CONTAMINANTS OF EMERGING CONCERN BEHAVIOR WITHIN WATER RENEWAL FACILITIES

by

Joshua C. Baker

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of the thesis submitted by

Joshua C. Baker

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The following individuals read and discussed the thesis submitted by Joshua C. Baker, and they evaluated the student's presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Sondra Miller, Ph.D.	Chair, Supervisory Committee
Kevin Roche, Ph.D.	Member, Supervisory Committee
Nick Hudyma, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Sondra Miller, Ph.D., P.E., Chair of the Supervisory Committee. The thesis was approved by the Graduate College.

DEDICATION

To my lovely wife Abby, daughters Aspen and Wren and my fur child Duke; ya'll got me through! I love you!

The LORD is my shepherd; I shall not want. He makes me lie down in green pastures. He leads me beside still waters. He restores my soul. He leads me in paths of righteousness for his name's sake. Even though I walk through the valley of the shadow of death, I will fear no evil, for you are with me; your rod and your staff, they comfort me.

– Psalm 23

"It is not the critic who counts; not the man who points out how the strong man stumbles, or where the doer of deeds could have done them better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood; who strives valiantly; who errs, who comes short again and again, because there is no effort without error and shortcoming; but who does actually strive to do the deeds; who knows great enthusiasms, the great devotions; who spends himself in a worthy cause; who at the best knows in the end the triumph of high achievement, and who at the worst, if he fails, at least fails while daring greatly, so that his place shall never be with those cold and timid souls who neither know victory nor defeat."

– Theodore Roosevelt

"Don't expect to be motivated every day to get out there and make things happen. You won't be. Don't count on motivation. Count on Discipline."

– Jocko Willink

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ABSTRACT

Emerging constituents (ECs), also called contaminants of emerging concern (CECs) are defined by the United States Environmental Protection Agency (U.S.EPA) as: "…pharmaceuticals and personal care products." This also includes per- and polyfluoroalkyl substances (PFAS), which are used in waterproofing and non-stick cooking products. It is highly likely that regulatory limits will be placed on many ECs because they tend to accumulate in the environment and biological tissues with little to no transformation. ECs pose a threat to the ecological systems of our nation and the fundamental need for clean water by all life on earth. Clean water is essential for food production, whether directly, through activities such as fishing, or secondarily, through irrigation for crop production. Research shows that ECs have affected the endocrine systems of certain fish species throughout the United States. Some studies indicate that upward of 85% of male fish sampled had eggs growing within their reproductive organs. ECs in the United States primarily enter water bodies through water renewal facilities, whether on-site (e.g. septic systems) or centralized municipal utilities (e.g. City of Boise's water renewal system). Research shows various psychotropic drugs, prescribed and illicit, are present in both receiving and discharge streams of many North American water renewal facilities. It is unclear the extent to which ECs are removed or accumulate through wastewater treatment processes. This is further exacerbated by the abundant release of ECs into collection systems across our nation, and the rate at which new ECs are being generated for personal care and medical uses.

This research examined a targeted set of ECs within the Lander Street Water Renewal Facility (LSWRF), the older of the City of Boise's two water renewal facilities. The research detected and mapped certain ECs as they processed through the LSWRF. Their paths through the facility, behavioral tendencies, and variations in concentration are presented here. While the concentrations detected are low in comparison to medical dosing concentrations, the accumulation potential of these substances in the natural receiving systems remains unknown. Water and soil must be clean for life to thrive. We have been given the responsibility by our creator to be good stewards of the earth and its resources. ECs pose a threat to life. We must continue conducting research to find a way to prevent ECs from causing harm to our natural systems. Research like this is the beginning of good stewardship.

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LIST OF ABBREVIATIONS

ABE	Aeration Basin Effluent
BSU	Boise State University
CEC	Constituent of Emerging Concern
EC	Emerging Constituent
EPA	Environmental Protection Agency
GBT	Gravity Belt Thickener
INF	Influent
LC/MS	Liquid Chromatography Mass Spectrometry
LSWRF	Lander Street Water Renewal Facility
MS	Mass Spectrometer
TDC	Thesis and Dissertation Coordinator
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PE	Primary Clarifier Effluent
PPCP	Pharmaceuticals and Personal Care Products
RAS	Return Activated Sludge
SCE	Secondary Clarifier Effluent
SPMD	Semi Permeable Membrane Device
WAS	Waste Activated Sludge
W3	Chlorinated Facility Effluent

CHAPTER ONE: INTRODUCTION

Overview

This paper focuses on contaminants of emerging concern specific to wastewater renewal facilities. A contaminant of emerging concern, referred to in this document as an emerging constituent (EC), is outlined by the US Environmental Protection Agency (EPA) as:

"Contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (PPCPs), are increasingly being detected at low levels in surface water, and there is concern that these compounds may have an impact on aquatic life. It is important for EPA to be able to evaluate the potential impact of CECs and PPCPs on aquatic life and have an approach for determining protective levels for aquatic organisms. These chemicals have features that require additional consideration when applying existing ambient water quality criteria for the protection of aquatic life, using EPA's 1985 Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Life and Their Uses." (United States Environmental Protection Agency, 2020)

ECs enter the environment in various ways. Pharmaceuticals and personal care products enter the wastewater collection system through residential drainage. Personal care products, for example, are applied in the shower and are washed off and down the drain. Pharmaceuticals are ingested and pass through the digestive system (McClements, 2018). A portion of the EC is carried out of the body as waste, it then moves through the collection system toward water renewal facilities. ECs may pass through a water renewal

facility and then enter the environment through the outfall. Residential septic systems may also pass ECs directly into the groundwater (James, Miller-Schulze, Ultican, Gipe, & Baker, 2016). In most cases, groundwater contamination will result in surface water contamination due to the direct connection between the two (Banks, Simmons, Love, & Shand, 2011).

The research team was awarded a seed grant from the College of Engineering at Boise State University to identify ECs in the Boise wastewater streams and to better understand the fate of ECs within water renewal facilities. ECs present a potential threat to human drinking water systems, human food production, and natural ecosystems through their continued uncontrolled distribution in the environment and subsequent accumulation.

Purpose

The fundamental questions that this research is considering are:

1. What is the fate and transport of ECs within water renewal facilities?
2. Are there specific unit processes that appear to have an impact on the concentrations of ECs within water renewal facilities?

These are then followed by two hypotheses:

1. Certain ECs will be treated within existing water renewal facilities.
2. Settling will be the primary method of removal for ECs within water renewal facilities.

Access to clean drinking water is a basic right of all humanity as it is a basis for life. Our entire worldwide food supply is tied to clean water whether the food is taken directly via fishing, water is used to grow food that humans eat, or to grow food that animals eat.

The ecological impacts of ECs must be fully understood. ECs have the potential to affect wildlife through acute toxicity, reproductive disruption, or longer-term effects through bioaccumulation (Nilsen et al., 2019). Of special importance is the possibility of endocrine system disruption through the release of synthetic proteins and hormones into natural systems (Matthiessen & Johnson, 2007). There are many unanswered questions that surround the impact of ECs on natural systems. What levels of ECs are dangerous? What effect does the accumulation of ECs have? The prevalence and rapid production of ECs also creates a continued need for analysis since each could have a different effect.

Water renewal facilities are instrumental in understanding the potential for treatment and removal of ECs. Public information campaigns exist to limit the disposal of ECs, especially pharmaceuticals, into the sewer system. These programs replaced decades old, misinformed advice to flush unused pharmaceuticals down the toilet. Unfortunately, this bad advice caused long lasting contamination of natural river systems. But now, nearly every law enforcement entity in the country accepts unused and expired medications for proper disposal. Landfills accept other household materials like antifreeze and paint for disposal. There is still a significant use of medications (Martin, Hales, Gu, & Ogden, 2019), personal care products, and other items that contain ECs that are sent down drains every day. It is our responsibility to understand the effects these chemicals have on our finite water supply and determine how we remedy those impacts.

Literature Review

Research has been conducted to determine the concentrations and possible treatment methods for ECs. This literature review focused primarily on the types of ECs that were tested for, the methods used to detect them, and tests showing effects in natural water bodies and water renewal facilities. An issue with water renewal facilities and treatment effectiveness is that each treatment facility is highly unique. Each facility has different flows, chemical loads, pH, and other characteristics that could interact with ECs in different ways. This research should not be considered a conclusive treatment approach. It is an indication of the types of treatment that show promise and could be further explored.

Research in Natural Systems

Studies have been conducted on natural systems regarding ECs. One such research was conducted by Metcalfe et al. (Metcalfe, Metcalfe, Bennett, & Haffner, 2000). This research focused primarily on the existence and concentration of polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) in the Detroit River. The focus was on the contamination of these chemicals in fish species. Researchers were not searching for pharmaceuticals, as is the topic of this research paper, the methods for data collection and detection of PCBs and PAHs are like ECs. Data collection was completed with the use of semi-permeable membrane devices (SPMDs) to collect sediment within the Detroit River. SPMDs are exceptional at collecting hydrophobic compounds, which many ECs, PCBs, and PAHs are.

The results determined there were various concentrations of PCBs and PAHs in the Detroit River system. In two out of the six sites the concentrations were high enough

to present possible lethal levels for aquatic life (Metcalf et al., 2000). The sampling method of using SPMDs to collect long term composite river samples could be applied to this research further if it is expanded to long term river monitoring.

Other research sought to determine the concentration of ECs within fish tissues in Canada. (O'Toole et al., 2006) examined the effect of salmon spawning on the water quality of Lake Ontario. A correlation was observed between salmon being contaminated in the Pacific Ocean and bringing those contaminants into various rivers and streams in Alaska. This is related to the life cycle of salmon, a species that spawns in rivers and streams, journeys downstream to the ocean, then returns to the same stream 3 years later to spawn and die (Groot & Margolis, 1998). Their carcasses release necessary nutrients to river systems across North America. Based on previous research, PCBs and other contaminants were known to accumulate to the mg/kg concentrations in salmon tissues. This raised the question whether decaying carcasses were releasing contaminants back into natural river systems.

Researchers found a correlation between salmon carcass decay and spiking concentrations of various ECs within Lake Ontario (O'Toole, Metcalfe, Craine, & Gross, 2006). This shows another method of contamination of river systems in addition to direct discharge by humans. The Boise River does not have an active salmon spawn, but this is certainly applicable to other reaches of the Columbia River system. The Boise River watershed does have a substantial fish population. As these fish die and decay their carcasses could likewise release concentrated ECs that have accumulated into the river system.

Accumulation of ECs within fish tissues shows possible effects that ECs have on fish species. Certain compounds have a direct effect on the endocrine system of fish. Personal care products contain synthetic musks and scents that are released into the environment via wastewater treatment facilities (O'Toole & Metcalfe, 2006). These compounds are found in soaps, cosmetics, laundry detergent, and other consumer products. Synthetic musks are designed to mimic natural human pheromones to increase sexual desire. While not designed specifically to mimic hormones in fish, synthetic musks and other ECs have been shown to disrupt fish reproduction by causing male fish to grow female eggs within them (Konkel, 2016).

Another study conducted by O'Toole et.al. (2006), various fish species were collected from Hamilton Harbor in Ontario, Canada, and their fat was tested for various musk compounds. The following data table was presented in the study:

Table 1.1 Selected Results from (O'Toole & Metcalfe, 2006)

	Mean Concentration µg/kg wet weight			
Musk	B. Bullhead (n=3)	Gizzard Shad (n=3)	White Perch (n=2)	Yellow Perch (n=3)
DPMI	nd	nd	nd	nd
ADBI	nd	4.9 (± 2.4)	6.5 (± 0.1)	nd
AHMI	nd	4.7 (± 8.2)	5.8 (± 3.3)	nd
ATII	0.6 (± 1.1)	8.9 (± 4.9)	9.1(± 0.8)	9.9 (± 8.4)
HHCB	5.9 (± 0.3)	141.4 (± 90.3)	84.4 (± 33.3)	391.1 (± 253.4)
AHTN	2.9 (± 0.2)	22.3 (± 13.5)	16.7(± 0.5)	96.8 (± 73.0)
M. XYLENE	nd	nd	nd	nd
M. AMBRETTE	nd	nd	nd	nd
M. MOSKENE	nd	nd	nd	nd
M. TIBETENE	nd	nd	nd	nd
M. KETONE	nd	nd	nd	nd
Total Musks	**9.5 (± 0.7)**	**182.2 (± 102.5)**	**122.5 (± 41.3)**	**498.0 (± 327.6)**
mean & range	**9.0–10.2**	**80.9–332.2**	**93.2–151.7**	**93.4–945.4**
% Lipid	1.86 (± 0.18)	6.86 (± 6.56)	2.58 (± 2.07)	0.47 (± 0.15)

The highest concentration compound from above is HHCB, a musk used in various perfumes developed in the 1950s and marketed under the brand name Galaxolide. This research shows that hydrophobic synthetic compounds other than PCBs and PAHs can accumulate in the tissues of fish.

Research into natural systems has provided the following major points:

- Various ECs are found in natural systems, indicating that human created compounds are escaping into the environment.
- ECs have caused endocrine disruption in fish affecting populations. They have the possibility of causing acute toxicity as well.
- ECs are accumulating in the tissues of aquatic organisms.

Understanding the impact ECs have on natural systems is important because most water renewal facilities discharge to a natural system of some kind. Research also shows the persistent state of ECs in natural systems. The focus of this paper is on the fate of ECs within water renewal facilities, with the understanding that the water renewal facility discharges have a direct effect on natural systems.

Research Within Water Renewal Facilities

Water renewal facilities are each uniquely designed to address different permit limits and utility preferences. Each facility receives a unique influent stream. Some influent loads are highly affected by industrial waste streams, while others are highly concentrated due to limited intrusion from ground water (Metcalf & Eddy, 1999). This means the detection and treatment for ECs cannot be standardized and requires a custom process for each facility. Several studies have been completed across the world looking at ECs coming in and going out of a water renewal facility, but very few focus on the treatment and fate of ECs within facilities. Methods for detection vary from study to study, but generally all the research uses some form of mass spectrophotometry. The method of sample purification is also different for various studies. (Asimakopoulos, Kannan, Higgins, & Kannan, 2017) focused on the analytical methods used to detect the

various constituents. Many studies separate the liquid and particulate fractions of the sample to prevent clogging of analytical equipment. This practice overlooks the potential for EC loading in the particulate fraction. (Asimakopoulos et al., 2017) created a sample preparation method to leave the particulate fraction in the solution to achieve more complete results. The method presented a more accurate representation of the concentration of ECs in wastewater. A few of the more well-known compounds examined are presented in the table below.

Table 1.2 Selected Results from (Asimakopoulos, Kannan, Higgins, & Kannan, 2017)

Compound	Influent Concentration (ng/L)	Effluent Concentration (ng/L)
Methamphetamine	14.9	3.0
Cocaine	225	12.9
Lidocaine	426	422
Hydrocodone	11.4	6.1
Risperidone	<LOD	<LOD
Citalopram	115	76.7
Caffeine	50,000	1110

This shows the variability in treatment of ECs within the same water renewal facility. Cocaine showed a significant reduction across the water renewal facility while lidocaine showed no real decline in concentration (Asimakopoulos et al., 2017). This article didn't outline the type of process units at the water renewal facility.

A Canadian study, (Hoque, et al., 2014), was conducted in Ontario at a dual lagoon water renewal facility. They showed similar results to (Asimakopoulos et al., 2017) with variations between EC removal efficiency. The figure below is taken from the study to illustrate the variations:

Table 1.3 Selected Results from (Hoque, et al., 2014)

Estimated mean concentrations of indicator compounds (POCIS) in Lakefield sewage lagoon during the summer, fall and winter sampling periods. Final effluents that show statistically significant (Mann Whitney *U* test; p = 0.001) differences in mean concentrations from the raw influent are indicated by an asterisk (*).

Compounds	Summer			Fall			Winter		
	L1 (raw influent) (ng/L)	L2 (aerated sewage) (ng/L)	L3 (final effluent) (ng/L)	L1 (raw influent) (ng/L)	L2 (aerated sewage) (ng/L)	L3 (final effluent) (ng/L)	L1 (raw influent) (ng/L)	Pre-UV (ng/L)	Post-UV (ng/L)
Carbamazepine	4.15 ± 0.90	4.85 ± 1.23	4.54 ± 0.51	22.6 ± 19.1	13.2 ± 0.21	12.3 ± 0.46*	7.37 ± 0.30	10.5 ± 0.17	11.4 ± 0.19*
Sulfamethoxazole	3.53 ± 0.03	0.58 ± 0.23	0.04 ± 0.01*	3.35 ± 0.14	1.81 ± 0.11	0.74 ± 0.11*	3.91 ± 0.87	0.73 ± 0.25	1.19 ± 0.28*
Trimethoprim	3.66 ± 0.70	1.11 ± 1.05	1.07 ± 0.25*	5.21 ± 1.19	10.5 ± 2.07	2.69 ± 0.34	9.72 ± 0.40	9.81 ± 1.27	8.36 ± 0.46
Gemfibrozil	0.06 ± 0.01	0.16 ± 0.00	0.08 ± 0.02	0.10 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	0.02 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Ibuprofen	7.54 ± 0.48	1.62 ± 0.40	0.64 ± 0.05*	60.3 ± 8.05	51.3 ± 5.07	11.4 ± 0.40*	4.38 ± 0.61	0.44 ± 0.02	0.95 ± 0.03*
Sucralose	18.2 ± 0.85	30.8 ± 5.02	35.2 ± 4.47*	11.5 ± 1.05	29.5 ± 1.78	47.8 ± 5.36*	11.1 ± 5.76	18.3 ± 0.07	18.2 ± 2.90

L1, L2 and L3 sites at raw sewage inflow (influent), aerated sewage and treated sewage outflow pipe (final effluent). Pre-UV = Before UV treatment. Post-UV = After UV treatment. For mean concentrations, n = 3; ±SD.

One study examined a conventional activated sludge water renewal facility in Quebec Canada that is similar in size to the LSWRF. The research looked specifically at different sampling techniques, but the results presented below do examine four different treatment process units within the water renewal facility (Rodayan, Majewsky, & Yargeau, 2014).

Table 1.4 Selected Results from (Rodayan, Majewsky, & Yargeau, 2014)

	Grab				POCIS				24 h composite			
	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW	Untreated WW	Influent to aeration	Influent to secondary clarifier	Treated WW
Cocaine and its metabolite												
Cocaine	869 ± 20	871 ± 20	83 ± 5	70 ± 9	1841 ± 28	1735 ± 14	367 ± 4	333 ± 8	903 ± 6	875 ± 9	83 ± 5	72 ± 5
Benzoylecgonine	1594 ± 58	1616 ± 66	367 ± 21	356 ± 15	78 ± 35	76 ± 28	19 ± 10	18 ± 25	1855 ± 24	1750 ± 18	330 ± 12	298 ± 11
Amphetamine-type stimulants												
Amphetamine	68 ± 8	74 ± 9	17 ± 2	18 ± 2	78 ± 7	76 ± 6	19 ± 3	18 ± 3	85 ± 5	78 ± 6	13 ± 3	10 ± 2
Methamphetamine	76 ± 8	79 ± 2	17 ± 2	16 ± 2	92 ± 9	83 ± 3	12 ± 2	9 ± 1	85 ± 5	80 ± 3	19 ± 2	14 ± 2
MDMA	177 ± 12	192 ± 10	148 ± 10	140 ± 2	196 ± 11	192 ± 10	101 ± 8	96 ± 5	216 ± 6	210 ± 4	109 ± 4	99 ± 6
Ephedrine	114 ± 5	136 ± 10	164 ± 27	141 ± 15	139 ± 10	136 ± 10	167 ± 27	165 ± 24	158 ± 5	140 ± 5	129 ± 3	118 ± 4
Opioid drugs												
Codeine	405 ± 26	464 ± 15	508 ± 10	477 ± 33	459 ± 20	460 ± 24	508 ± 10	497 ± 9	494 ± 7	456 ± 3	478 ± 8	519 ± 4
Acetylcodeine	45 ± 8	46 ± 11	48 ± 7	55 ± 3	–[a]	–	–	–	70 ± 6	60 ± 8	43 ± 3	40 ± 3
Morphine	42 ± 5	48 ± 5	45 ± 3	45 ± 4	56 ± 6	56 ± 6	43 ± 4	43 ± 5	76 ± 4	70 ± 3	54 ± 3	46 ± 2
Acetylmorphine	137 ± 12	144 ± 8	98 ± 7	99 ± 8	–	–	–	–	142 ± 4	132 ± 3	91 ± 3	88 ± 2
Methadone	42 ± 5	34 ± 3	54 ± 4	69 ± 4	40 ± 5	36 ± 4	55 ± 5	62 ± 4	63 ± 4	52 ± 4	59 ± 3	63 ± 2
Tramadol	68 ± 12	58 ± 6	128 ± 8	125 ± 10	50 ± 5	41 ± 4	68 ± 7	73 ± 4	62 ± 4	48 ± 5	44 ± 5	67 ± 3
Ketamine	67 ± 9	71 ± 7	22 ± 7	20 ± 2	84 ± 7	78 ± 8	22 ± 3	20 ± 2	90 ± 5	78 ± 3	21 ± 5	18 ± 2
Oxycodone	65 ± 6	55 ± 5	52 ± 4	52 ± 4	69 ± 6	62 ± 2	52 ± 4	52 ± 3	78 ± 4	79 ± 3	65 ± 5	58 ± 2
EDDP	93 ± 6	103 ± 9	131 ± 12	144 ± 5	107 ± 5	103 ± 9	125 ± 5	130 ± 5	107 ± 4	103 ± 2	94 ± 3	87 ± 5

[a] Dashes represent values that were not obtained for compounds whose sampling rates are not available.

Based on the literature review conducted for this thesis, the mechanisms and fate of ECs remains elusive. The most promising method for removal in non-filtered water renewal facilities is through settling. Most facilities are designed for settling other contaminants such as phosphorus and metals. Water renewal facilities generally function on the principal of separating the solids from the liquids (Metcalf & Eddy, 1999). This is accomplished through multiple settling steps. There are certain facilities that operate, chemically or biologically, enhanced nutrient removal through the addition of metal salts or creation of bioreactors to remove targeted nutrients like ammonia and phosphorus. How ECs behave in these is unknown. UV disinfection is another possible mechanism for EC treatment. UV is the most common disinfection technology in the United States. The typical power design point is 35 mj/cm^2 to meet requirements for pathogen reduction (Metcalf & Eddy, 1999). This is much lower than the Title 22 requirement for re-use in California that dictates a dose of 100 mj/cm^2 (State of California, 2018). This higher dose is used as this design points because its where chemical bonds begin to break due to the radioactive energy. However, the effect of UV disinfection on ECs is unknown and exceedingly difficult to predict due to the variation in chemical form for each EC. A research study was conducted in Quebec to examine the effectiveness of ozonation on a select few ECs (Rodayan, Segura, & Yargeau, 2013). Ozonation is another form of disinfection treatment, where an electrical arc is used to ionize air and create ozone (O_3) that is collected, pressurized, and diffused into the effluent stream of a facility. It is a form of oxidation reaction that reduces pathogen loads and has the potential to oxidize other compounds (Gunten, 2003). Research from other sources indicated that the use of chlorine or ozone for oxidation of ECs may present a different problem in that the ECs

are simply transformed and not destroyed or disposed of (Rodayan et al., 2013). In (Rodayan et al., 2013), individual compounds in pure water, mixed compounds in pure water and mixed compounds in wastewater were ozonated. The results are provided in the figure below for the various compounds selected and their removal percentage after ozonation.

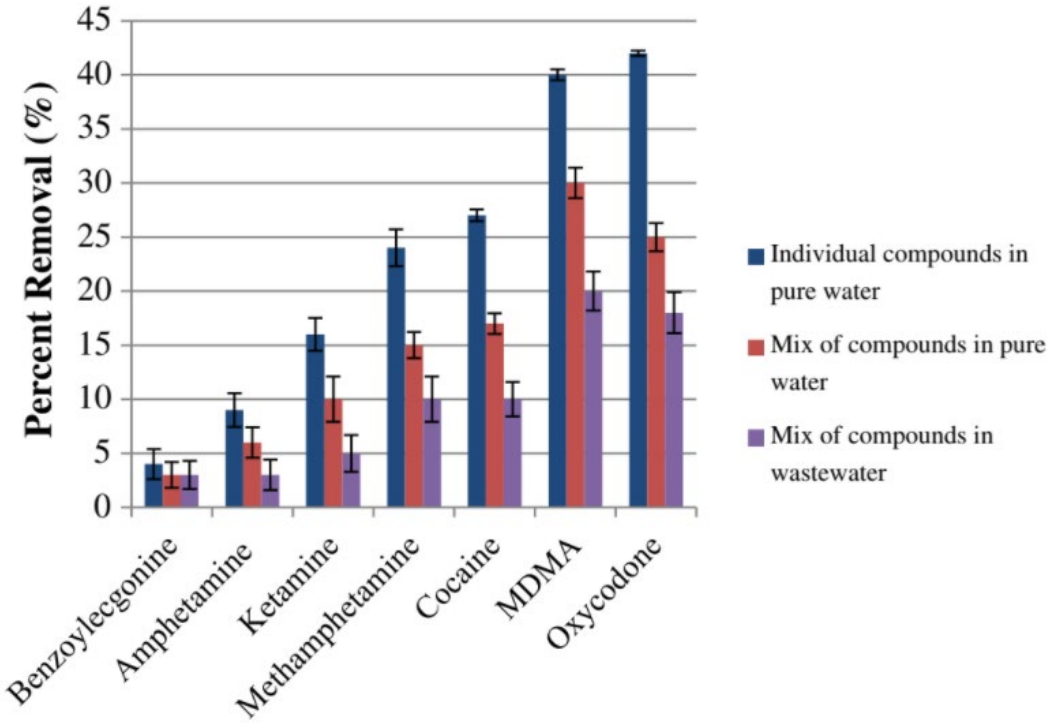

Figure 1.1 Ozone Treatment Effectiveness Chart from (Rodayan, Segura, & Yargeau, 2013)

Removal percentages vary between ECs. The data shows that with a very robust disinfection process such as ozonation, the efficiency of the process is affected by the wastewater itself. Thus, the lower the quality the effluent of a facility, the lower removal percentage any advanced oxidation reaction or other disinfection step would have.

Literature Review Summary

It is important to consider the work of predecessors to better understand ECs in water renewal facilities. The studies presented here form a picture of how ECs interact in a water renewal facility and in natural systems. The potential for bio-accumulation of ECs and pharmaceuticals should be further explored. It is clear that hydrophobic compounds such as PCBs, PAHs, and various synthetic compounds do bio-accumulate in various fish species. It is also apparent that direct discharge is not the only means for contamination because a correlation has been found between salmon carcass decay and contaminant concentration spikes in river systems (O'Toole et al., 2006). Through decay of aquatic species, the problem will be exacerbated because fish that have been exposed to hundreds of miles of river systems, die, and release their contaminated flesh back into the streams. This poses a way for ECs to enter portions of rivers that don't receive discharge from water renewal facilities.

A myriad of ECs exist and new ones are always in development. The research focused on over-the-counter painkillers, birth control, artificial sweeteners, illicit drugs, prescription pain killers, and mental health related drugs. No pattern emerged to show correlation between the type of EC to removal efficiency. Chemical makeup of ECs vary greatly. Some, such as synthetic musks, are designed to act like human hormones and have a structure like them (O'Toole & Metcalfe, 2006). Others, like PFAS are structured differently. It is unclear how they would be treated/broken-down and removed.

From the research, there is no obvious detection method other than LC-MS to detect ECs. Finding other detection methods will be a critical step forward in determining the best way to isolate treatment processes that are effective against ECs. An important

first step will be to measure the base concentrations of various chemicals and determine if an indicator compound(s) could be found. The location of the community plays a role in the concentrations of chemicals present. For example, the (Rodayan et al., 2014) study found incoming concentrations of cocaine at a medium sized city (population 225,000) in Quebec had a concentration of 869 ng/L where the (Asimakopoulos et al., 2017) study found an incoming concentration at a similar sized city in New York state to be 225 ng/L. Socioeconomic, legal differences between areas, and the type of treatment system affects EC concentrations. A combined sewer and storm water treatment system would have a lower concentration due to the storm water dilution. Many of the studies were conducted in straightforward lagoon style treatment. Lagoon treatment is common in rural areas, but many urban areas have more advanced types of treatment facilities like the LSWRF. Tertiary treatment, such as filtration or chemical addition may have a greater effect on ECs. This highlights another reason for a non-LC/MS way to detect ECs. Treatment processes are custom designed based on the wastewater facility's specific loading criteria.

In the (Hoque et al., 2014) study it was noted that there were detectable levels of carbamazepine upstream of the wastewater treatment facility. This is significant because the Otonobee River is in a remote region of Ontario with very few small communities upstream of the sampling location. The research suggested that the contamination may be caused by septic tanks along the water bodies that feed into the Otonobee (Hoque et al., 2014). This research underscored the need to further examine the fate and transport of ECs within the natural river systems. Each major river system is unique and will require its own research method and approach.

Possible Treatment Methods

Some forms of treatment are being developed to combat the persistence of ECs. There are two main types; the first is the use of oxidation reactions to drive destruction and the second is physical separation in the form of filtration (Rizzo et al., 2019). Oxidation is usually coupled in some way with disinfection and the systems typically include extremely high UV doses, addition of hydrogen peroxide upstream of UV, combined hydrogen peroxide and chlorine upstream of UV, and ozonation. These are all meant to drive oxidation reactions that will destroy ECs and pathogens (Gao, Deng, & Zhao, 2009). The issue with these is that as described in (Rodayan et al., 2013) it is unclear if this approach simply transforms the ECs into other compounds that are also hazardous. Not knowing the breakdown of all the different types of compounds makes their treatment difficult.

The most effective method for physical separation of ECs is reverse osmosis (RO) membrane filtration (Alturki et al., 2010). RO filtration systems remove all dissolved and particulate matter from a water stream leaving only pure water. Some downsides of RO are that it is expensive to build and has a very high operation and maintenance (O&M) cost component. RO is cost prohibitive for many municipalities. Standard wastewater membrane filtration combined with chemical addition may help to bind some ECs, but more research is required. Dual media (sand/anthracite) type facilities also may offer benefits due to the ability of carbon filters to bind dissolved components (Metcalf & Eddy, 1999). An issue with most tertiary filtration at wastewater facilities is that, unless the compounds eventually get into the solids side for disposal, they will continue to accumulate within the facility. This is due to the design of tertiary filtrations systems.

Once the filters have run for some time, they are cleaned and the water along with all the captured particles are pumped back to the head of the facility to go through the treatment steps again (Metcalf & Eddy, 1999). For nutrients this works well because the other treatment processes will get another chance to remove the nutrients through the solids system. ECs pose an issue if they don't ever get into the solids system to be disposed of.

CHAPTER 2 RESEARCH METHODS AND RESULTS

Overview

Emerging Constituent Selection

This research focused on a select few ECs to understand what may exist in the Boise treatment system and at what concentrations. This is not an exhaustive list but is to determine relative concentrations in the waste stream and determine if additional studies are warranted. The compounds selected are shown in the following table:

Table 2.1 ECs selected for testing

CEC Chemical Name	Trademark Name (s)	Typical Adult Dose*
Ibuprofen	Motrin, Advil, Nuprin	1,200 mg/day
Methlphenidate HCl	Ritalin	20-30 mg/day
Citalopram	Celxa	20-40 mg/day

*Typical Doses are provided for information only and in no way substitute for healthcare provider recommended values.

Ibuprofen is an over-the-counter painkiller and fever reducer. Methlphendidate HCl is an attention deficit hyperactivity disorder (ADHD) and attention deficit disorder (ADD) treatment drug that increases focus and is often prescribed to children and adults (Sherzada, 2011). Citalopram is a selective serotonin re-uptake inhibitor. It is used to treat anxiety and depression in adults (Milne & Goa, 1991). These ECs represent a broad spectrum of possibilities that are in the system and were selected because of their general widespread use across the United States. Research has not shown whether ECs are

destroyed in a drinking water treatment process or what the effect they may have on the human body in diluted concentrations of drinking water sources.

Water Renewal Facility Overview

The City of Boise owns and operates two water renewal facilities treating an average of approximately 30 million gallons per day (MGD). Both facilities are activated sludge secondary treatment facilities with UV disinfection. The Lander Street Water Renewal Facility (LSWRF) is the subject of this research.

Figure 2.1 LSWRF Aerial Photograph (courtesy of the City of Boise)

The facility is located on Lander Street in Boise, Idaho adjacent to the intersection of Veterans Memorial Parkway and the Boise River. The LSWRF is a secondary treatment activated sludge facility that was designed in 1948 and commissioned in 1950, predating the Clean Water Act (US EPA, 2022). The LSWRF primarily treats flows from north of the Boise River. Some flows from south of the Boise River are treated at

LSWRF through a series of siphon lines that run under the river from the South Boise Interceptor. The figure below shows the primary collection area for LSWRF.

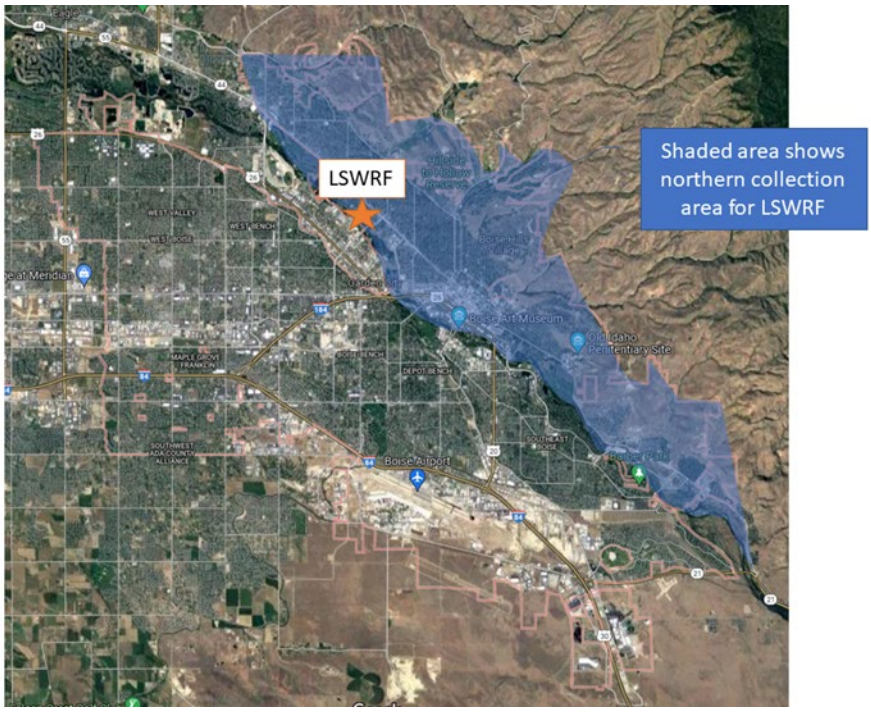

Figure 2.2 LSWRF Collection Area (image from Google Maps)

This research focused on the fate and transport across the LSWRF for ECs. Understanding where, or if, ECs are removed across a water renewal facility will influence the way ECs are treated in the future. Samples were collected after every major treatment step within the facility. These processes are discussed below in greater detail.

The primary removal mechanisms for a water renewal facility are (Metcalf & Eddy, 1999):

1. Transformation/Destruction
    a. This can be in the form of off-gassing such as in the nitrogen removal within the activated sludge system, during which

ammonia is transformed into nitrogen gas and released into the atmosphere.

b. Various chemicals are injected into the process including metal salts and pH stabilizers, these can potentially interact with various chemicals within the waste stream causing a chemical transformation.

c. Destruction occurs of certain chemicals and organisms depending on UV intensity. The typical dose of UV radiation at water renewal facilities is approximately 35mJ/cm^2 (Metcalf & Eddy, 1999). The dose is much higher at LSWRF at approximately 70 – 100 mJ/cm^2 (Hartman, 2021).

2. Physical Removal

a. This is the most common form of removal for a water renewal facility and usually involves either sludge collection and/or filtration.

b. Solids are removed at the primary and secondary clarifiers through settling and sludge collection at the LSWRF. Solids are then sent to the digesters for volatile solids reduction.

c. Treated solids for the City of Boise are sent to the Twenty Mile South Biosolids Application Site for use as Class B biosolid fertilizer to grow crops for cattle consumption.

Observing a reduction across the clarification processes would indicate physical removal via settling within the clarifiers. This would lead to ECs being removed from the

effluent but indicate that ECs are accumulating within the solids system. If a reduction is seen across a treatment process such as the activated sludge system or UV, it would indicate transformation/destruction.

Analysis Approach

The procedures summarized here have been described in detail elsewhere (Asimakopoulos et al., 2017). The approach is a series of sample collections, stabilizations, and concentration steps leading to LC/MS analysis. Samples are concentrated in a controlled manner to make the detection by the LC/MS more effective. The process is generally as follows:

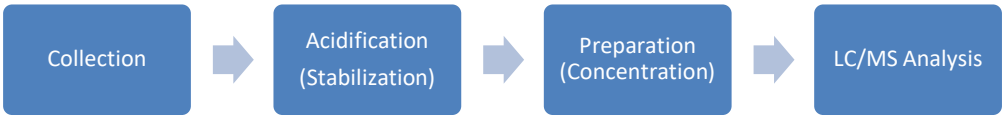

Figure 2.3 Sample Analysis Flow Chart

Sampling Procedures

All samples were collected as grab samples either using a grab sample bottle or a measured grab sample through a composite sampler. Grab samples were utilized for this research as they are commonly used at water renewal facilities and are a good procedure to determine a starting point for research at the LSWRF.

The figure below outlines a process flow diagram for the LSWRF. PS1 – PS3 are the primary sampling locations that are used by City of Boise operations staff for processes control.

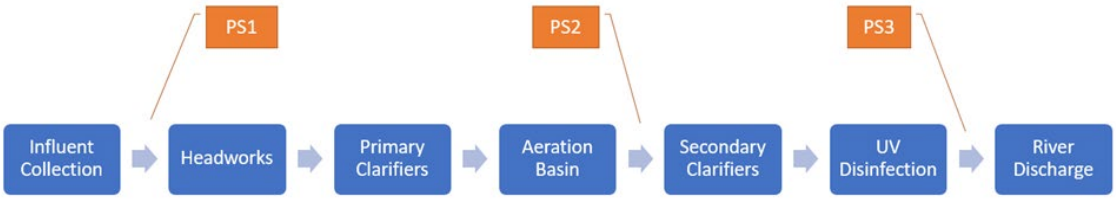

Figure 2.4 LSWRF Liquid Process Flow Diagram

The six sampling locations within the facility were:

1. Influent: Samples were collected from the main influent manhole, which is the same location used by the City of Boise. Samples were collected utilizing the automatic sampler at the facility. The sampler has a filter on the suction side of the pump that prevents large solids from entering. The opening size is approximately 1mm.
2. Primary Clarifier Effluent: Samples were pulled immediately after the Primary clarifier and after the addition of sodium hydroxide for pH control within the aeration basins.
3. Aeration Basin Effluent: Samples were pulled from the aeration basin effluent channel at the same location City of Boise operations samples for the mixed liquor concentrations.
4. Secondary Clarifier Effluent: Samples were pulled from the effluent launder of secondary clarifier number one.
5. UV: Samples were pulled using the automatic sampler used for final effluent compliance at the post aeration facility. This sample was collected after post-aeration, a process unit that adds dissolved oxygen to the

effluent for salmonid species. This represents the LSWRF Effluent to the Boise River.

6. W3: W3 water is facility effluent that is slightly chlorinated. Samples were pulled at yard W3 hydrant. This sample was intended to look at the potential effect of chlorine on EC concentration.

The figure below shows a satellite photograph with the sampling locations (orange) and liquids process units (blue) both throughout the LSWRF.

Figure 2.5 LSWRF Overview with sample locations

Sample Preparation

Sample preparation of the grab samples collected were performed in the laboratory according to the procedures described in (Asimakopoulos et al., 2017). The procedures included two different sample preparation methods (deemed method A and method B). Method A concentrated target analytes by applying vacuum and heat

followed by filtration (instrument protection) while method B extracted target analytes based on differential solubility. Sample preparation method A was selected for this research.

Sample Collection

Samples were all collected on weekdays at approximately the same time, between 3:00 PM and 6:00 PM. The following outlines the specific ways in which the samples were collected at each of the locations:

- Influent: A 500 mL sample was drawn using the HACH automatic sampler at the influent sampling location. The sample was transferred into a glass jar. A duplicate sample was pulled for every test at this location for QC purposes.
- Primary effluent: Grab samples were collected after the primary clarifier in the primary clarifier effluent channel downstream of the sodium hydroxide dosing point. The channel is approximately 4 feet wide and 10 feet deep. The sampler is only used for primary clarifier effluent. And is made with a plastic jar attached to a long pole. It is plunged to 5 feet below the surface of the channel and the grab sample is collected. The sample was then transferred into a glass jar.
- Aeration basin effluent: Grab samples were collected in the aeration basin effluent (ABE) channel immediately prior to the secondary clarifier influent splitter box. Samples were collected using a similar apparatus to the primary clarifier sampler that is reserved for the aeration basin effluent sampling location.

- Secondary clarifier effluent: Grab samples were collected in the effluent launder of secondary clarifier one. The glass jar was lowered into the effluent launder by hand for sample collection.
- UV Disinfection: Grab samples were collected for the UV effluent using the HACH composite sampler located at the post aeration facility. A 500 mL sample was collected into the glass jar.
- W3: Grab samples were collected for the W3 system using the W3 hose bib outside of the UV facility. The valve was opened and W3 ran onto the ground for approximately 1 minute before a sample was collected into the glass jar.
- Blank: The blank sample was collected in a glass bottle upon arrival back at the Environmental Research Lab. RO water available on tap within the room was used for the blank.

Acidification

Samples were immediately acidified after collection to prevent the need for freezing them. A solution of diluted hydrochloric acid and RO water was used. The beginning pH for samples ranged from 6.5 to 7.5. 75 mL was drawn from the sample jar into a cleaned beaker. The beaker was placed onto a mixing table and a magnetic stirrer was added into the sample. The HCl solution was placed into a titration burette and slowly added to the solution to lower the pH to 2.5 (+/- 0.5). pH was constantly monitored during titration using a HACH pH probe placed into the solution.

Samples were added to cleaned amber glass jars following acidification and stored in a refrigerator until sample preparation. All the amber glass jars used for storage were sealed with paraffin film.

Sample Concentration

Sample concentration followed method A from (Asimakopoulos et al., 2017) which involved the following steps:

1. Samples were combined as 25 mL of sample with 25 mL of Methanol with surrogate solution into a rotary evaporator flask.
2. Volume reduction by the rotary evaporator to approximately 2-5 mL
    a. rotary evaporator speed: 250 rpm
    b. rotary evaporator water bath temperature: 60 ºC
    c. rotary evaporator vacuum: ~40 torr
    d. The time for the rotary evaporator fluctuated from 9 to 15 minutes depending on the solution. Most times were approximately 12 minutes. It was noted that the primary effluent and raw influent samples tended to take the most time. Due to the high solids concentration of the ABE samples it was noted that solids were still present on the side of the flask after the solution had reached the desired volume.
    e. The condenser cooling water was maintained at 5 ºC by a chiller and constantly circulated with a pump.
3. Samples were transferred to a 15 mL glass sample tube and 10 mL of ethyl acetate was added.

4. A gentle stream of N_2 was used to evaporate the samples.
    a. Samples varied in final volume but were between 0.4 mL and 0.5 mL.
    b. Duration for the evaporation varied from 30 mins to 2 hrs depending on the samples. Raw influent and primary effluent typically took the longest time.
    c. Samples were placed in a warm bath at approximately 40 ^oC while being evaporated by a gentle stream of N_2.
5. Samples were diluted to 1 mL using pure methanol.
6. The extracted sample was placed into an amber vial to await instrument analysis. The sample vial was sealed with paraffin film and stored in a refrigerator until analysis by the LC – MS team.
7. Prior to instrument analysis the samples were filtered using a vacuum and filter. This step was to protect the equipment

LC-MS Analysis Procedure

The LC-MS procedure can be found in Appendix E and all activities associated with LC-MS were conducted by Dr. Xinzhu Pu at the Boise Sate Biomolecular Research Center.

Quality Control/Assurance

Quality control (QC) was of the utmost importance during sampling and analysis. The following QC practices were used:

Sampling/Preparation

Sampling QC focused on a few major areas:

- Sample collection methods:
    - Sample collection were all grab samples. Literary research indicated that grab samples generally show less concentration than composite samples. This could be due to the diurnal patterns that water renewal facilities experience.
    - All glassware was thoroughly cleaned prior to use in the field and after each step.
    - Each sample was placed in its own amber glass container.
    - Grab sample apparatus is the same apparatus that is used by City of Boise staff to collect process samples throughout the facility. Safety practices dictated that samples needed to be collected using sampling tools provided by the facility staff. The sampling apparatuses used were reserved for the sampling point and were not used in any other locations. Composite samplers have foam filters on them to prevent large solids from entering and fouling the sampler. Some samples were filtered prior during collection, and some weren't:
        - Raw influent: filtered by sampler
        - Primary effluent: unfiltered
        - Aeration basin effluent: unfiltered
        - Secondary clarifier effluent: unfiltered

        - UV effluent: filtered by sampler
        - W3: unfiltered
    - No food or drink are allowed within the LSWRF boundary thus cross contamination from food or drink isn't possible.
    - Latex gloves were worn by testers at every sampling location and throughout sample preparation.
    - Samples were immediately sealed after collection in cleaned glass amber jars.
- Sample Acidification:
    - All glassware was thoroughly cleaned prior to and after each sample was acidified.
    - The pH probe was thoroughly cleaned between each sample.
    - The pH probe was allowed to stabilize for each sample prior to recording the final pH reading.
    - Each sample was placed into a thoroughly cleaned amber sample jar with a piece of paraffin wax film under the cap to make an airtight seal.
    - All samples were maintained in the refrigerator until being removed for sample preparation.
- Sample Preparation
    - Rotary evaporator settings were duplicated for each sample.
    - The rotary evaporator flask was thoroughly cleaned after every sample using DI water within the lab.

- Each sample was placed into its own 15 mL sample tube after rotary evaporation and prior to N_2 blowdown to minimize the risk of cross contamination.
- After N_2 blowdown each sample was placed into its own cleaned small amber vial with a piece of paraffin wax film under the cap to make an airtight seal.
- All samples were maintained in the refrigerator until being removed for instrument analysis.

Surrogate Selection

Selection of surrogates to verify the sample preparation method is difficult with so many and varied ECs. Dihydro-carbamazepine was selected due to its similar chemical structure to both citalopram and ritalin. The surrogate was dosed into methanol and reduced to a concentration of 80 ng/L in each of the samples prior to preparation. This concentration was then used in the LC/MS software to automatically correct the data based on the calibration curves. Doing this allows for the detection of instrument drift along with issues in sample preparation. Dr. Xinzhu Pu recommended this approach.

Water Renewal Facility Conditions

The LSWRF facility operation is an ever-changing process like all water renewal facilities. On sampling days, it was verified with operations that no out of the ordinary chemical dosing or other upsets were taking place. This research is applied research and the sampling area is a working water renewal facility. Conditions, patterns, uses, and unknown loadings are a constant issue at these facilities (Wolff et al., 2018). The utmost care was taken during the sample preparation and laboratory side of the data collection,

and as much care as possible was taken at the LSWRF. This is an active water renewal facility, and many substances, conditions, or phenomena can affect the results.

Results

Data

Complete MS results for each of the samples can be found in Appendix A. Parameters of the sample preparation can be found in Appendix B for each of the samples that were prepared. Statistical analyses were conducted on the data set using a quartile method (Langford, 2006) to correct the data. The analysis can be found in appendix D.

There were 3 different samples taken at each location over three different months on April 22, 2021, July 24, 2021, and August 26, 2021. The influent was sampled twice during each sampling event as a quality control measure. The following shows the average concentrations of ECs examined at the various sampling points throughout the facility.

Table 2.2 Average Concentration of ECs detected within the LSWRF

	Methylphenidate (ng/L)	Citalopram (ng/L)	Ibuprofen (ng/L)
Influent	41	149	6,000
Primary Effluent	59	284	4,269
Aeration Basin Effluent	41	242	1,211
Secondary Clarifier Effluent	43	145	409
UV Disinfection Effluent	42	119	398
W3 Chlorinated UV Effluent	42	65	157
Blank	37	ND	ND

The data for Citalopram and Ibuprofen shows a general decline across the LSWRF. Methylphenidate did not show any significant decrease across the facility. The figures below illustrate the average concentrations across the facility.

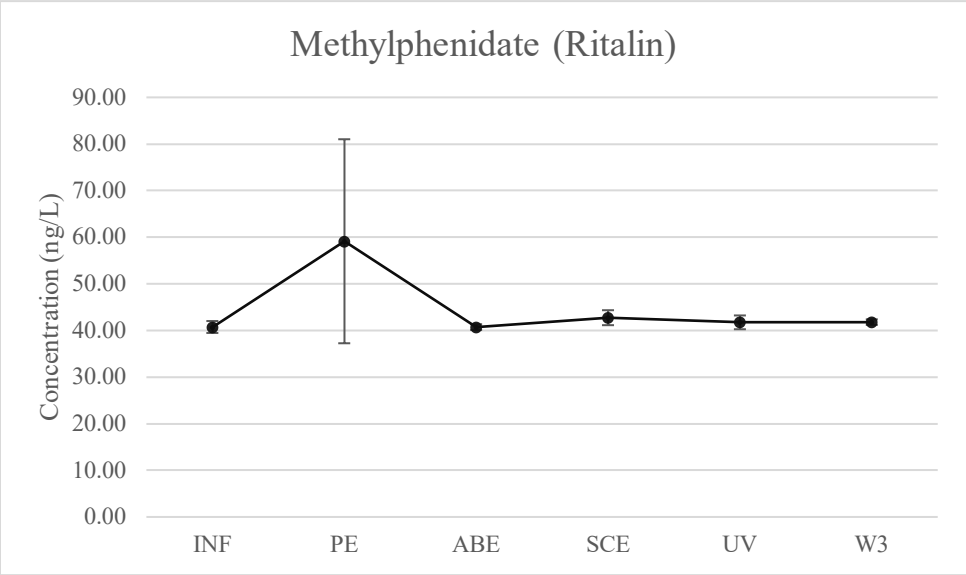

Figure 2.6 Methylphenidate concentration across the LSWRF

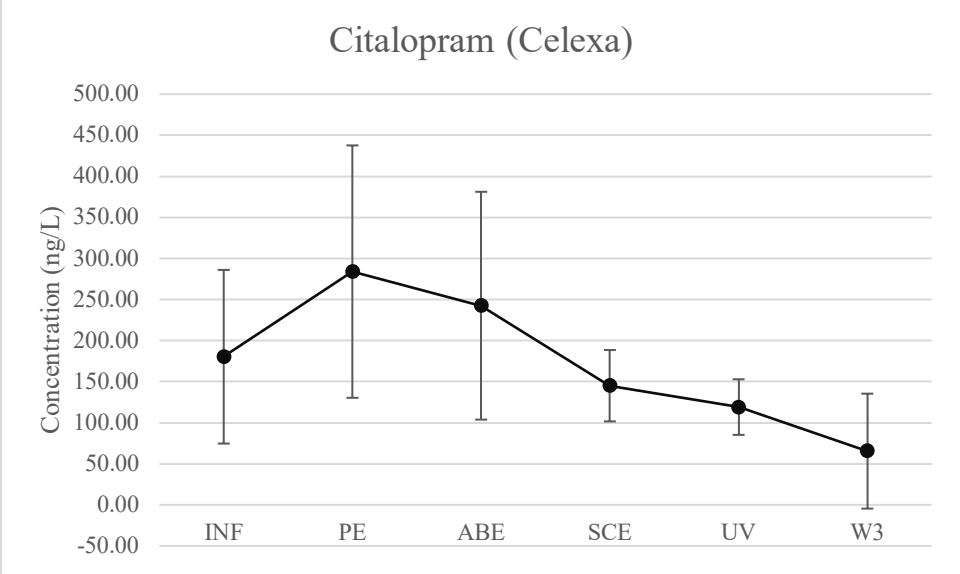

Figure 2.7 Citalopram concentration across the LSWRF

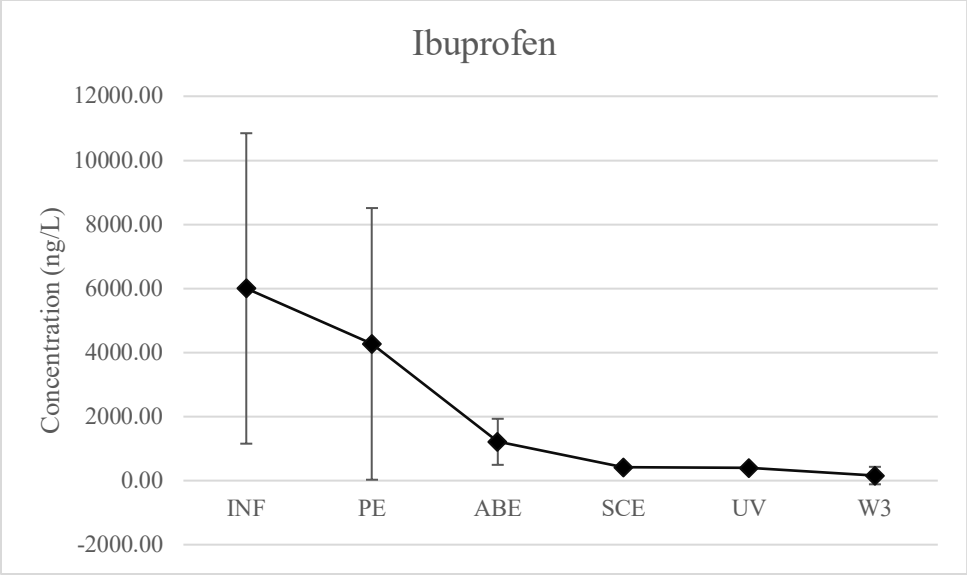

Figure 2.8 Ibuprofen concentration across the LSWRF

To better understand the potential impact the average concentrations were converted into pounds per year based on an average flow of 11 million gallons per day (Hartman, 2021) and are presented in the table below.

Table 2.3 Pounds per year at the LSWRF

	Methylphenidate (lbs/year)	Citalopram (lbs/year)	Ibuprofen (lbs/year)
INF	1	5	204
PE	2	10	145
ABE	1	8	41
SCE	2	5	14
UV	1	4	14
W3	1	2	5

Representing this graphically, the figure below shows the various loadings at each of the sampling locations.

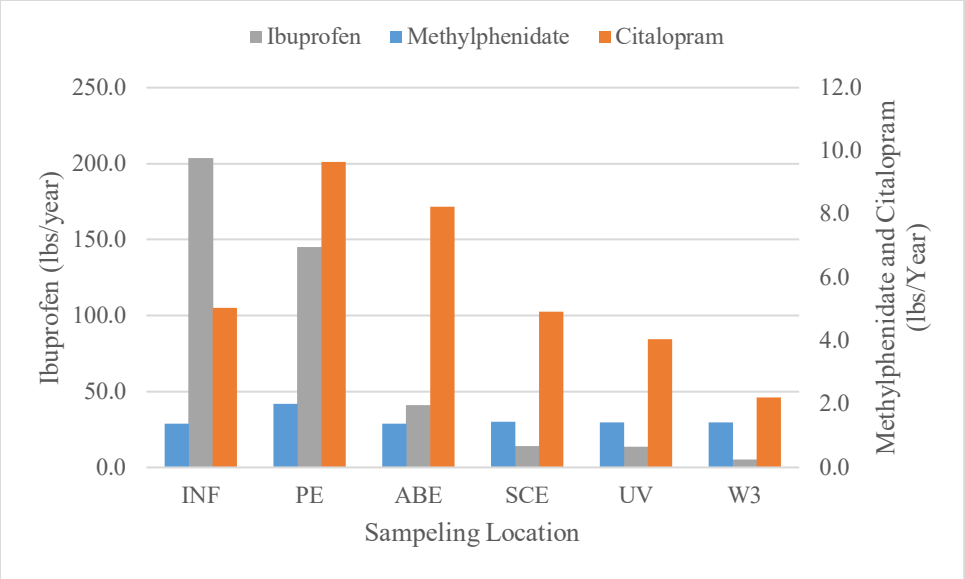

Figure 2.9 EC loading in pounds per year across the LSWRF

Observations

Ibuprofen showed a 97% decrease across the LSWRF, citalopram showed a 56% decrease while methylphenidate effectively showed no change in concentration across the facility.

The data reveals that ECs are present at the LSWRF. This is not unexpected, given the prevalence of their consumption in the United States (Martin et al., 2019). All three of the ECs tested in this project behaved differently. Each has a unique incoming concentration and appears to react to the different water renewal processes in different ways. Comparing studies is difficult due to the varied nature of the incoming wastewater and the unique characteristics of the water renewal facilities themselves.

Variation in the data was noted and is presented in Appendix C. Variation also enters the study due to the range of compounds present in wastewater, from hydrocarbons to solvents to ECs (Gardner et al., 2012). Each of these substances has the potential to alter the results through their unknown effects on ECs.

CHAPTER 3 CONCLUSIONS AND RECOMMENDATIONS

Emerging Constituents (ECs) are prevalent in the products Americans consume. Products like soaps, perfumes, clothing waterproofing, non-stick cookware, and pharmaceuticals all contain ECs that have impacts on water systems (United States Environmental Protection Agency, 2020). Research has shown adverse effects such as male fish growing eggs within their reproductive organs (Konkel, 2016). It has also been shown that bioaccumulation has the potential to increase concentrations of ECs in areas where they are not directly discharged into (O'Toole et al., 2006).

Limited research has been performed on the effectiveness of water renewal facilities to treat ECs, but it appears that the effectiveness of the process is dependent on the EC itself (Asimakopoulos et al., 2017). The process technology of ozonation has shown to be mildly effective to treat ECs, but its full-scale application is not yet fully understood (Rodayan et al., 2013).

This project focused on the effectiveness of various treatment processes at a conventional activated sludge water renewal facility. The LSWRF data showed several key trends that correspond to the literature review:

1. Some ECs are shown to have a decreasing concentration gradient across the facility while others do not. This may indicate that treatment effectiveness is dependent on the EC itself.
2. Concentrations were like those found in other studies.
3. Certain process units can affect EC concentrations.

The research completed at LSWRF revealed certain levels of treatment for ECs at certain process units within the facility. One trend was the increase in concentration of methylphenidate and citalopram at the primary effluent. The LSWRF has an internal recycle stream in the facility's drain system. The facility drain is introduced into the flow path downstream of the influent sampling point but prior to the primary clarifiers. It consists of various process drains, stormwater, and restrooms throughout the facility. There were no storm events during sampling so stormwater effects can be ruled out. One of the process drains is from the gravity belt thickener (GBT) where waste activated sludge (WAS) from the secondary clarifier sludge is thickened prior to entering the digester. This is the likely source of the concentration spike at the primary clarifier effluent sampling point. As a part of the GBT process the WAS is thickened using polymer and a drain system where the sludge goes from approximately 1% solids to about 5% solids. The excess water from this process is sent down the facility drain to be treated with the rest of the water. It is common to see concentration spikes in the primary clarifiers for other monitored nutrients such as phosphorus (Hartman, 2021). It follows that some of the EC concentration removed between the aeration basin effluent and secondary clarifier effluent sampling points is sent back to the headworks as a recycle. Examining the data for citalopram, the average influent concentration is only slightly higher than the secondary clarifier concentration. This indicates that a large portion, if not all of the citalopram being removed in the secondary system is being recycled to the head of the facility.

In contradiction to this result, ibuprofen shows a decline across every treatment process. The drop in concentration between influent and primary effluent indicate that

settling of the ibuprofen is taking place within the primary clarifiers and is entering the digesters through primary sludge removal. Decline in concentration between the primary effluent and aeration basin influent indicate that some fraction of ibuprofen is being removed and sequestered in the biological process. The biological process involves the rapid increase in concentration of microorganisms by creating an optimal environment for them to grow (Metcalf & Eddy, 1999). This process does involve the addition of a base to increase the pH of the wastewater stream. After discussions with City of Boise operations, it was learned that sodium hydroxide (caustic) is added to the basin to raise the basin influent from 7 to 7.5. It is not anticipated this has a significant effect on ECs since the pH change is so small. Certain facilities can also add sodium hypochlorite to control the growth of filaments in the return activated sludge (RAS) system. RAS and WAS are both secondary sludge from the bottom of the secondary clarifiers. It was verified with LSWRF operations that there was no addition of sodium hypochlorite during the sampling period for this project. This leaves the decrease between primary effluent and aeration basin effluent concentration likely due to the sequestration of ibuprofen into the biomass that is removed to the digesters in WAS.

All the ECs measured showed a decrease across the UV system. methylphenidate and ibuprofen showed very slight differences in the average concentrations, indicating no real effect on the concentrations for these ECs. The required dosage for water renewal facilities for pathogen reduction is 35 mj/cm^2 (Metcalf & Eddy, 1999). This is a small dose compared to the Title 22 reuse requirement of 100mj/cm^2 (State of California, 2018). The LSWRF dose varies and is higher than the 35 mj/cm^2 (Hartman, 2021). It is unknown if a higher dose of UV would be effective in the removal of ECs. Citalopram

did have a reduction in concentration across the UV, indicating that the UV system influenced the concentration. Destruction of ECs within the UV system may be due to the radioactive energy being imparted into the water that would cause the destruction of the chemical bonds that hold ECs together (Sgroi, Anumol, Vagliasindi, Snyder, & Roccaro, 2021). The process of EC transformation is a target for more research to understand. It is unclear what the breaking of the chemical bonds does and what the components of the ECs form into.

Citalopram and ibuprofen did show a decrease in concentration from the UV to W3 set. W3 is facility utility water that is dosed with sodium hypochlorite. This water is used across the facility for wash water, spray bar water, irrigation within the facility boundary and other miscellaneous uses. The sodium hypochlorite dose is minimal and only used to prevent algae growth. It appears that chlorine injection influences the concentration of some ECs. This is likely due to the destruction of the chemical bonds with the addition of the sodium hypochlorite (Cerreta, Roccamante, Oller, Malato, & Rizzo, 2019).

This research shows that the removal efficiency of ECs within water renewal facilities is dependent on the EC itself and the process units present at the facility. Each water renewal facility is unique both in influent and in treatment. The research questions studied in this project and their brief answer are presented below:

1. What is the fate and transport of ECs within water renewal facilities?
    a. This is dependent on the EC itself and the treatment processes of the water renewal facility. It appears that ECs that are removed go

into the solids systems within the facility. The potential transformation of ECs needs to be further studied.

2. Are there specific unit processes that appear to have an impact on the concentrations of ECs within water renewal facilities?
    a. Based on this project's data, it appears that settling, biological processes and destructive methods (UV and chlorine addition) all can influence EC concentrations. This is dependent on the EC itself, but it appears that conventional water renewal facilities can influence some ECs.
    b. A statistical analysis is presented in Appendix D that outlines the various confidence intervals to support the assertion that settling is influencing the concentrations of ECs within the water renewal facility. The confidence interval that sedimentation has a reducing effect on EC concentration across the secondary clarifier is:
        i. Ibuprofen: 87%
        ii. Citalopram: 90%
        iii. Methylphenidate: 85%

These are then followed by two hypotheses:

1. Certain ECs will be treated within existing water renewal facilities.
    a. Confirmed: ibuprofen showed a reduction in concentration across the facility. The amount of treatment is dependent on the treatment process and the EC.

2. Settling will be the primary method of removal for ECs within water renewal facilities.
    a. Settling does play a role in the removal of ECs within a water renewal facility. Other treatment processes have an effect such as chlorine UV radiation and chlorine injection.

Recommendations

The mass balance for the LSWRF must be completed. This study did not determine concentrations within the solids system of the facility. A more complete understanding of the treatment of ECs will come when the concentrations in the solids system can be determined. A reliable means of sampling solids will need to be determined and then the results analyzed with the liquids stream data.

Samples should be taken over a greater time to better understand the trends in the data in future studies. This project's budget was limited by the grant funding and the research relied on an expensive testing method. Additional samples would have diminished concerns over data quality and helped to reduce the variability in data from an active water renewal facility. Future studies will continue to have varied data due to the unique environment of an active water renewal facility.

Lab based studies with known EC concentrations and modeled process units would be an important step to understand which process units have the most effect. Water renewal facilities are unique and subject to rapid changes in concentrations without warning. These factors make sampling and deciphering data difficult. Modeling process units in a lab to understand the effect of each will be important. Other substances in the

wastewater influence the treatment of ECs making treatment effectiveness difficult to compute (Roayan et al., 2013).

This project was an applied research endeavor. Its purpose is to instruct the reader on the treatment of ECs within a functioning water renewal facility. For this work to be continued in the future a more reliable and fast means of determining EC concentrations and treatments needs to be developed that can be run by any municipal lab in the country. Operations staff and laboratory staff work very closely to determine the effectiveness of water renewal facility operation. Lab samples are taken every day and analyzed for the LSWRF at the City of Boise's Lab. These results inform operators how the facility is operating and any modifications they should make. Using sample preparation and LC/MS, as performed for this project, is not a feasible long-term way to determine EC concentration in water renewal facilities. Finding indicator compounds or rapid tests for ECs will be critical to implementing real EC limits in permits.

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APPENDIX A

Results Data Table

Table A.1 Results Data Table

Sample Name	Sample Collection Date	Acq. Date-Time	Methylphenidate		Citalopram		Ibuprofen	
			RT (min)	Final Conc. (ng/ml)	RT (min)	Final Conc. (ng/ml)	RT (min)	Final Conc. (ng/ml)
INF-1	4/22/2021	10/29/2021 18:48	2.18	1.01	2.93	2.70	4.48	34.67
INF-3	4/22/2021	10/29/2021 21:16	2.18	1.01	2.93	2.77	4.48	18.67
PE-5	4/22/2021	10/29/2021 22:09	2.18	2.07	2.93	9.52	4.48	52.86
ABE-7	4/22/2021	10/29/2021 20:44	2.18	1.02	2.93	2.26	4.48	36.64
SCE-9	4/22/2021	10/29/2021 21:48	2.18	1.11	2.93	3.38	4.48	9.84
UV-11	4/22/2021	10/29/2021 22:41	2.18	1.08	2.93	3.20	4.53	9.98
W3-13	4/22/2021	10/29/2021 21:58	2.18	1.06	2.93	1.42		ND
BLNK-19	4/22/2021	10/29/2021 19:09	2.18	0.92		ND		ND
INF-21	7/24/2021	10/29/2021 21:26	2.18	0.98	2.93	3.29	4.48	277.47
INF-22	7/24/2021	10/29/2021 22:51	2.18	1.06	2.93	7.27	4.48	160.43
PE-23	7/24/2021	10/29/2021 23:12	2.18	1.00	2.93	2.67	4.48	38.42
ABE-24	7/24/2021	10/29/2021 20:12	2.17	1.00	2.93	6.87	4.48	44.21
SLE-25	7/24/2021	10/29/2021 19:30	2.18	1.03	2.93	2.68	4.50	9.66
UV-26	7/24/2021	10/29/2021 20:02	2.18	1.01	2.93	2.04	4.48	9.89
W3-27	7/24/2021	10/29/2021 19:41	2.18	1.04		ND		ND

INF-31	8/26/2021	10/29/2021 20:55	2.18	1.04	2.93	2.55	4.48	258.70
INF-32	8/26/2021	10/29/2021 18:58	2.18	1.26	2.93	8.47	4.48	747.05
PE-33	8/26/2021	10/29/2021 23:33	2.18	1.37	2.93	9.11	4.48	228.86
ABE-34	8/26/2021	10/29/2021 21:37	2.18	1.03	2.93	9.05	4.48	9.98
SLE-35	8/26/2021	10/29/2021 19:20	2.18	1.07	2.93	4.81	4.48	11.18
UV-36	8/26/2021	10/29/2021 23:55	2.18	1.03	2.93	3.69	4.48	9.96
W3-37	8/26/2021	10/29/2021 22:19	2.18	1.03	2.93	3.48	4.46	11.81
BLNK-30	8/26/2022	10/29/2021 21:05	2.18	0.92		ND		ND

APPENDIX B

Sample Preparation Data Table

The information presented in the table below shows all the data that was collected on each sample during preparation. Not every prepared sample was analyzed through the LC/MS. Some duplicate samples were held in reserve if the MS had an issue running the samples. The sample numbers outlined in the first column of Appendix A show all the samples that were run through the MS while the table in this appendix show all the samples that were collected and prepared.

Table B.1 Sample Preparation Data Table

Sample Type	Date of Sample Collection	Date of sample preparation	Sample Number	Sample Location	Sample Volume	Sample pH	Methanol Dilution 1 volume (mL)	Pre-rotovap volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate volume(mL)	Pre N2 volume (mL)	Post N2 Volume (mL)
Influent 1	4/22/2021	6/12/2021	1	INF	25	2.6	25	50	5	10	15	0.5
Influent 1	4/22/2021	6/12/2021	2	INF	25	2.6	25	50	4.3	10	14.3	0.48
Influent 2	4/22/2021	7/3/2021	3	INF	25	2.57	25	50	2.9	10	12.9	0.5
Influent 3	4/22/2021	7/3/2021	4	INF	25	2.56	25	50	4.39	10	14.39	0.45
Primary effluent 1	4/22/2021	7/3/2021	5	PE	25	2.66	25	50	4	10	14	0.3
Primary Effluent 2	4/22/2021	7/3/2021	6	PE	25	2.54	25	50	4.6	10	14.6	0.5
Aeration Basin Effluent 1	4/22/2021	7/3/2021	7	ABE	25	2.47	25	50	4.8	10	14.8	0.5
Aeration Basin Effluent 2	4/22/2021	7/3/2021	8	ABE	25	2.46	25	50	2.8	10	12.8	0.3
Secondary Clarifier Eff 1	4/22/2021	7/3/2021	9	SCE	25	2.48	25	50	3.49	10	13.49	0.5

Secondary Clarifier Eff 2	4/22/2021	7/3/2021	10	SCE	25	2.48	25	50	2.7	10	12.7	0.5
UV Disinfection Effluent 1	4/22/2021	7/3/2021	11	UV	25	2.49	25	50	3.9	10	13.9	0.4
UV Disinfection Effluent 2	4/22/2021	7/3/2021	12	UV	25	2.48	25	50	4.9	10	14.9	0.5
W3 1	4/22/2021	7/3/2021	13	W3	25	2.46	25	50	4.4	10	14.4	0.5
W3 2	4/22/2021	7/3/2021	14	W3	25	2.43	25	50	4.5	10	14.5	0.4
Upstream 1	4/22/2021	7/3/2021	15	URV	25	2.4	25	50	3.6	10	13.6	0.5
Upstream 2	4/22/2021	7/3/2021	16	URV	25	2.6	25	50	3.8	10	13.8	0.45
DownStream 1	4/22/2021	7/3/2021	17	DRV	25	2.41	25	50	3.7	10	13.7	0.4
Downstreatm 2	4/22/2021	7/3/2021	18	DRV	25	2.5	25	50	3.2	10	13.2	0.4
Blank DI	7/3/2021	7/3/2021	19	BLNK	25	2.59	25	50	4.3	10	14.3	0.4
Blank DI	7/24/2021	8/14/2021	20	BLNK	25	2.5	25	50	3.3	10	13.3	0.5
Influent 1	7/24/2021	8/14/2021	21	INF	25	2.59	25	50	4.1	10	14.1	0.55
Influent 2	7/24/2021	8/14/2021	22	INF	25	2.56	25	50	4.1	10	14.1	0.52
Primary Effluent	7/24/2021	8/14/2021	23	PE	25	2.54	25	50	4.4	10	14.4	0.45

Aeration Basein Effluent	7/24/2021	8/14/2021	24	ABE	25	2.62	25	50	3.9	10	13.9	0.42
Secndary Clarifier Effluent	7/24/2021	8/14/2021	25	SCE	25	2.56	25	50	4.5	10	14.5	0.51
UV disinfection Effluent	7/24/2021	8/14/2021	26	UV	25	2.59	25	50	4	10	14	0.49
W3	7/24/2021	8/14/2021	27	W3	25	2.58	25	50	3.6	10	13.6	0.5
Upstream	7/24/2021	8/14/2021	28	URV	25	2.55	25	50	2.9	10	12.9	0.42
DownStream	7/24/2021	8/14/2021	29	DRV	25	2.54	25	50	3.5	10	13.5	0.4
Blank DI	8/26/2021	9/3/2021	30	BLNK	25	2.53	25	50	2.5	10	12.5	0.5
Influent 1	8/26/2021	9/3/2021	31	INF	25	2.53	25	50	5	10	15	0.5
Influent 2	8/26/2021	9/3/2021	32	INF	25	2.42	25	50	4.8	10	14.8	0.49
Primary Effluent	8/26/2021	9/3/2021	33	PE	25	2.56	25	50	4.9	10	14.9	0.5
Aeration Basein Effluent	8/26/2021	9/3/2021	34	ABE	25	2.49	25	50	3.3	10	13.3	0.5

Secndary Clarifier Effluent	8/26/2021	9/3/2021	35	SCE	25	2.42	25	50	3.8	10	13.8	0.41
UV disinfection Effluent	8/26/2021	9/3/2021	36	UV	25	2.53	25	50	3.5	10	13.5	0.5
W3	8/26/2021	9/3/2021	37	W3	25	2.56	25	50	3.9	10	13.9	0.5
Upstream	8/26/2021	9/3/2021	38	URV	25	2.55	25	50	3.1	10	13.1	0.49
DownStream	8/26/2021	9/3/2021	39	DRV	25	2.53	25	50	3.7	10	13.7	0.43

APPENDIX C

Standard Deviation Results

Table C.1 Standard deviation calculations for all sample locations full data set

	Methylphenidate		Citalopram		Ibuprofen	
	Standard Deviation (ng/L)	Average (ng/L)	Standard Deviation (ng/L)	Average (ng/L)	Standard Deviation (ng/L)	Average (ng/L)
INF	4.15	42.37	119.77	180.32	10,670.17	9,980.00
PE	21.88	59.14	174.08	283.97	4,241.06	4,268.53
ABE	0.62	40.72	157.10	242.47	719.11	1211.13
SCE	1.60	42.74	49.20	145.05	33.17	409.15
UV	1.48	41.74	38.34	119.04	1.92	397.79
W3	0.60	41.79	79.27	65.38	272.64	157.41
Blank	0.13	36.89	0.00	0.00	0.00	0.00

Table C.2 Standard deviation calculations for all sample locations data set with outliers removed

	Methylphenidate		Citalopram		Ibuprofen	
	Average Concentration (ng/L)	Standard Deviation (ng/L)	Average Concentration (ng/L)	Standard Deviation (ng/L)	Average Concentration (ng/L)	Standard Deviation (ng/L)
INF	40.74	1.28	148.63	80.23	5,999.61	4,846.59
PE	59.14	21.88	283.97	153.66	4,268.53	4,241.06
ABE	40.72	0.62	242.47	138.67	1,211.13	719.11
SCE	42.74	1.60	145.05	43.43	409.15	33.17
UV	41.74	1.48	119.04	33.84	397.79	1.92
W3	41.79	0.60	65.38	69.97	157.41	272.64

Table C.3 Standard deviation calculations influent duplicates

Influent	Ibuprofen			
	S1	S2	Average	StDev
April	1,386.86	746.76	10,66.81	320.052
July	11,098.95	6,417.29	8,758.12	2,340.828
Aug	10,348.19	29,881.94	20,115.07	9,766.878
Influent	Citalopram			
	S1	S2	Average	StDev
April	108.14	110.78	109.46	1.322
July	131.54	290.76	211.15	79.612
Aug	101.92	338.78	220.35	118.432
Influent	Methylphenidate			
	S1	S2	Average	StDev
April	40.27	40.20	40.24	0.034
July	39.25	42.57	40.91	1.66
Aug	41.42	50.51	45.97	4.544

APPENDIX D

Statistical Analysis

Statistical calculations for standard deviations can be found in Appendix C. The sample set is small for this project due to the cost and complexity related to testing for ECs. It is not uncommon in the water renewal industry to have few samples to represent large populations. It also appears that the standard deviations are greater in the higher solids sampling locations such as the influent and primary effluent.

Outlier determination is also a common way to determine if wastewater data should be removed because it often varies due to unpredictable nature of the facilities. A quartile approach was used for this data set to determine outliers (Langford, 2006). The data and table calculations for this can be found in Appendix D. First, the first and third quartiles were calculated, Q_1 and Q_3 respectively, using the following Excel function:

=Quartile.inc(range,quart)

Second, the interquartile range (IQR) was calculated by subtracting Q_1 from Q_3. Third the lower limit was calculated as follows:

$=Q_1 - (1.5 \times IQR)$

This represents the lower limit of the data that would be considered a non-outlier. Lastly the upper limit of the data that would be considered a non-outlier was calculated as follows:

$=Q_3 + (1.5 \times IQR)$

Data for each constituent and each sampling point was examined to see which, if any, would be considered outliers once the upper and lower limits were determined. Note that the sample had to exceed 1.5 times the interquartile range to be considered as an outlier. Only two values were determined to be outliers. Sample INF – 32 showed outlier values for the methylphenidate and ibuprofen. Both values failed on the upper limit.

Because the value was for the same sample, and the citalopram value was also the highest of all the influent (although the citalopram value was below the upper limit threshold) INF -32 was thrown out as an outlier completely.

Corrected Data

The following table outlines both the complete dataset average calculations and the average calculations with the outliers removed.

Table D.1 Average concentration of ECs both corrected and complete data for the LSWRF at sampling locations.

	Methylphenidate (all data)(ng/L)	Methylphenidate (outlier removed)(ng/L)	Citalopram (all data)(ng/L)	Citalopram (outlier removed)(ng/L)	Ibuprofen (all data)(ng/L)	Ibuprofen (outlier removed)(ng/L)
INF	42.37	40.74	180.32	148.63	9,980.00	5,999.61
PE	59.14	59.14	283.97	283.97	4,268.53	4,268.53
ABE	40.72	40.72	242.47	242.47	1,211.13	1,211.13
SCE	42.74	42.74	145.05	145.05	409.15	409.15
UV	41.74	41.74	119.04	119.04	397.79	397.79
W3	41.79	41.79	65.38	65.38	157.41	157.41

Only the influent values were affected by the outlier data. Calculations for the standard deviation of the corrected data can be found in Appendix C. The following figures outline the influent gradients across the LSWRF with the outlier data removed.

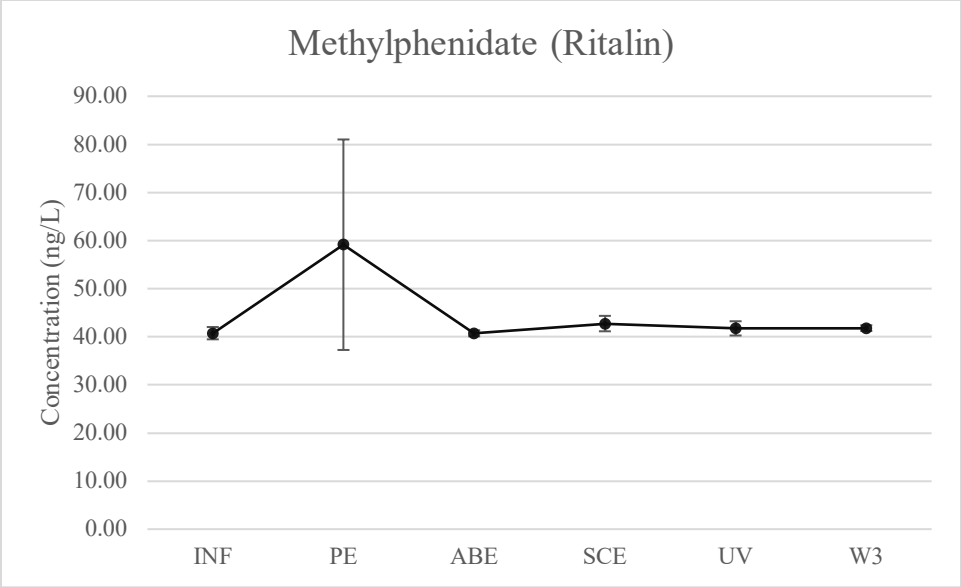

Figure D.1 Methylphenidate concentration across the LSWRF with outlier data removed

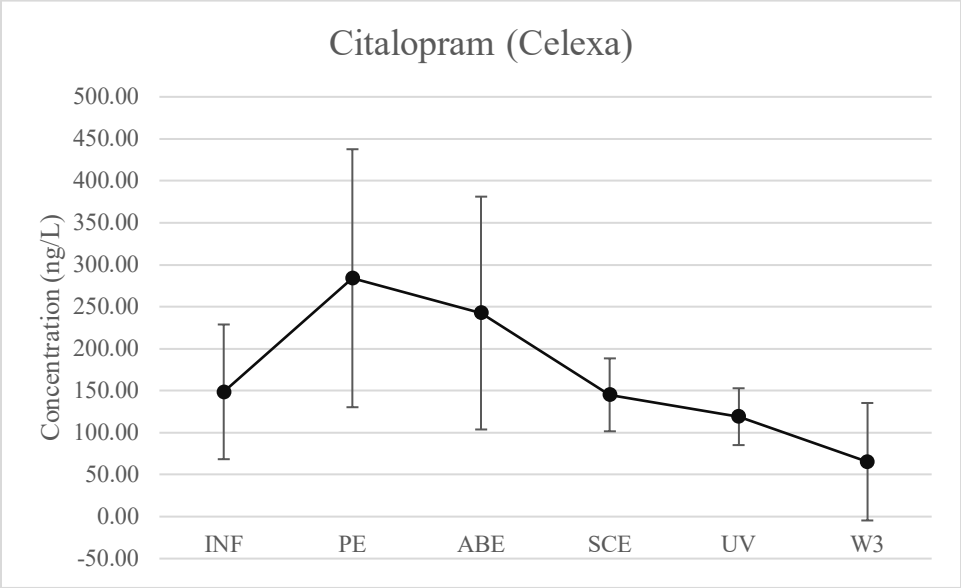

Figure D.2 Citalopram concentration across the LSWRF with outlier data removed

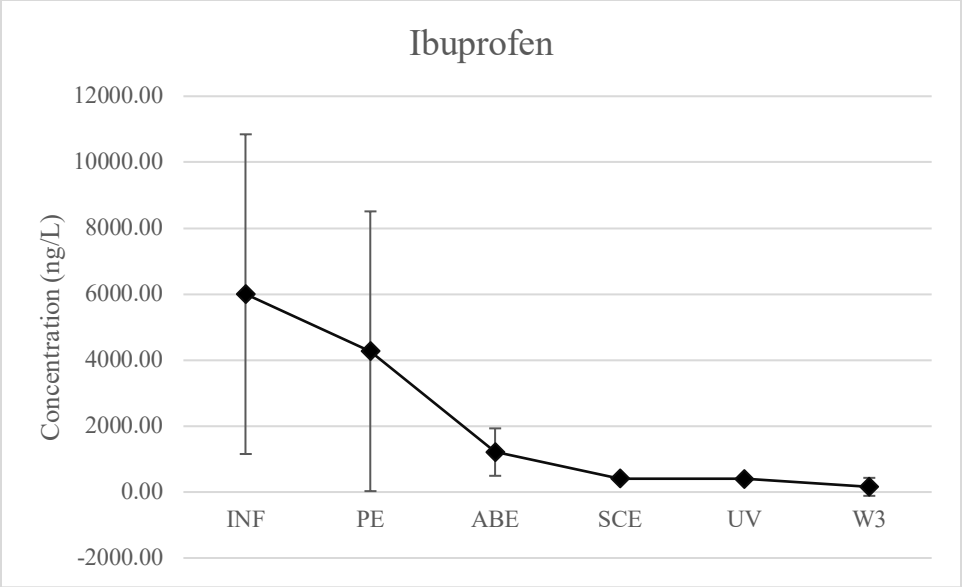

Figure D.3 Ibuprofen concentration across the LSWRF with outlier data removed

Error bars for these values are based on the standard deviation of the data collected. Error bars are large, but the general trend is still seen even if the concentration varies. The general trend for all the ECs examined also holds with the error bars considered. Error is likely introduced into this data set due to the limited number of grab samples and the characteristics of water renewal facilities. The following tables are taken from excel and show the statistical analysis and results.

Table D.2 Table showing calculations of upper and lower limit for outlier determination

Ibuprofen					
	Q1	Q3	IQR	Lower limit	Upper Limit
INF	2,644.47	10,911.26	8,266.79	-9,755.717	23,311.443
PE	1,825.63	5,634.35	3,808.72	-3,887.456	1,1347.44
ABE	932.49	1,617.00	684.51	-94.278	2643.77
SCE	390.12	420.51	30.39	344.531	466.091
UV	397.04	398.87	1.83	394.297	401.617
W3	0.00	236.11	236.11	-354.16793	590.27999
Citalopram					
	Q1	Q3	IQR	Lower limit	Upper Limit
INF	108.80	250.95	142.16	-104.4385	464.1895
PE	235.65	372.56	136.91	30.278	577.926
ABE	182.69	318.48	135.79	-20.987	522.157
SCE	121.30	163.89	42.59	57.412	227.78

UV	104.81	137.75	32.94	55.395	187.163
W3	28.48	98.07	69.59	-75.90495	202.45497
Methylphenidate					
	Q1	Q3	IQR	Lower limit	Upper Limit
INF	40.22	42.29	2.06	37.125	45.381
PE	47.26	68.80	21.54	14.946	101.106
ABE	40.39	41.01	0.62	39.468	41.932
SCE	41.93	43.53	1.60	39.522	45.938
UV	40.93	42.38	1.45	38.761	44.545
W3	41.47	42.06	0.59	40.584	42.952

Table D.3 Table showing calculations of upper and lower limit for outlier determination

Sample	Final Conc. (ng/L)	Sample	Final Conc. (ng/L)	Sample	Final Conc. (ng/L)	Ibuprofen Outliers	Citalopram Outliers	Methylphenidate Outliers
ABE-7	40.644	ABE-7	90.44	ABE-7	1465.58	FALSE	FALSE	FALSE
ABE-24	40.140	ABE-24	274.94	ABE-24	1,768.42	FALSE	FALSE	FALSE
ABE-34	41.372	ABE-34	362.02	ABE-34	399.40	FALSE	FALSE	FALSE
INF-1	40.272	INF-1	108.14	INF-1	1,386.86	FALSE	FALSE	FALSE
INF-3	40.204	INF-3	110.78	INF-3	746.76	FALSE	FALSE	FALSE
INF-21	39.252	INF-21	131.54	INF-21	11,098.95	FALSE	FALSE	FALSE
INF-22	42.572	INF-22	290.76	INF-22	6,417.29	FALSE	FALSE	FALSE
INF-31	41.424	INF-31	101.92	INF-31	10,348.19	FALSE	FALSE	FALSE
INF-32	50.512	INF-32	338.78	INF-32	29,881.94	TRUE	FALSE	TRUE
PE-5	82.900	PE-5	380.61	PE-5	2114.39	FALSE	FALSE	FALSE
PE-23	39.820	PE-23	106.78	PE-23	1,536.87	FALSE	FALSE	FALSE
PE-33	54.692	PE-33	364.51	PE-33	9,154.32	FALSE	FALSE	FALSE

SCE-9	44.368	SCE-9	135.24	SCE-9	393.80	FALSE	FALSE	FALSE
SLE-25	41.160	SLE-25	107.36	SLE-25	386.44	FALSE	FALSE	FALSE
SLE-35	42.696	SLE-35	192.54	SLE-35	447.22	FALSE	FALSE	FALSE
UV-11	43.372	UV-11	127.99	UV-11	399.27	FALSE	FALSE	FALSE
UV-26	40.480	UV-26	81.62	UV-26	395.61	FALSE	FALSE	FALSE
UV-36	41.380	UV-36	147.51	UV-36	398.47	FALSE	FALSE	FALSE
W3-13	42.428	W3-13	56.96	W3-13	0.00	FALSE	FALSE	FALSE
W3-27	41.700	W3-27	0.00	W3-27	0.00	FALSE	FALSE	FALSE
W3-37	41.244	W3-37	139.18	W3-37	472.22	FALSE	FALSE	FALSE

Methylphenidate

Methylphenidate blank values were not 0 ng/L as the other ECs measured but the value was lower than the other average concentrations measured. There are various reasons that this could have happened including QC issues, instrument variation/contamination and/or detection interference. The same QC procedures were followed for all samples and the same blank samples showed 0 ng/L values for ibuprofen and citalopram. This indicates that there is likely interference in the instrument with the detection of methylphenidate. Dr. Xinzhu Pu agreed that there was likely detection of methylphenidate but likely there was some interference for the instrument. The blank sample was approximately 10% lower than the average incoming concentration indicating there still was detection of methylphenidate at the LSWRF.

Statistical Significance

A t-test was utilized to test the hypothesis that settling did influence the concentrations of ECs. Values from the primary clarifier effluent and the secondary clarifier effluent were considered because these are the points during the treatment process at which liquids are separated from solid through settling. Primary performance was not determined because the gravity belt underflow concentrations were not known. A one tail t-test was performed looking for the decrease in concentration of the EC across the secondary clarifier. All the tests below were completed using the excel t-test function. The following table outlines the t-test for ibuprofen:

Table D.4 Ibuprofen t-test results

	PCE	SCE
Mean	4268.527	409.1493
Variance	17986614	1100.346
Observations	3	3
Pearson Correlation	0.999074	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.588584	
P(T<=t) one-tail	0.126545	
t Critical one-tail	1.06066	

The date presented in the above table indicates that there is approximately an 87% confidence that settling will have a reducing effect on the concentration of ibuprofen.

The following table outlines the t-test for Citalopram:

Table D.5 Citalopram t-test results

	PCE	SCE
Mean	283.9667	145.048
Variance	23610.08	1886.226
Observations	3	3
Pearson Correlation	0.715919	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.905733	
P(T<=t) one-tail	0.09848	
t Critical one-tail	1.06066	

The date presented in the above table indicates that there is approximately an 90% confidence that settling will have a reducing effect on the concentration of citalopram.

The following table outlines the t-test for Methylphenidate:

Table D.6 Methylphenidate t-test results

	PCE	SCE
Mean	59.13733	42.74133
Variance	478.7923	2.574357
Observations	3	3
Pearson Correlation	0.988411	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.399165	
P(T<=t) one-tail	0.148343	
t Critical one-tail	1.06066	

The date presented in the above table indicates that there is approximately an 85% confidence that settling will have a reducing effect on the concentration of citalopram.

APPENDIX E

LC-MS Procedures and QC

LC-MS Settings

LC-MS/MS analysis was performed using an Agilent 1290 Infinity II LC consisting of an Agilent 1290 Infinity II multisampler (G7167B), an Agilent 1290 Infinity II high speed pump (G7120A), and an Agilent 1290 Infinity II multicolumn thermostat (G7116B) coupled to an Agilent 6470B triple quadrupole LC/MS system. Instrument control, data acquisition, qualitative and quantitative data analysis, and reporting were done using Agilent MassHunter workstation software. Dihydrocarbamazepine was used as an internal standard for quantification of citalopram, ibuprofen, and methylphenidate. The following tables outline the settings and conditions of the LC/MS analysis

Table E.1 Chromatographic Conditions

Parameter	**Setting**
Guard Column	Agilent ZORBAX RRHD StableBond Aq, 2.1 mm, 1.8 µm
Analytical Column	Agilent ZORBAX RRHD StableBond Aq, 2.1 x 100 mm, 1.8 µm
Column Oven	40 ±2 °C
Injection Volume	5 µL
Run Time	10 minutes
Autosampler Temperature	12 ±2 °C
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile

Table E.2 Gradient Settings

Time (min)	Flow (mL/min)	%A	%B
0.0	0.40	90	10
5.0	0.40	20	80
6.0	0.40	20	80
7.0	0.40	90	10
10.0	0.40	90	10

Table E.3 MS Parameters

Parameter	Setting
MS Acquisition	dMRM
Ion Source Type	Agilent Jet Stream electrospray ionization
Drying Gas Temperature	350 °C
Drying Gas Flow	10 L/min
Nebulizer	45 psi
Sheath Gas Heater	400 °C
Sheath Gas Flow	11 L/min
Capillary	4,000 V
Nozzle Voltage	0 V
Precursor Ion and Production Ion Resolution	Unit

Table E.4 Compound-Specific Conditions: precursor-to-product ion transitions, fragmentor, collision energies (CE), cell accelerator voltage (CAV), and retention times (RT).

Compound	Polarity	Precursor Ion (m/z)	Product Ion (m/z)	RT (min)	Fragmentor (V)	CE (V)	CAV (V)
Citalopram	Positive	325.2	109.0	2.90	131	24	5
Dihydrocarbamazepine	Positive	239.1	194.0	3.45	126	28	5
Ibuprofen	Negative	205.1	161.1	4.50	67	4	5
Methylphenidate	Positive	234.2	84.1	2.20	109	32	5

LC-MS QC/QA

The following figures show the calibration curves for each of the ECs that were considered in this study:

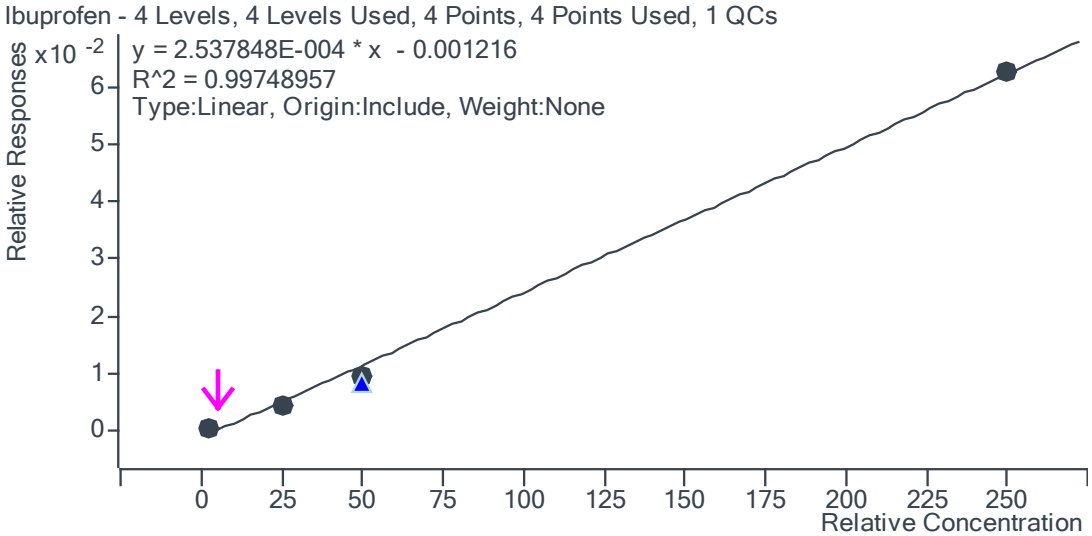

Figure E.1 Ibuprofen Calibration Curve

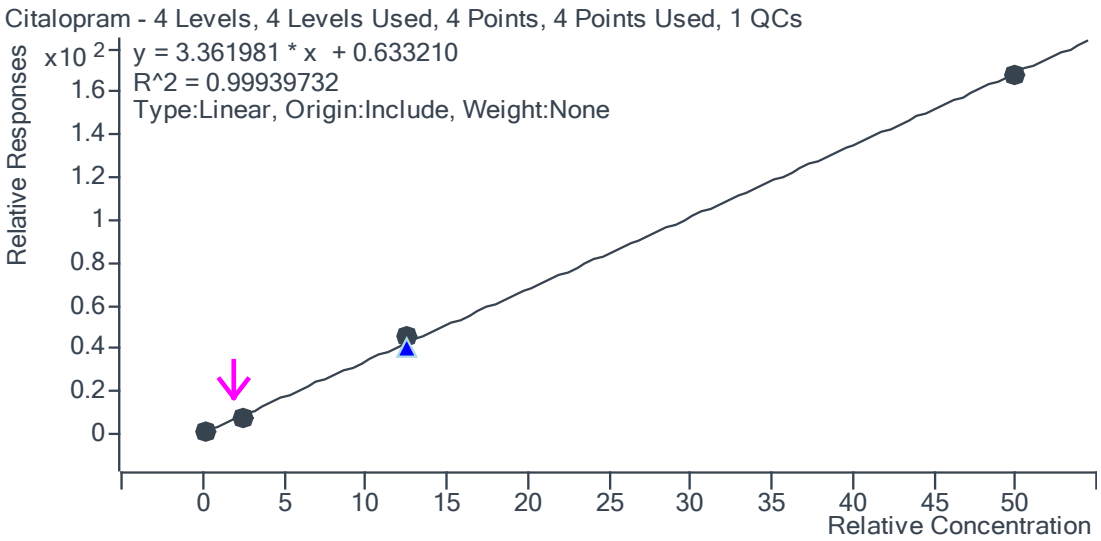

Figure E.2 Citalopram Calibration Curve

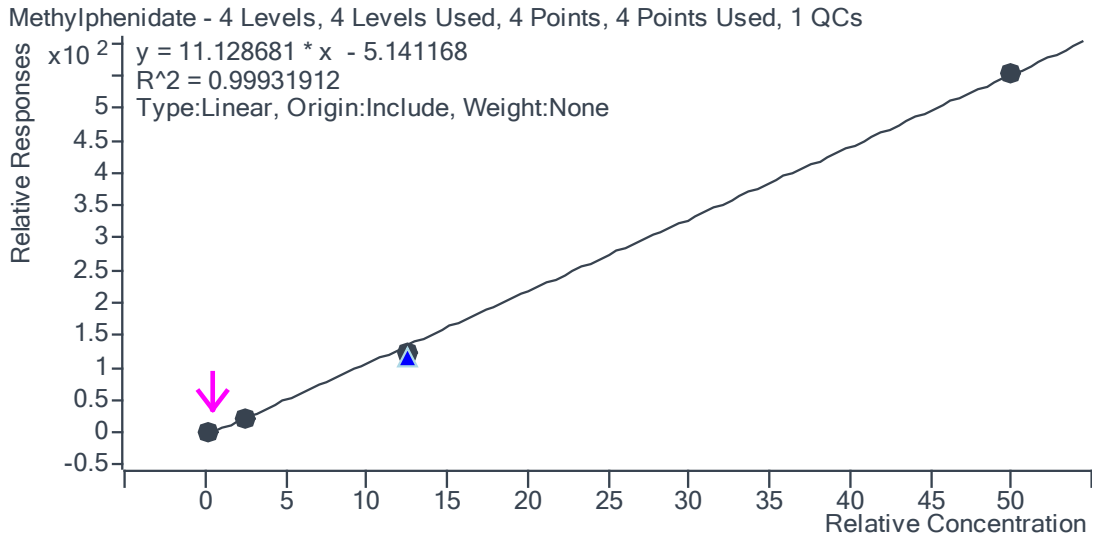

Figure E.3 Methylphenidate Calibration Curve

The following figures outline the standard chromatograms of each of the ECs that were used. These were generated using pure forms of the ECs:

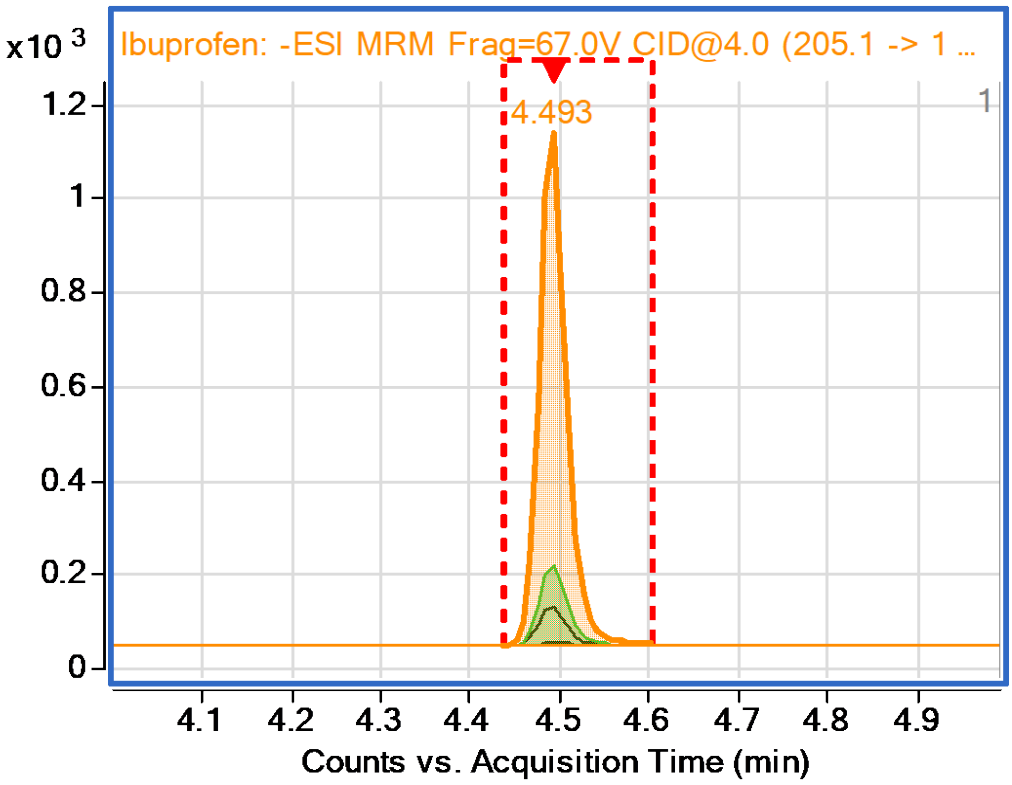

Figure E.4 Ibuprofen Standard Chromatogram

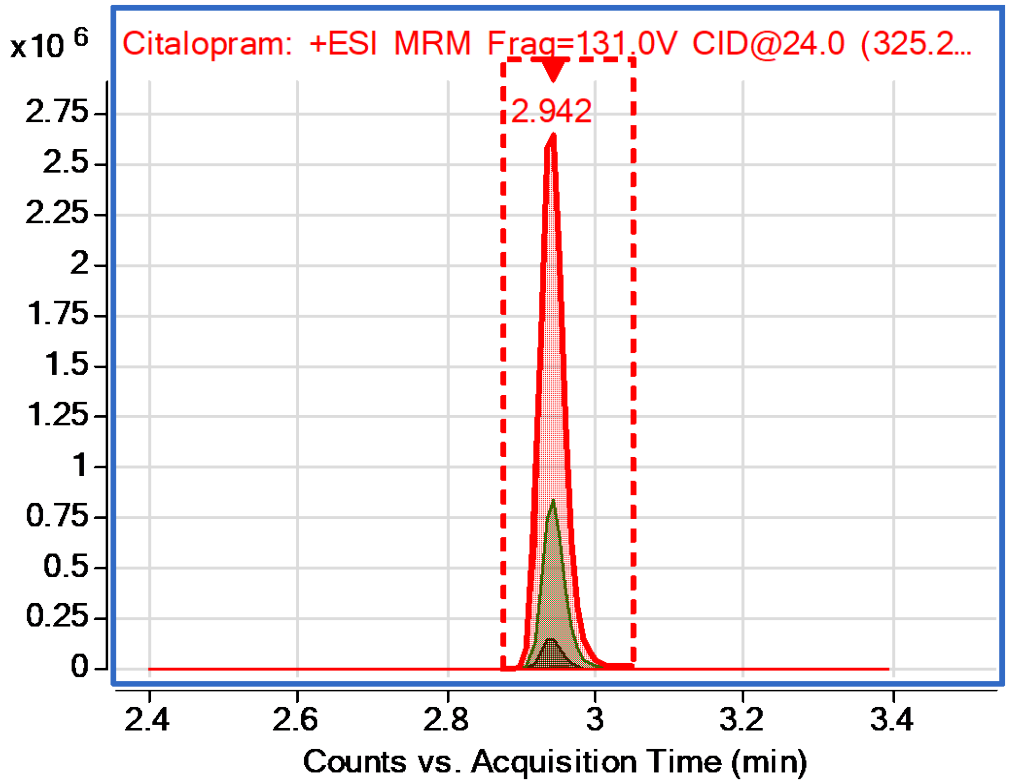

Figure E.5 Citalopram Standard Chromatogram

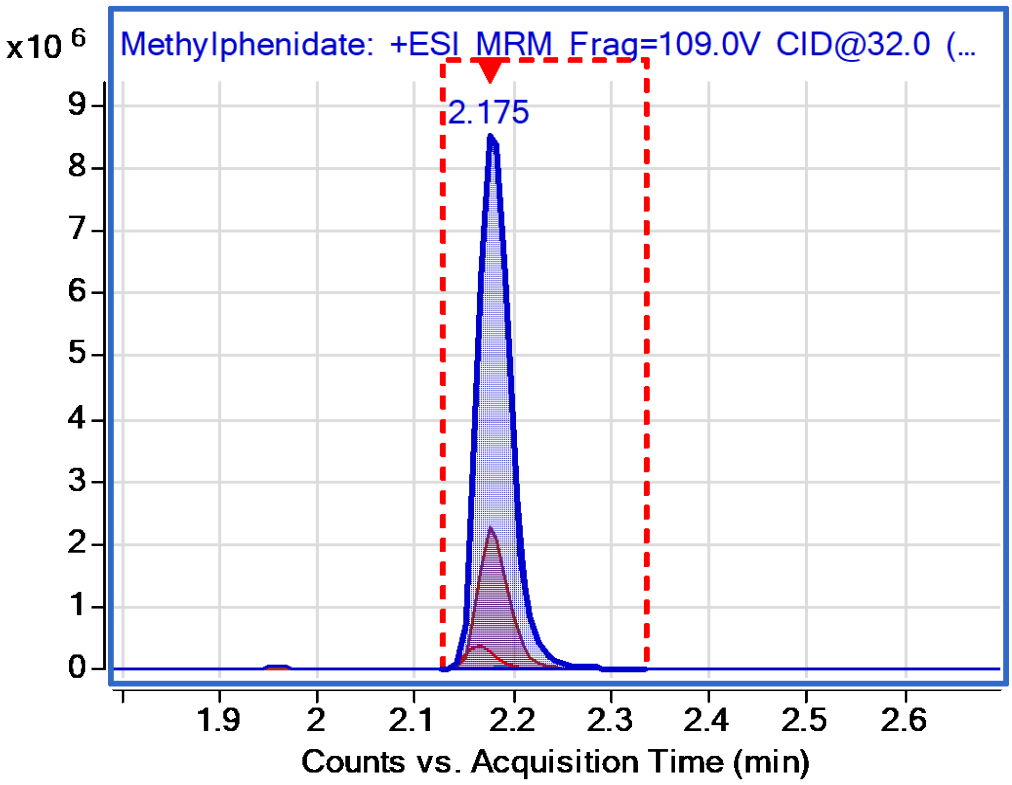

Figure E.6 Methylphenidate Standard Chromatogram

Pure forms of the ECs to be analyzed were procured and provided to the MS team. The units were calibrated to the standards. Blank solvent samples were also periodically run through the LC/MS devise to clean the sensor and reduce instrument drift.