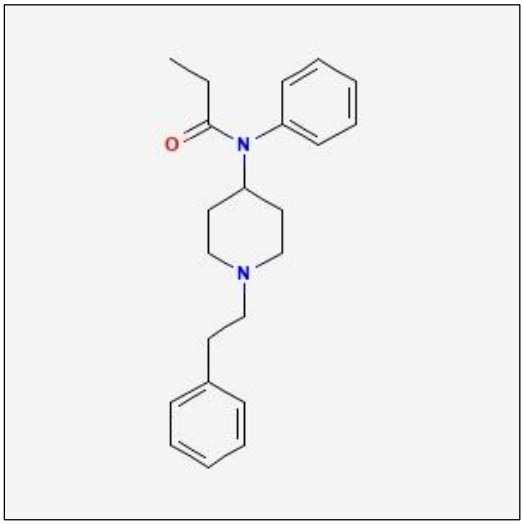


Chromatogram of enrofloxacin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1 and red line is Q2.

Fentanyl



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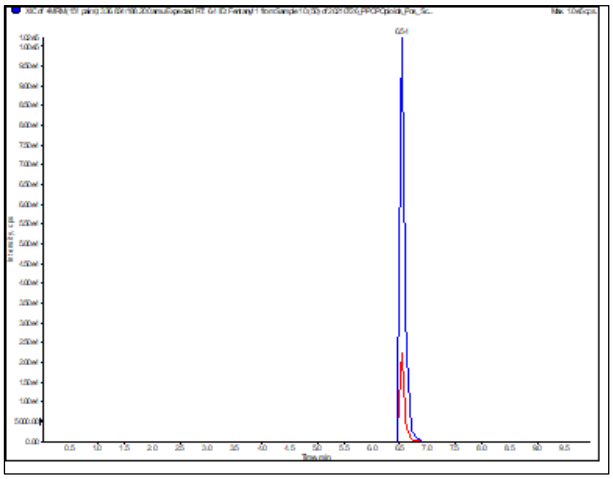
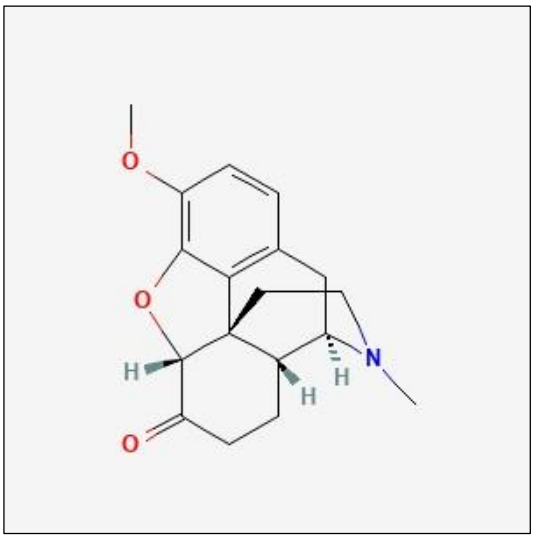


Chromatogram of fentanyl showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Hydrocodone



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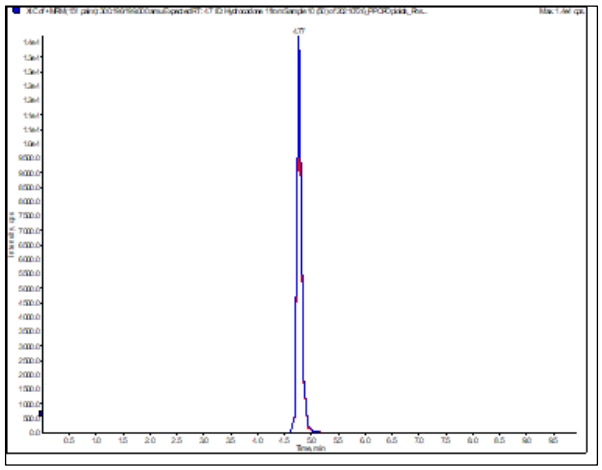
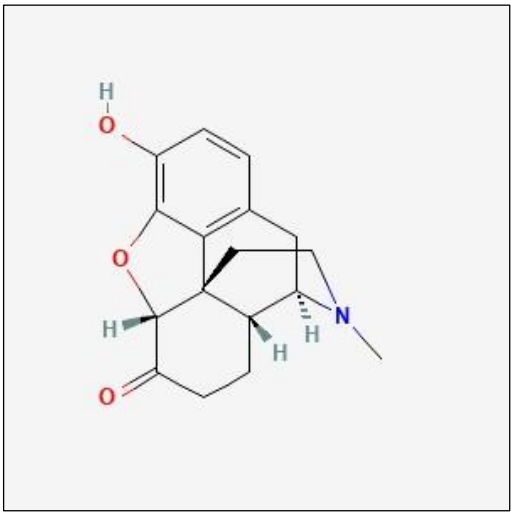


Chromatogram of hydrocodone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Hydromorphone



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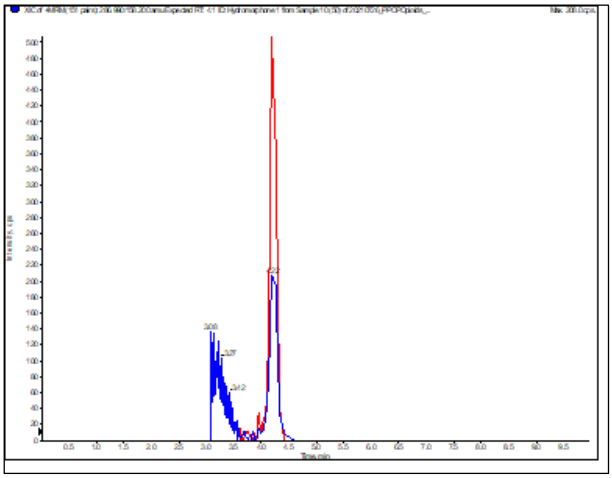
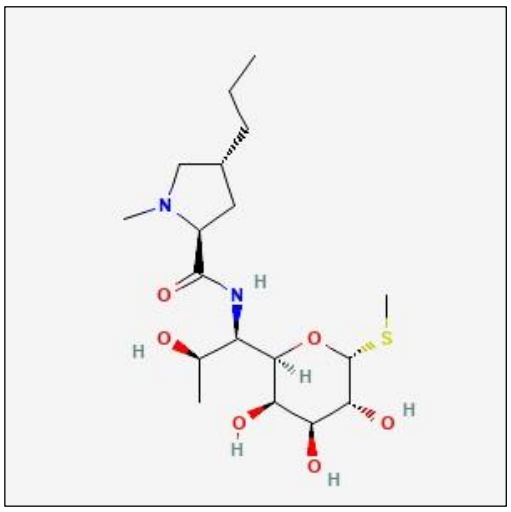


Chromatogram of hydromorphone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Lincomycin



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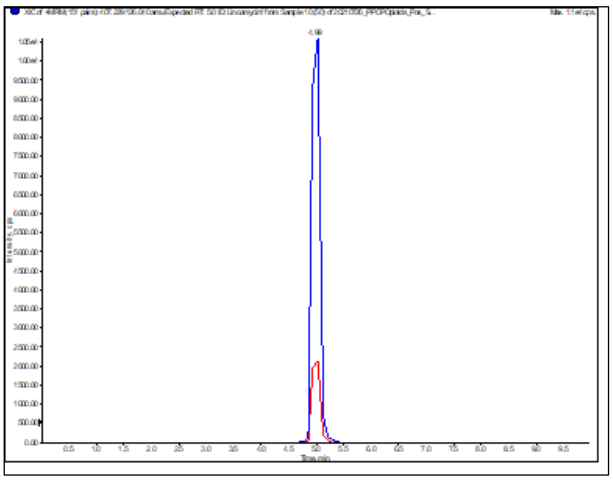
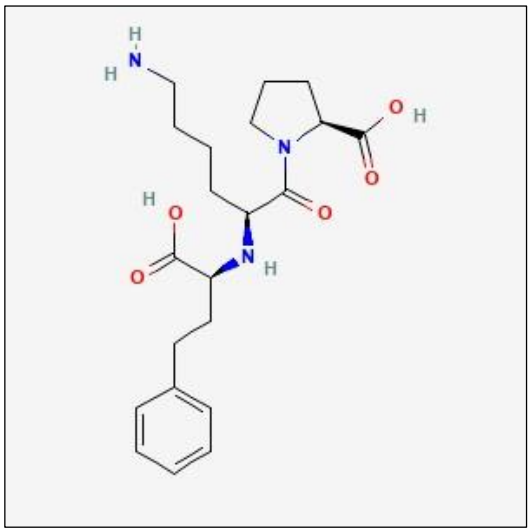


Chromatogram of lincomycin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Lisinopril



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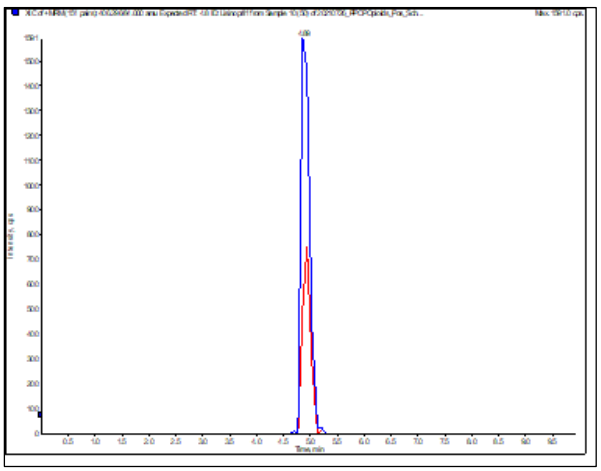
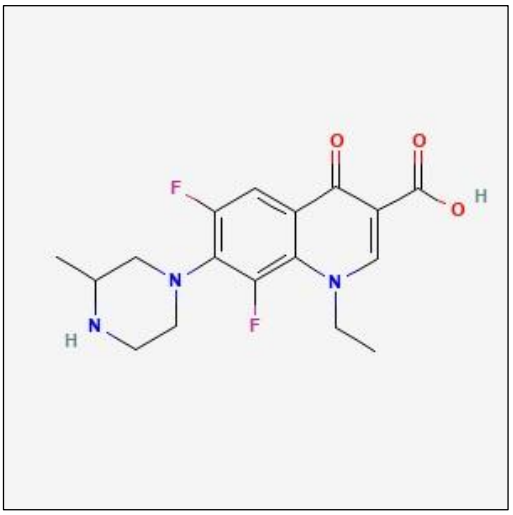


Chromatogram of lisinopril showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Lomefloxacin



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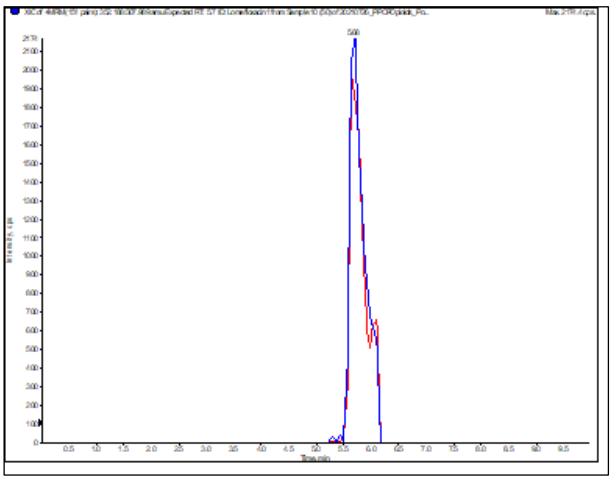
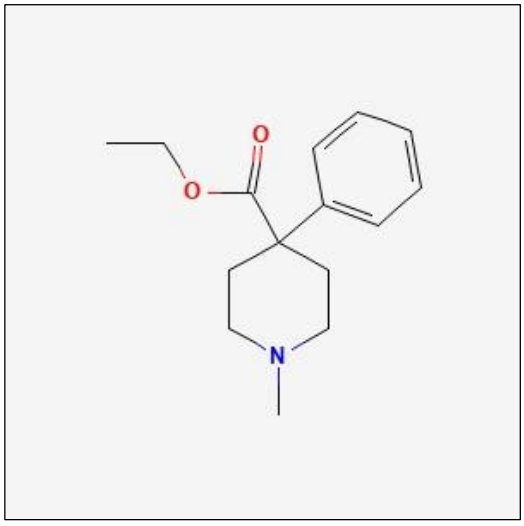


Chromatogram of lomefloxacin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Meperidine



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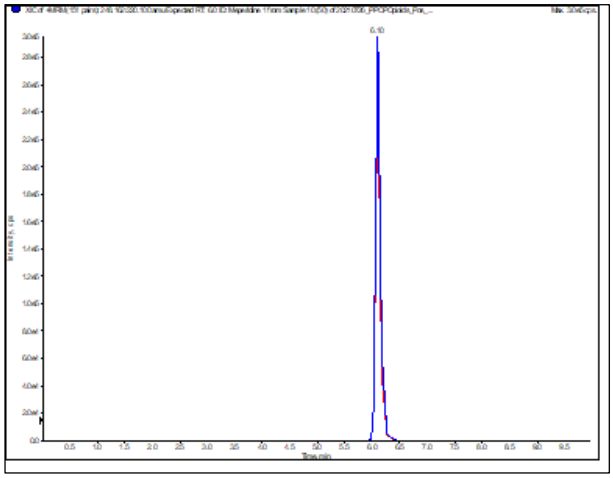
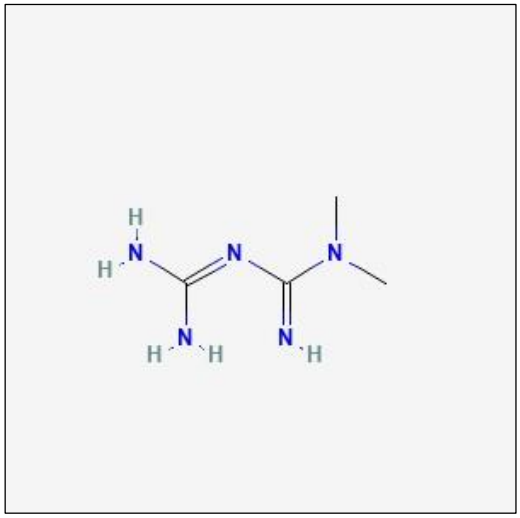
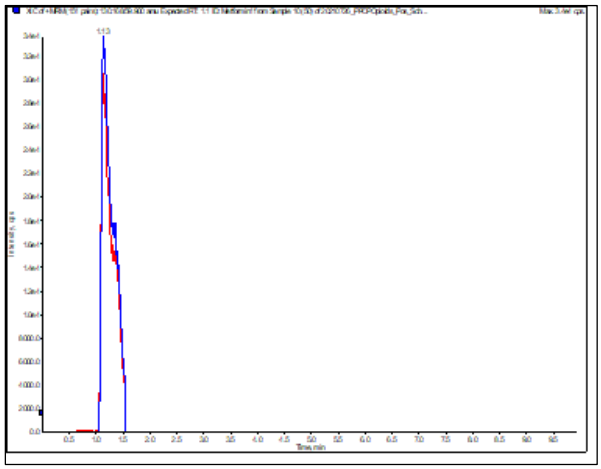


Chromatogram of meperidine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Metformin

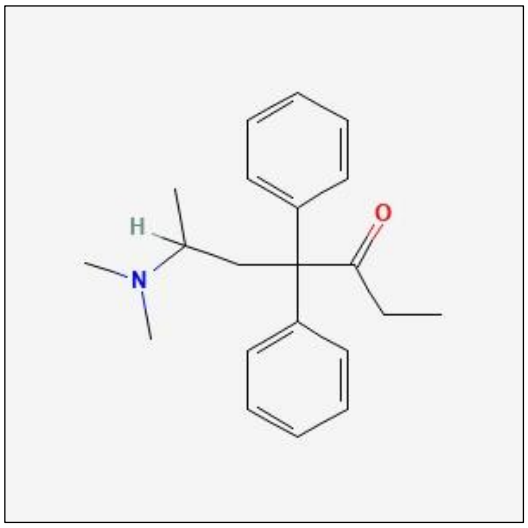


pubchem.ncbi.nim.nih.gov

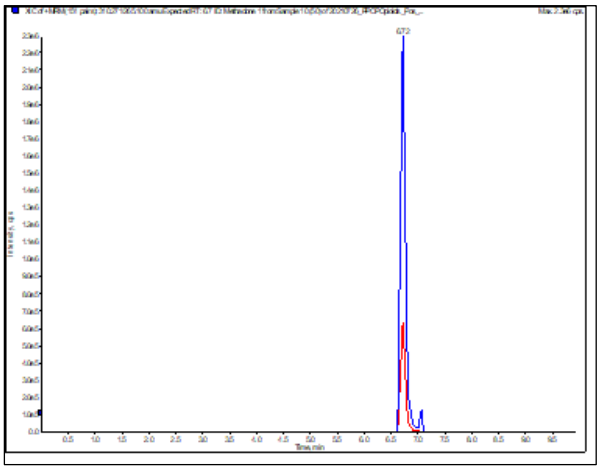


Chromatogram of metformin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Methadone

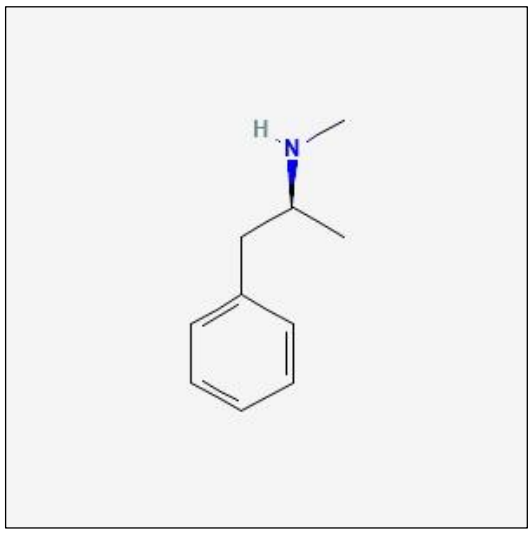


pubchem.ncbi.nim.nih.gov

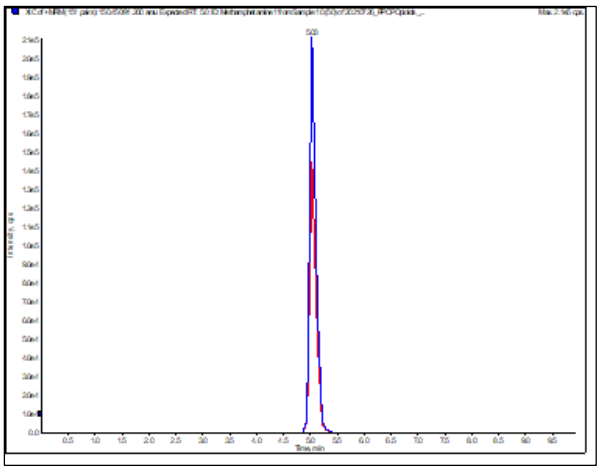


Chromatogram of methadone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line isQ1 and red line is Q2.

Methamphetamine

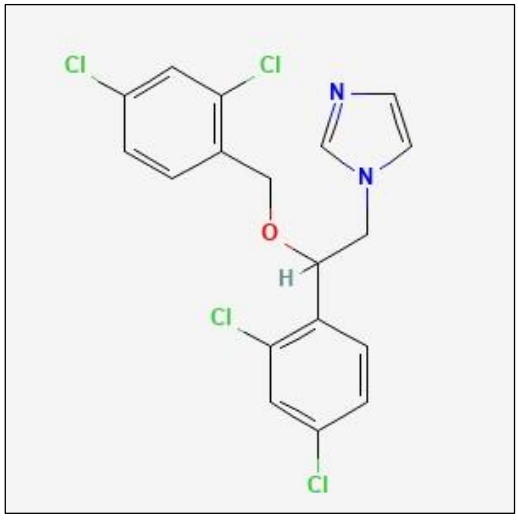


pubchem.ncbi.nim.nih.gov

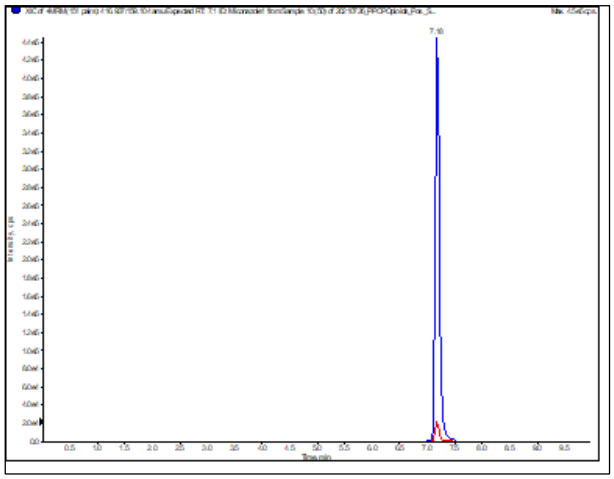


Chromatogram of methamphetamine showing Q1 and Q2 peaks generated from LC - MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Miconazole

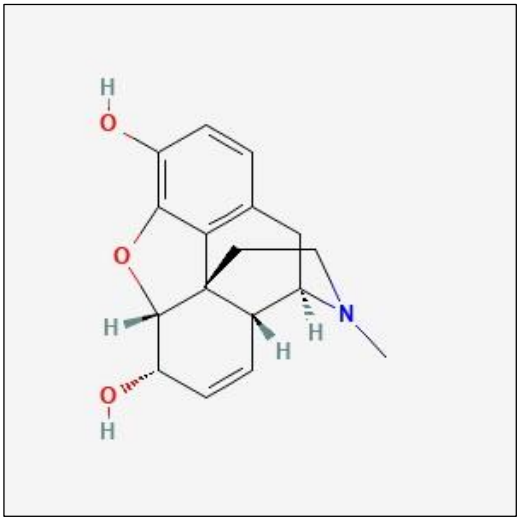


pubchem.ncbi.nim.nih.gov

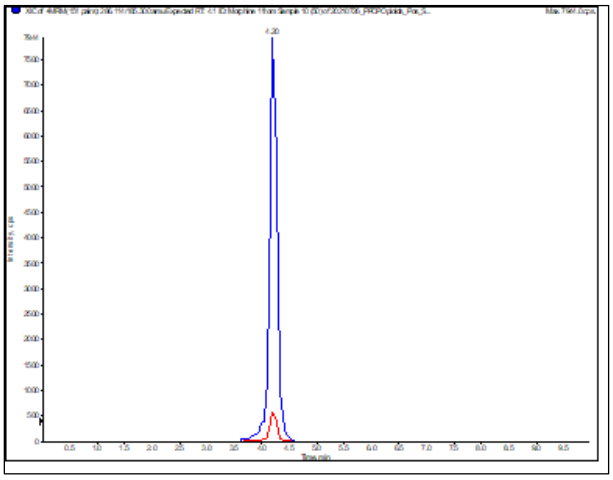


Chromatogram of miconazole showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Morphine

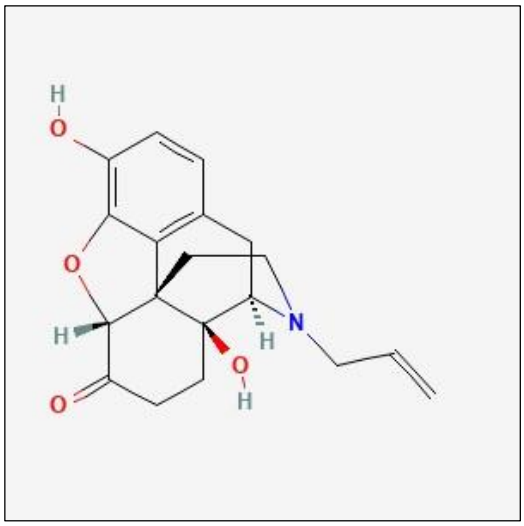


pubchem.ncbi.nim.nih.gov



Chromatogram of morphine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Naloxone



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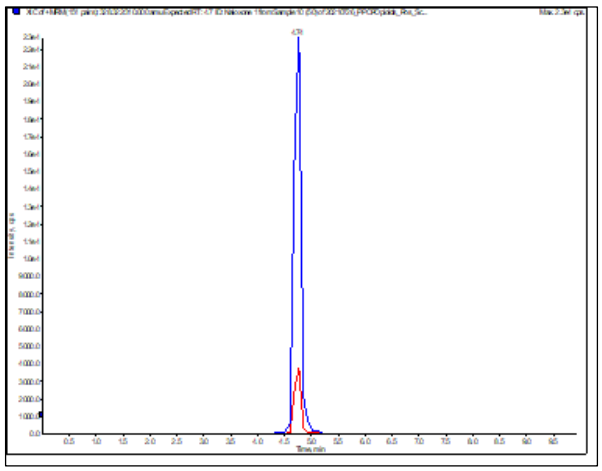
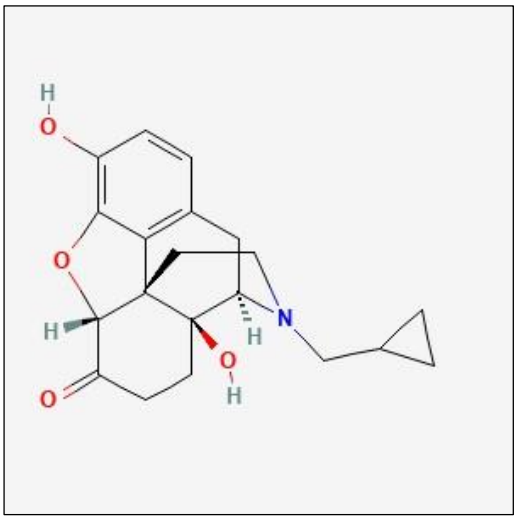


Chromatogram of naloxone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Naltrexone



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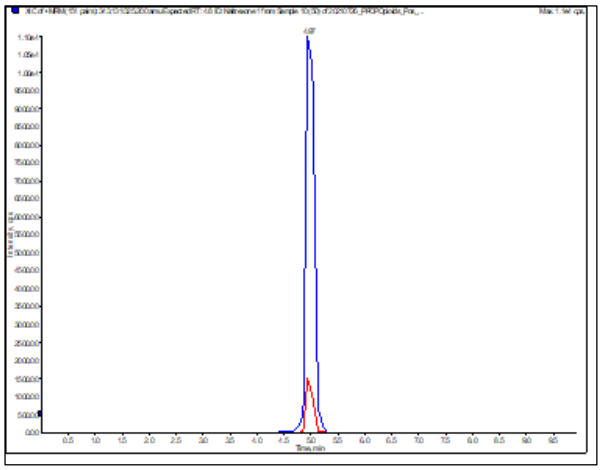
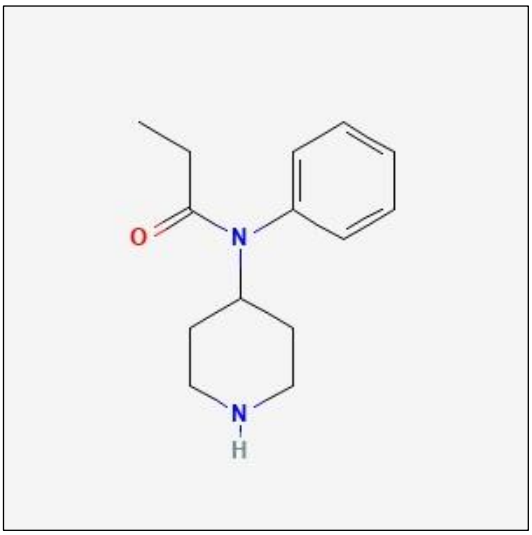


Chromatogram of naltrexone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Norfentanyl



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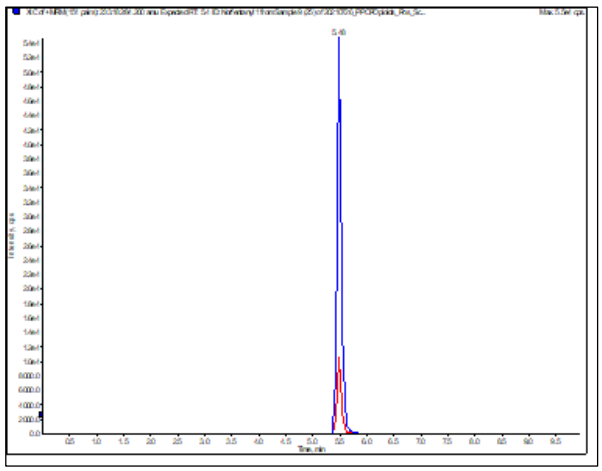
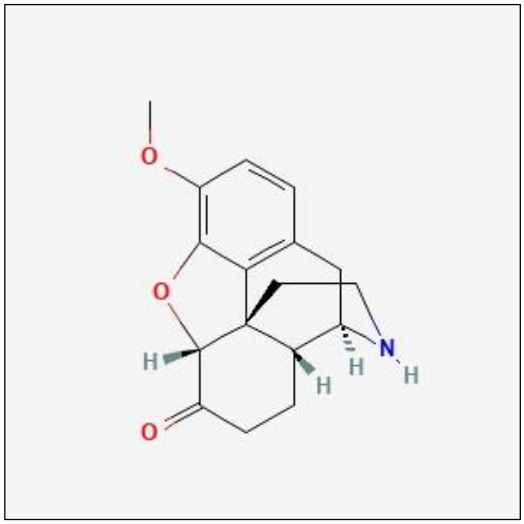


Chromatogram of norfentanyl showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Norhydrocodone



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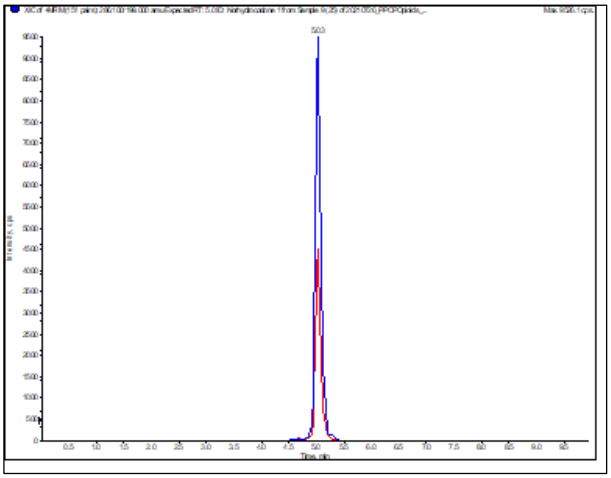
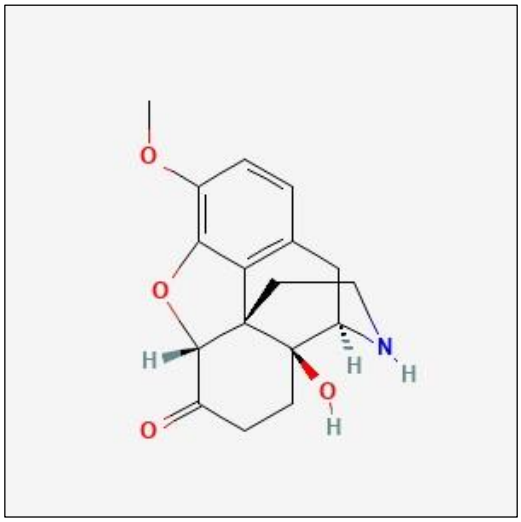


Chromatogram of norhydrocodone showing Q1 and Q2 peaks generated from LC - MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Noroxycodone



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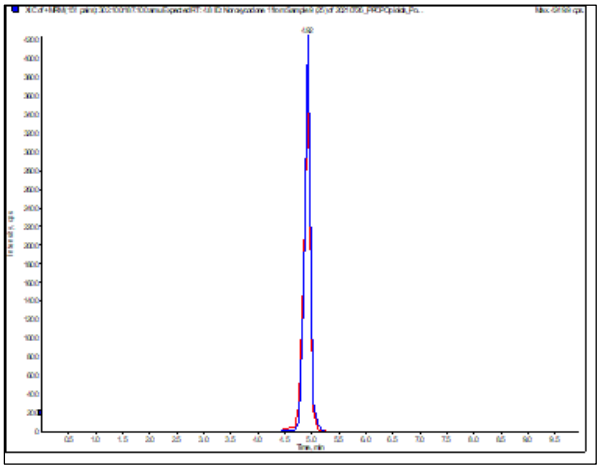
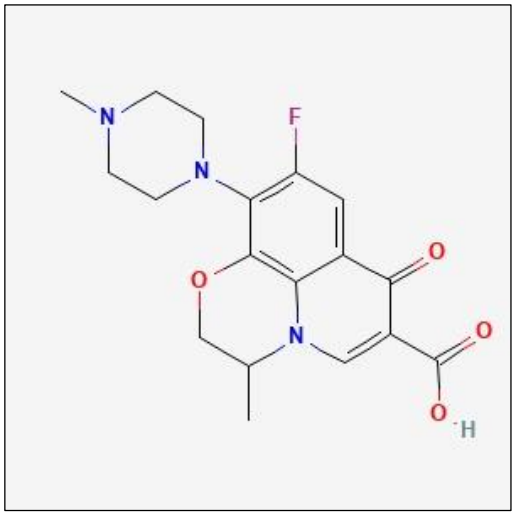


Chromatogram of noroxycodone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Ofloxacin



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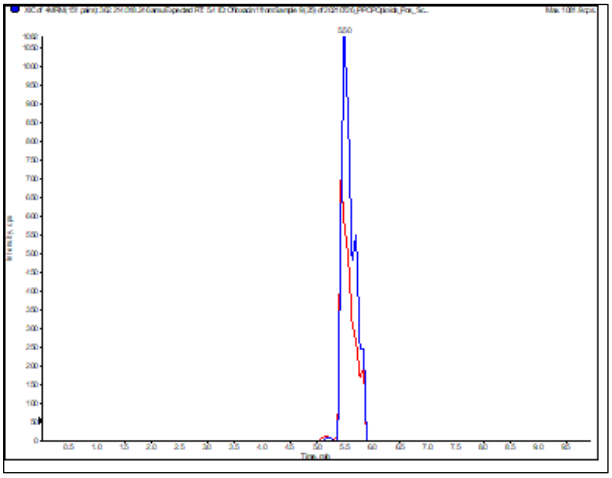
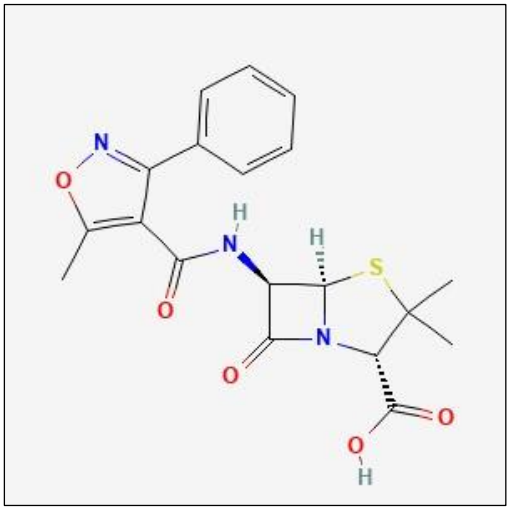


Chromatogram of ofloxacin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Oxacillin



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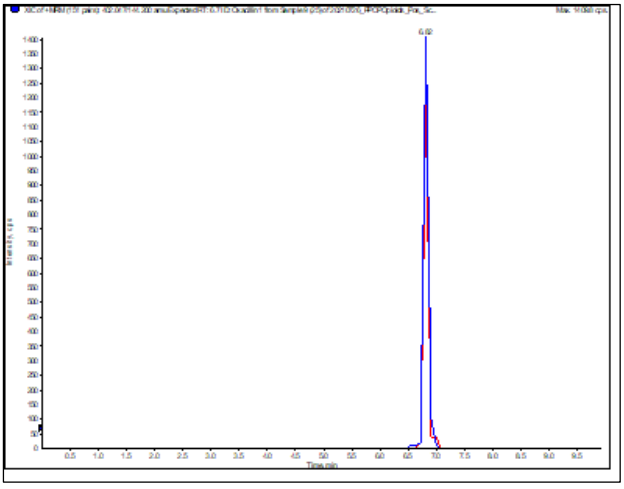
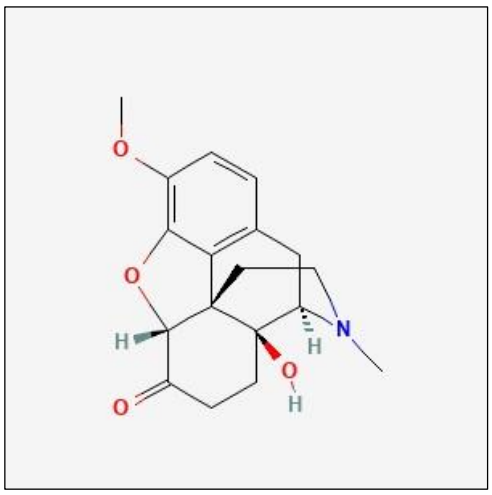


Chromatogram of oxacillin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Oxycodone



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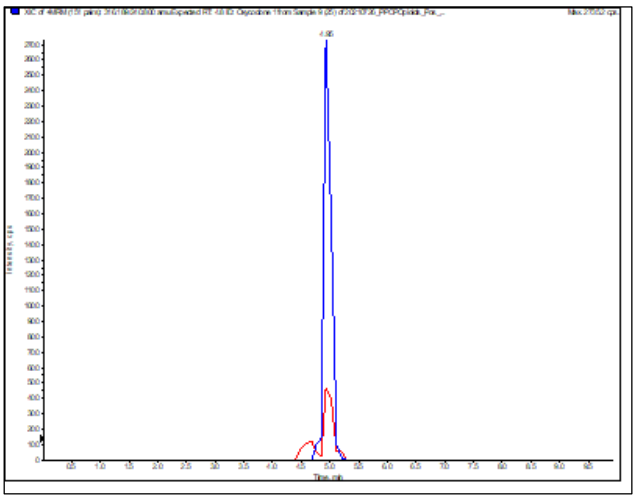
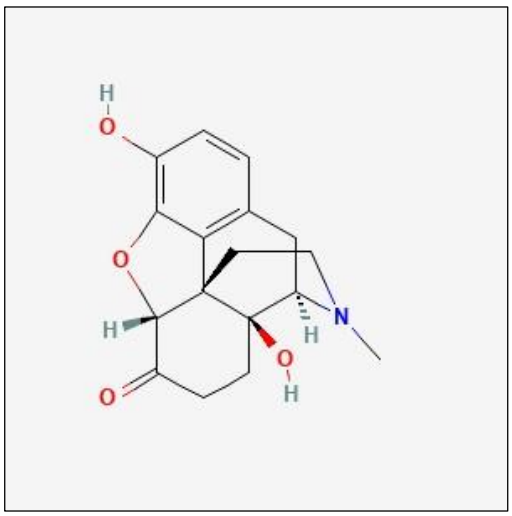


Chromatogram of oxycodone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Oxymorphone



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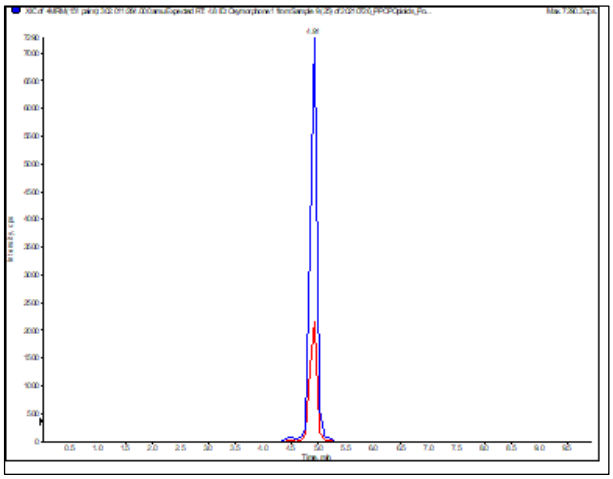
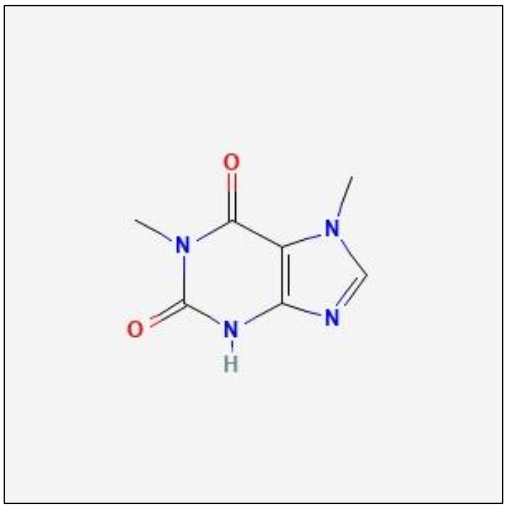
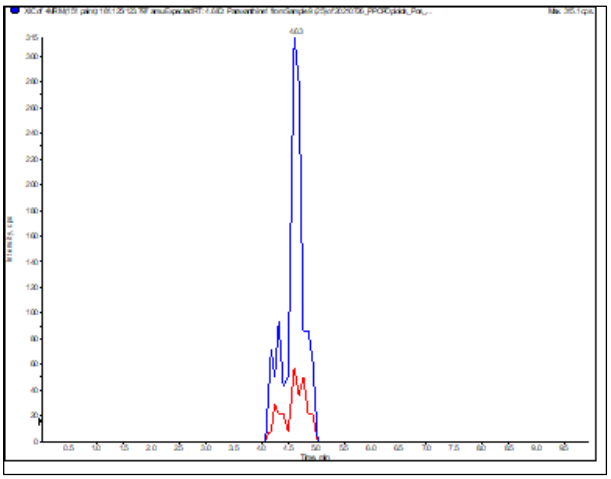


Chromatogram of oxymorphone showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Paraxanthine

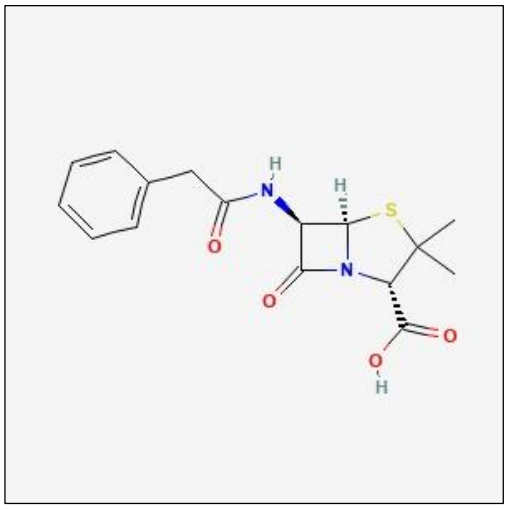


pubchem.ncbi.nim.nih.gov

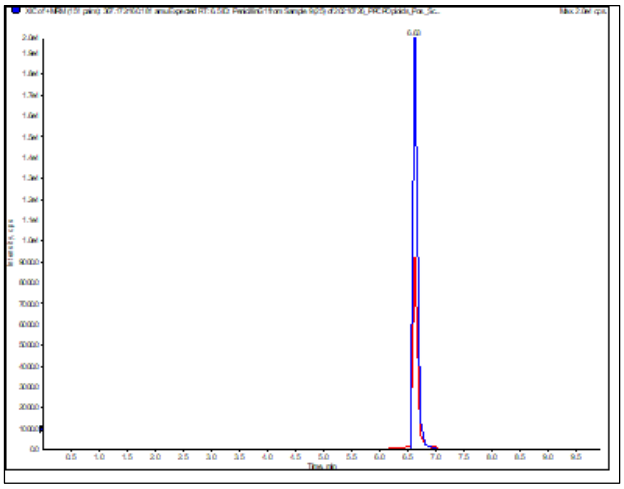


Chromatogram of paraxanthine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Penicillin G

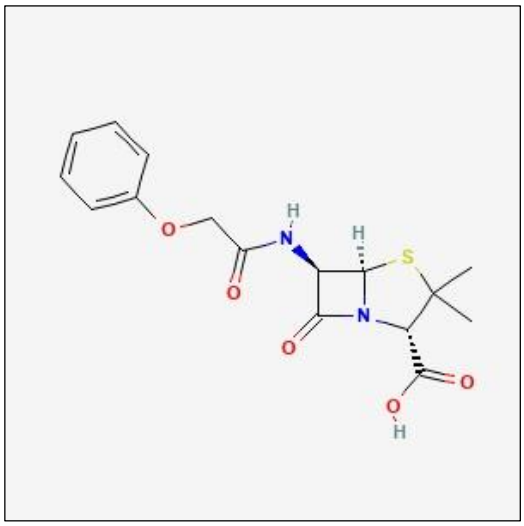


pubchem.ncbi.nim.nih.gov

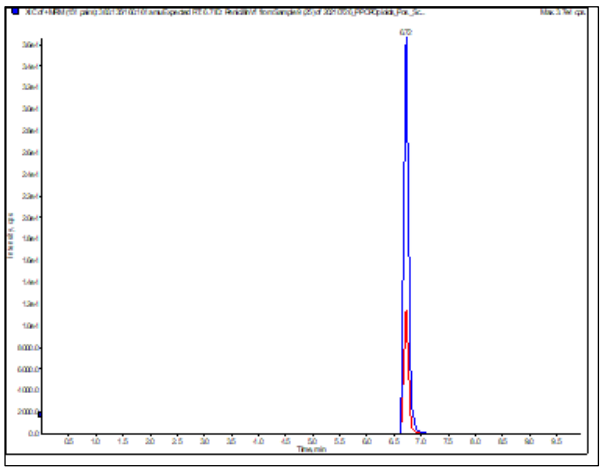


Chromatogram of penicillin G showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Penicillin V

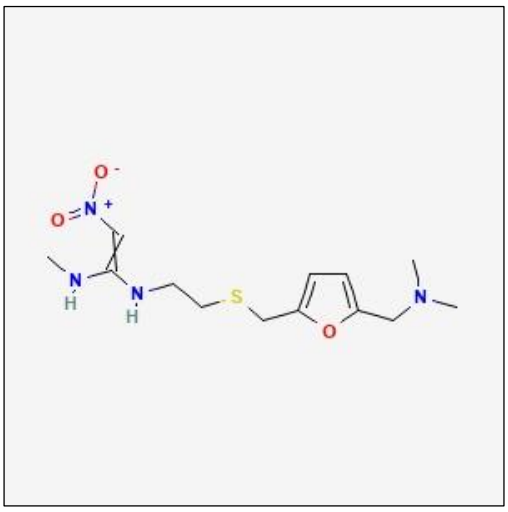


pubchem.ncbi.nim.nih.gov

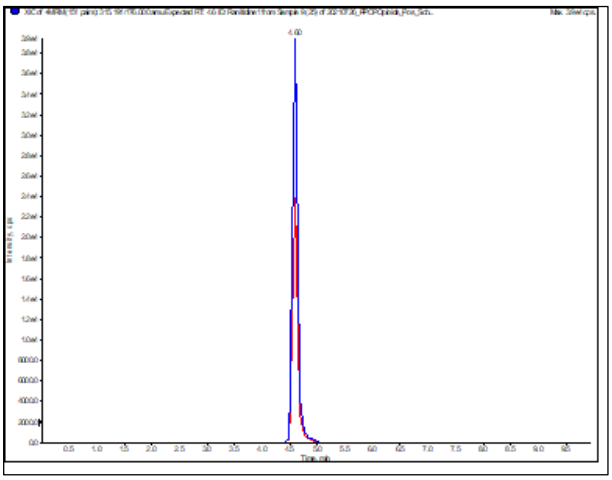


Chromatogram of penicillin V showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Ranitidine

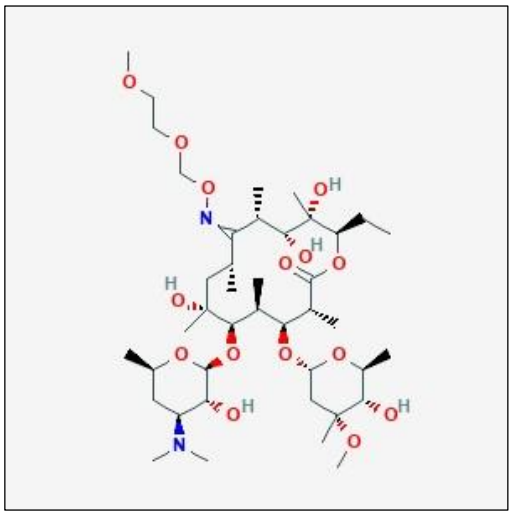


pubchem.ncbi.nim.nih.gov

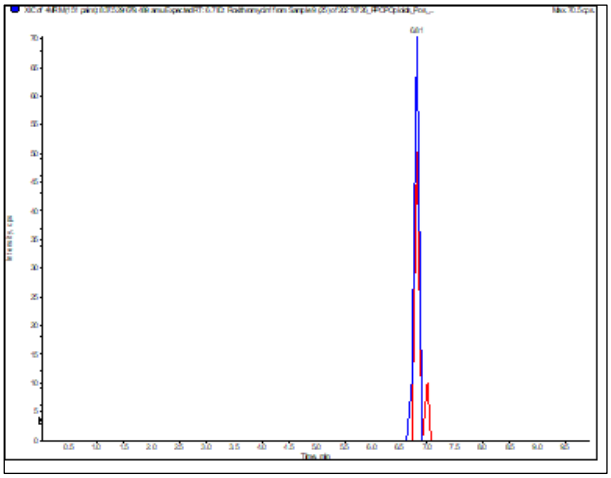


Chromatogram of ranitidine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Roxithromycin

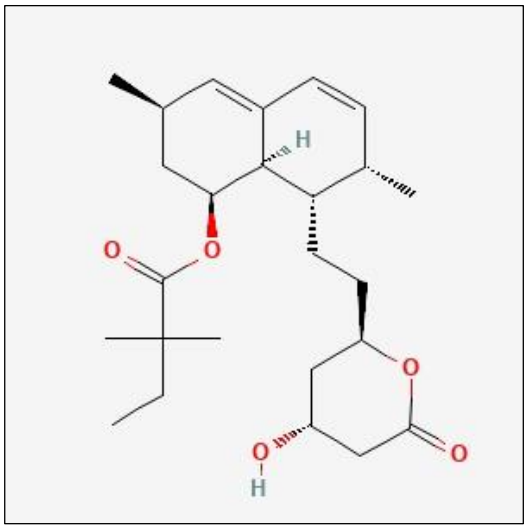


pubchem.ncbi.nim.nih.gov



Chromatogram of roxithromycin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Simvastatin



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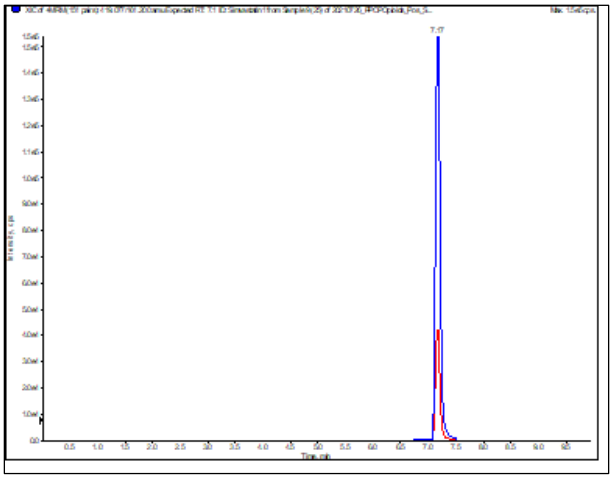
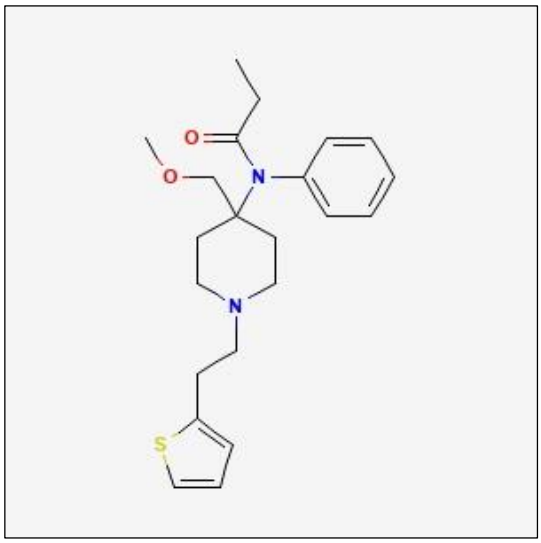


Chromatogram of simvastatin showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sufentanil



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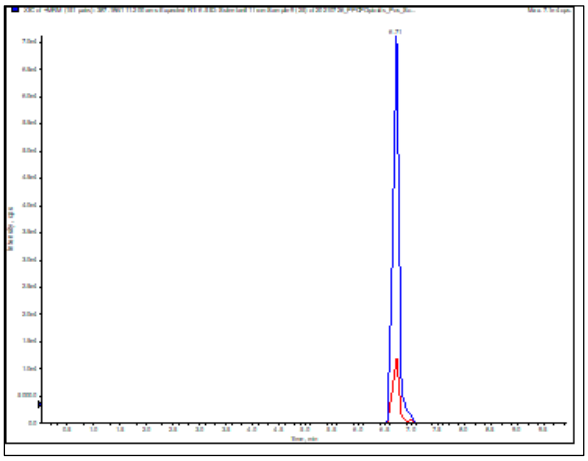
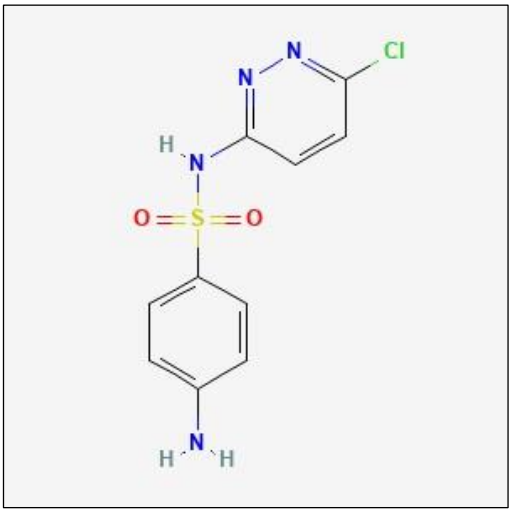


Chromatogram of sufentanil showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfachloropyridazine



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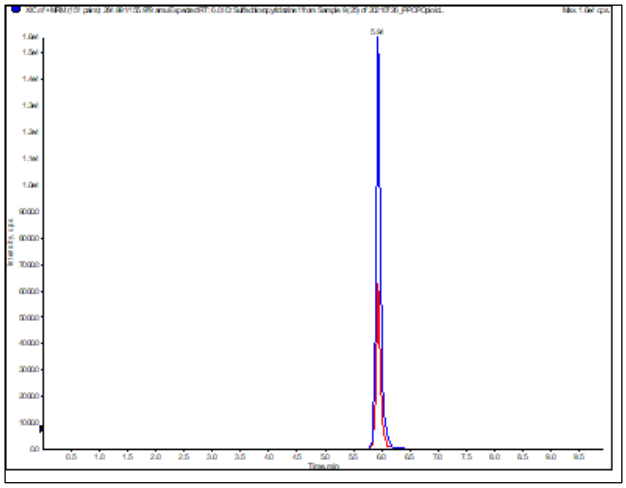
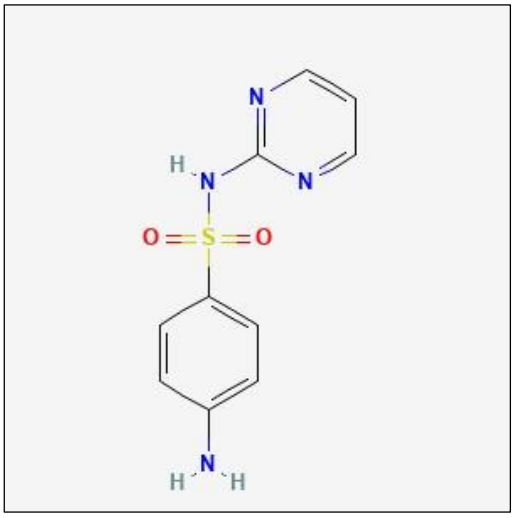


Chromatogram of sulfachloropyridazine showing Q1 and Q2 peaks generated from LC-MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfadiazine



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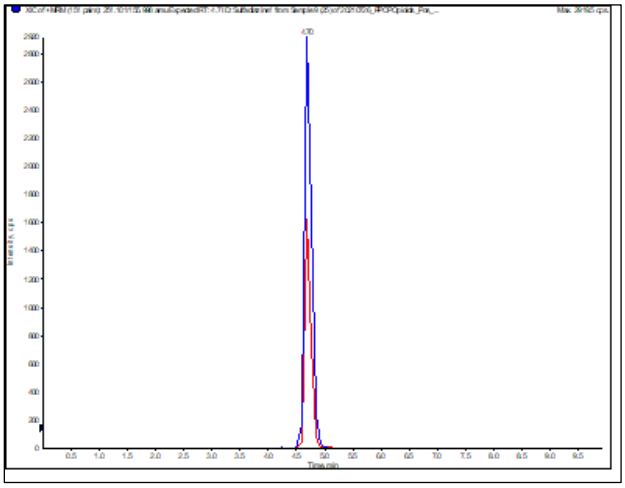
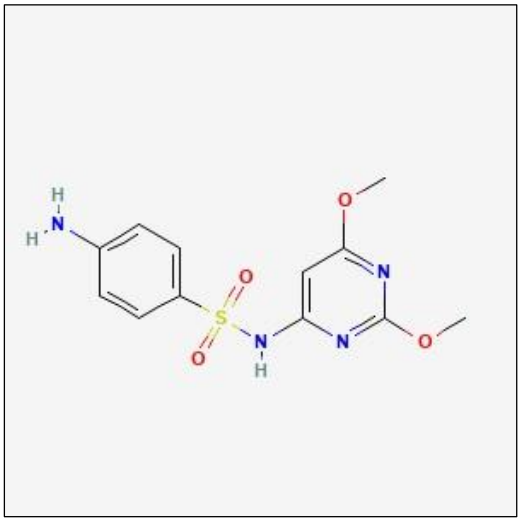


Chromatogram of sulfadiazine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfadimethoxine



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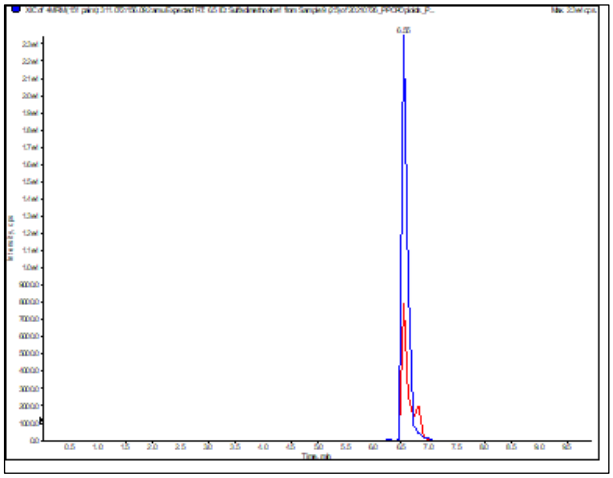
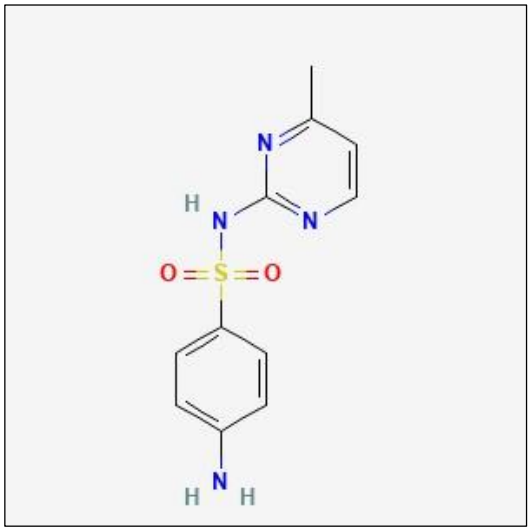


Chromatogram of sulfadimethoxine showing Q1 and Q2 peaks generated from LC - MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfamerazine



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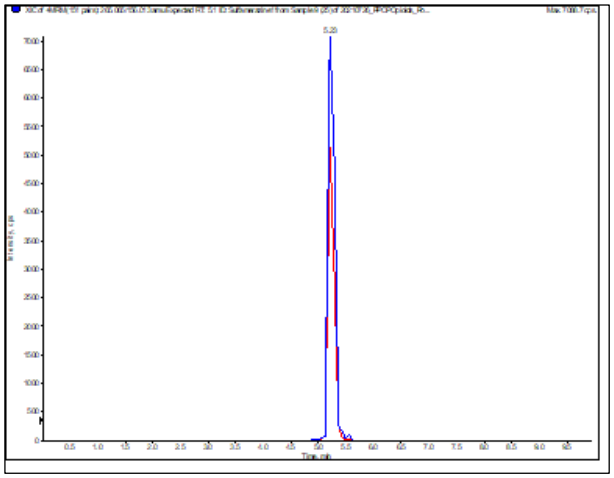
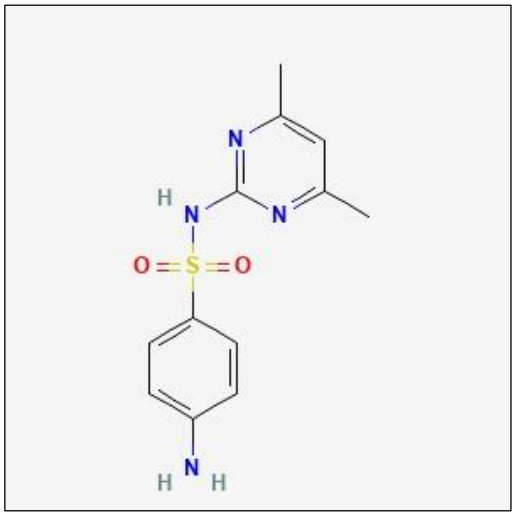


Chromatogram of sulfamerazine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfamethazine



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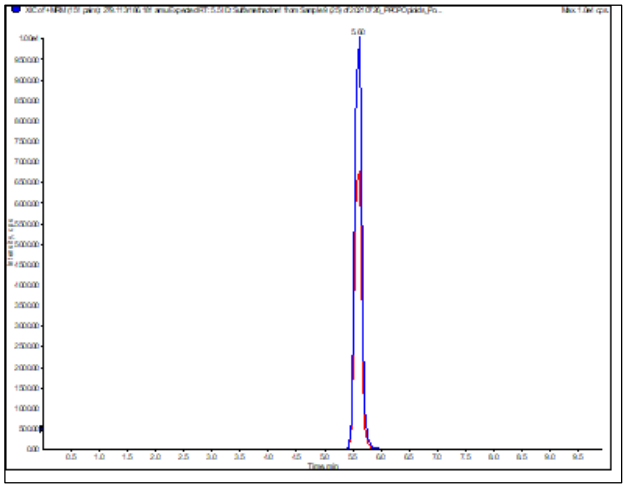
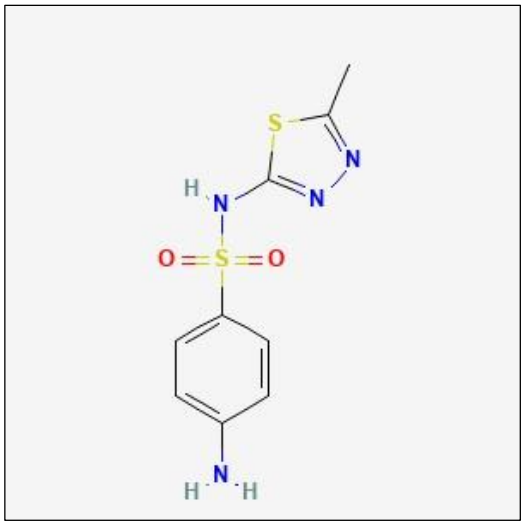


Chromatogram of sulfamethazine showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfamethizole



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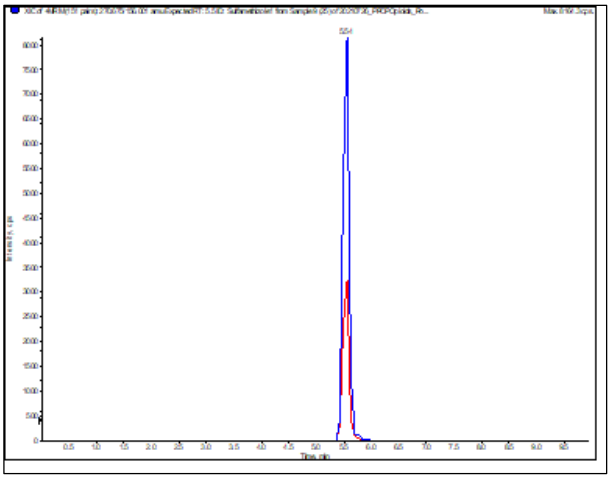
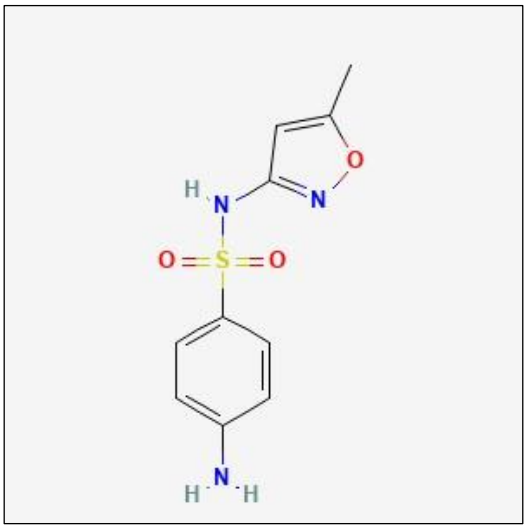


Chromatogram of sulfamethizole showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfamethoxazole



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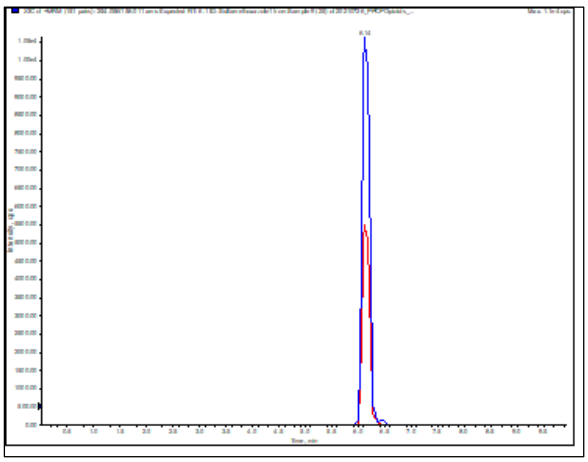
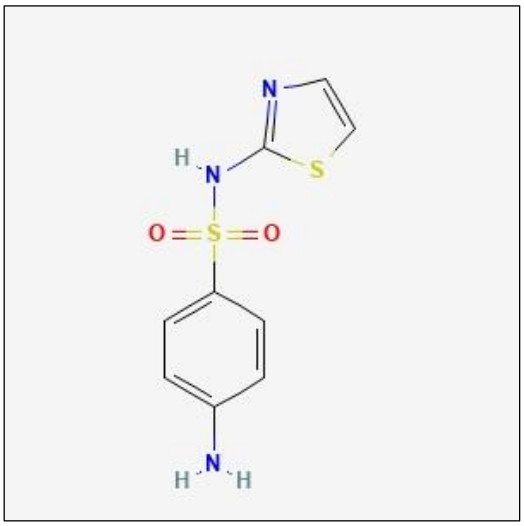


Chromatogram of sulfamethoxazole showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Sulfathiazole



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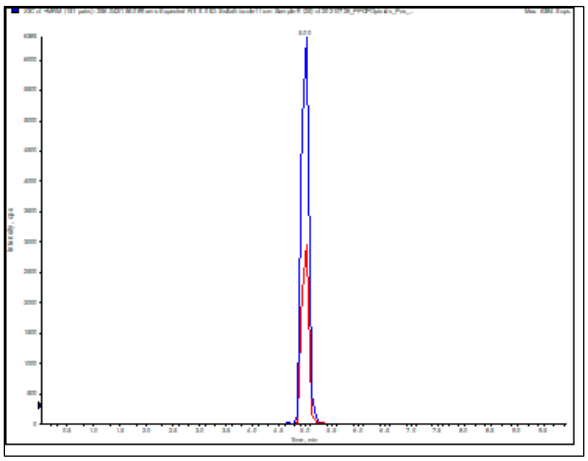
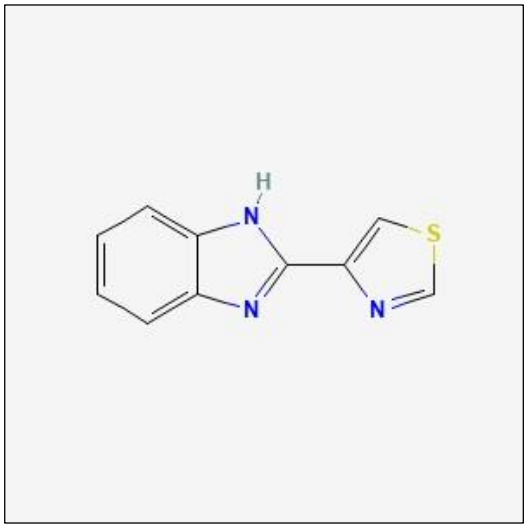


Chromatogram of sulfathiazole showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Thiabendazole



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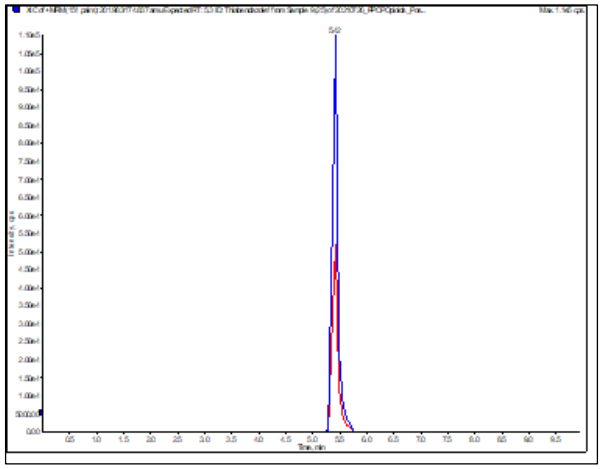
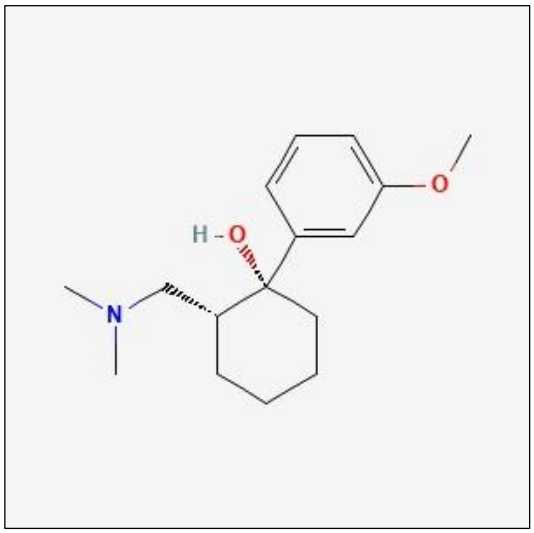


Chromatogram of thiabendazole showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Tramadol



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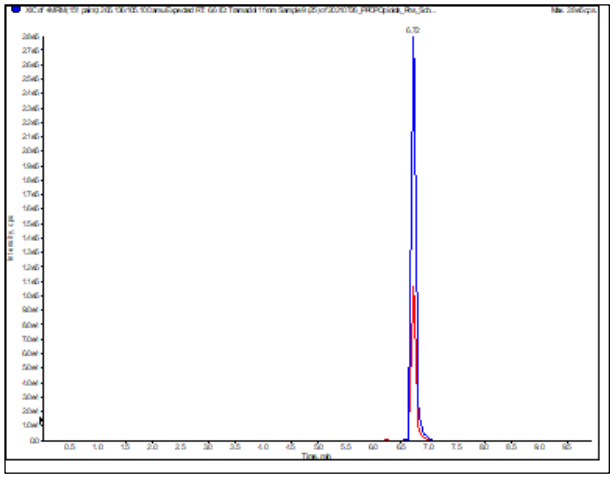
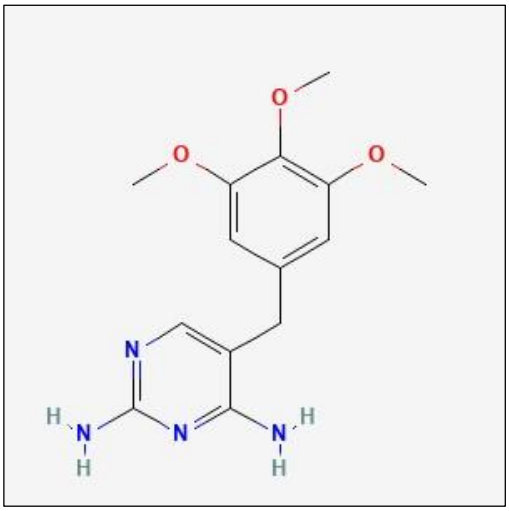


Chromatogram of tramadol showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

Trimethoprim



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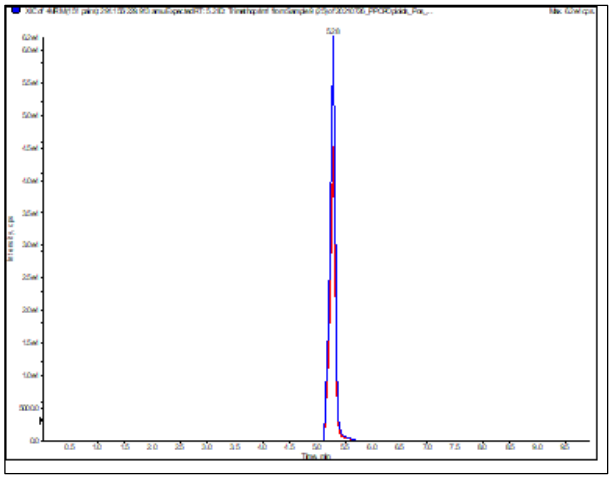

Chromatogram of trimethoprim showing Q1 and Q2 peaks generated from LC -MS/MS with y-axis counts per second (intensity) and x-axis time in minutes. Blue line is Q1, and red line is Q2.

CURRICULUM VITAE

Mary L Seaman

Education

Doctor of Philosophy, Environmental Health Sciences *(in progress)* August 2023

University of Wisconsin, (UW) Milwaukee, Milwaukee, WI

Dissertation: "Detection of Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Treatment Plants and Urban River Systems"

Master of Science, Biological Sciences May 1998

University of Wisconsin, Oshkosh, WI

Bachelor of Science, Biological Sciences and Psychology May 1995

University of Wisconsin, Oshkosh, WI

Research Experience

Researcher University of Wisconsin, Milwaukee	2011 to present
Research AssistantU.S. Antarctic Research Program	1996-1997
Researcher for REU Program UW Milwaukee	1994

Professional Development

Vilas County Public Health Department	2005-2015
McNair Scholars Director	2008-2016
Lab Technician, City of Oshkosh WWTP	2001-2002
Lab Technician, City of Fond du Lac WWTP	2000
Autopsy Assistant, St. Elizabeths & Theda Clark Hospitals	2014
Wrote two Anatomy & Physiology lab manuals	2016-2017
Dissected pig hearts for Appleton Medical Cardiac Conference	2016

Undergraduate Research Task Force	2007-2016

Teaching Experience

Senior Lecturer University of Wisconsin Oshkosh	1998-2017
Instructor Fox Valley Menasha - one semester	2009
Visiting Instructor Ripon College	2016-2017
CAPP liaison for Neenah and Ripon High Schools	2013-2017

Grants

Two McNair Scholars grants both funded for 1.2 million	2008-2016
Teaching grant to dissect cadaver funded for $6,000	2014
Solid Waste Research Grant funded for $30,000	2008-2009
Sturgeon for Tomorrow Small grant	1994-1995

Publications

Beversdorf, LJ., K. Rude, C.A. Weirich, S.L. Bartlett, **M. Seaman**, P. Biese, T. Gosz, M. Suha, C. Stempa, C. Shaw, and T.R. Miller. Indicators of cyanobacterial peptide levels in raw drinking water, in Water Research

Manuscripts in Preparation

Mary L. Seaman, * and Todd R. Miller

A Survey of Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs from Influent, Effluent and Surface Water from Two Wastewater Treatment Plants

In preparation for Science of the Total Environment

Mary L. Seaman, and Todd R. Miller

Mass Balance, removal and seasonality of sixty pharmaceuticals and personal care products (PPCPs), and illicit drugs from two wastewater treatment plants (WWTPs). *In preparation for Science of the Total Environment*

Mary L. Seaman, and Todd R. Miller

Survey of Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs from six urban river systems and Seasonality Differences.

In preparation for Water Research

Conferences

AGU Fall meeting, 2022, Chicago, IL	December 12-16
Ninth National Conference on Undergraduate Research	1995
Schenectady, New York	