ANALYSIS OF EMERGING CONSTITUENTS IN AN ACTIVATED SLUDGE WASTEWATER TREATMENT FACILITY

by

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DEDICATION

I want to dedicate this to my family. To my husband, Jon. To my parents, John and Linda. To Bethany and Carlos, Hannah and Sai, Joy, Ron and Deb, and Liz. To all my friends that are actually more like family. All of you have helped me get here today, and you should celebrate this accomplishment as your own because I could not have done it without you all.

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ABSTRACT

Emerging constituents (ECs), or contaminants of emerging concern (CECs), are pharmaceuticals and personal care products (Contaminants of Emerging Concern including Pharmaceuticals and Personal Care Products | US EPA, 2023). The primary source of ECs in surface waters is the discharge from wastewater treatment facilities (WWTF) (Kumar et al., 2022). Little is known about the effects treatment processes have on ECs. Research regarding ECs has increased due to the threats they pose to the environment and human health (Khan et al., 2021). This research aimed to expand understanding ECs' fate through an activated sludge WWTF. This was accomplished by studying concentrations present in the wastewater after each treatment step and testing for statistically significant removals and temporal variation. Eleven constituents from varying drug classes and personal care products were analyzed. The selected ECs were categorized into three groups (1) over-the-counter drugs and personal care products (acetaminophen, ibuprofen, and salicylic acid); (2) common prescription drugs (albuterol, cimetidine, methylphenidate, and theophylline); and (3) specialized prescription drugs (citalopram, primidone, sulfamethoxazole, and warfarin).

The sampling process was completed over six months, from February to July 2022, at the Lander Street Water Renewal Facility (LSWRF). This research is unique in that each step in the treatment process was analyzed, including the solids treatment process. EC concentrations were analyzed by liquid chromatography and mass spectrometry (LC/MS).

Three major trends in the liquid and solids results were identified: significant reductions, sequestering and release, and continuous release. Significant reductions were

exhibited by six constituents: acetaminophen, ibuprofen, salicylic acid, theophylline, and sulfamethoxazole. Reductions were primarily from the biological treatment, and the net reductions ranged from 90.7% to 99.8%. Sequestering and release were exhibited by three constituents (albuterol, cimetidine, and primidone) in that concentrations were reduced following biological treatment, increased after secondary clarification, and varying degrees of reduction through the remaining treatment processes. It is believed that microorganisms sequester and are unable to uptake constituents as they do with phosphorus. Subsequently, the microorganisms release constituents in the secondary clarifier. This resulted in minimal net reductions of the constituents ranging from 13.6% to 58.6%. The continuous release was exhibited by methylphenidate and citalopram from the influent to secondary clarification, which is believed to be due to parent compounds breaking down and releasing them into the system. The solids results did not mirror the liquid's and exhibited a continuous reduction across solids sample locations. This reduction is believed to be due to constituent break down to a metabolite or being biologically transformed into the liquid phase. Citalopram exhibited little net reduction (0.75%), and methylphenidate exhibited a 53.3% net increase. Warfarin was not detected in liquid or solids samples due to low LC/MS sensitivity.

Student’s t-tests of temporal variations found four ECs with significantly different liquid concentrations (albuterol, cimetidine, citalopram, and primidone and one EC with statistically different solids concentrations (acetaminophen). It was concluded that temporal variations in albuterol and acetaminophen were likely due to seasonal usage. It is difficult to conclude if the variation is due to wastewater variability or from the temporal variation with this limited dataset. There are no direct indicators for temporal variations of cimetidine,

citalopram, and primidone. It was concluded that the ECs' variation is due to the variability of the wastewater.

This research expanded the understanding of ten detected ECs in the LSWRF. It determined four constituents with significant reductions and identified ECs with increased concentration through the facility. Conclusions of temporal variations were also formulated. It is recommended that further research be conducted concerning ECs due to the threat they pose to the environment and human health despite this study answering the identified research questions.

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LIST OF ABBREVIATIONS

ABE	Aeration Basin Effluent
BLK	Blank Sample
BSU	Boise State University
DS	Digested Solids
DWN	Downstream Boise River Sample
EC	Emerging Constituent
EPA	Environmental Protection Agency
GBTU	Gravity Belt Thickener Underflow
INF	Influent
LC	Liquid Chromatography
LSWRF	Lander Street Water Renewal Facility
PCE	Primary Clarifier Effluent
PCS	Primary Clarifier Solids
PPCP	Pharmaceuticals and Personal Care Products
SCE	Secondary Clarifier Effluent
UP	Upstream Boise River Sample
UV	Ultraviolet Disinfected Facility Effluent
WAS	Waste Activated Sludge

W3	Chlorinated Facility Effluent
WWTF	Wastewater Treatment Facility

CHAPTER 1: INTRODUCTION

Overview

Chapter 1 will introduce the topic of emerging constituents (ECs) in wastewater. It will cover why this topic has gained interest and concern due to the potential effects of ECs on the environment and human health. This chapter's literature review section will also summarize the previous studies regarding EC research in the environment and wastewater treatment facilities (WWTFs).

Purpose

This research was funded by the City of Boise, whose purpose was to further examine ECs present within the Lander Streat Water Renewal Facility (LSWRF). ECs are becoming increasingly important as the U.S. Environmental Protection Agency and the Idaho Department of Environmental Quality are starting to investigate regulating constituents. This research aimed to better understand the effects on ECs from an activated sludge wastewater treatment facility. This research aimed to find general trends of constituent reductions in liquid and solids treatment processes. The city would be able to plan for potential regulations once the constituents within the facility are understood.

General trends would illustrate the need for specialized research into specific constituents, removal processes, and constituents' phases. Solids analysis was included in this research because research previously conducted by Baker (2022) identified it as the next step required to provide a complete analysis. Potential temporal variation was researched in

addition to reduction analysis. This research provided insight into the different trends present due to the sampling period and frequency.

This research aimed to determine ECs' fate and transport through the LSWRF. ECs, also commonly known as compounds of emerging concern, consist of various drugs and personal care products. These compounds range from over-the-counter pain relievers such as ibuprofen to illicit drugs (US EPA(a), 2023). ECs include personal care products such as perfumes, detergents, and soaps (US EPA(b), 2023). Wastewater treatment facilities (WWTF) currently have no regulations regarding the treatment or observance of ECs (US EPA(c), 2022). It has been found that some ECs can pass through the LSWRF relatively unchanged and are then discharged into the Boise River (Baker, 2022).

The presence of ECs in the environment dramatically threatens our ecosystem (Kumar et al., 2022). Studies regarding ECs have increased due to their impact on aquatic ecosystems, including behavioral changes, reproduction decline, and even acute toxicity. While seemingly insignificant, behavioral changes could affect aquatic species' sociality, risk assessment, and even reproduction, ultimately changing how a species interacts with its environment (Brodin et al., 2014). Bioaccumulation is another potential effect that has cause for concern. Bioaccumulation is the process by which a substance’s concentration increases in biological tissue (Chojnacka & Mikulewicz, 2014). This effect can magnify concentrations in higher trophic levels (Chojnacka & Mikulewicz, 2014). There is the potential for bioaccumulation to affect humans if concentrations increase in fish. A common example of bioaccumulation in fish is the magnification of heavy metals such as mercury in albacore tuna (Stamatis et al., 2019). Other impacts that would affect humans include soil contamination from using contaminated water sources. This has led to the uptake of ECs in

crops affecting the agricultural food chain (Christou et al., 2019). Research on ECs has continued to gain interest in understanding and preventing ECs from contaminating surface waters and soils, with their full effects on the environment still unknown.

Eleven ECs were selected and monitored at the LSWRF for six months during this research. The research questions that were posed were:

1. What are the EC concentrations entering the LSWRF's system?
2. How are the EC concentrations being affected by the LSWRF's system?
3. Are EC concentrations varying temporally within the LSWRF's system?

These research questions were posed to support a better understanding of the behavior of ECs in WWTFs. More proactive measures can be taken to reduce the discharge of ECs into surface waters once a better understanding of their behavior is gained.

Background

This section will provide fundamental background information on the LSWRF and its treatment processes, where all the samples were collected. The facility is an activated sludge treatment process, and Figure 1 illustrates the treatment train and the sampling locations, which are discussed below.

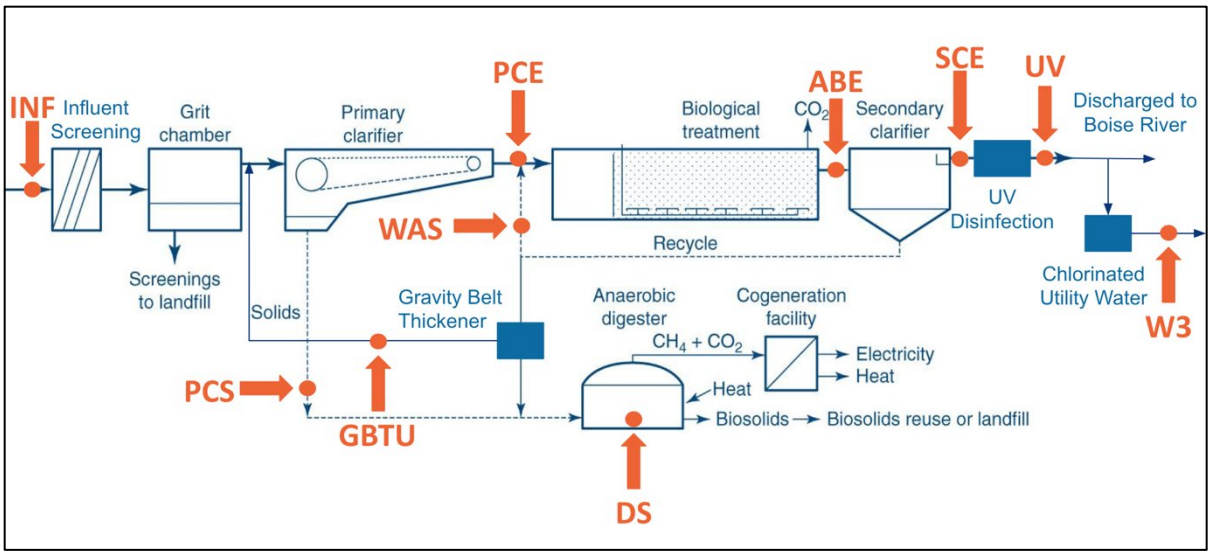

Figure 1: Water Renewal Facility Treatment Process Diagram (Modified from Mihelcic & Zimmerman, 2021)

Large debris is removed once wastewater enters the headworks, then continues to the grit chamber, where large particles are removed. This is followed by primary clarification, where the flow decreases to allow smaller particles to settle. The liquid treatment continues to aeration basins, where biological treatment reduces biochemical oxygen demand, total suspended solids, and dissolved solids such as ammonia and phosphorus, followed by secondary clarification. The final liquid process is UV disinfection immediately before discharge into the Boise River. The LSWRF also chlorinates a portion of water after UV disinfection, which is not discharged. It is used as needed within the LSWRF for spray bar water to reduce foaming created by biological treatment, irrigation, and other miscellaneous uses (Baker, 2022).

The gravity belt thickener processes secondary clarifier solids and then combined with primary clarifier solids (PCS). Liquid removed from the solids during the gravity belt thickening process is collected and redirected to the LSWRF headworks immediately downstream of the influent sampling location. Solids treatment continues to digesters, where

they are stabilized for approximately 30 days. Stabilized sludge is pumped approximately eight miles to the West Boise Water Renewal Facility for dewatering. Final treated solids are then sent to the Twenty Mile South Farm located outside the City of Kuna, Idaho, and are used as fertilizer for feed crops.

The daily volume and characteristics of the wastewater treated at LSWRF can vary widely. The facility receives primarily residential waste, though not solely human waste. The LSWRF receives anything disposed of within the facility's receiving lines, which includes oil, antifreeze, and cleaning solutions. Additionally, the facility receives waste from downtown Boise, Idaho, where several breweries are located and contains high amounts of organic waste that the LSWRF treats. The LSWRF also sees changes in loading when Boise State University is in session.

Literature Review

The literature review section will discuss preceding research efforts on the presence of ECs in natural systems such as rivers and streams and their presence in WWTFs (engineered systems). The previous findings and research that have been conducted have allowed this study to delve deeper into the research topic to provide data for the local community. In addition, these studies supported the development of sample collection and analysis methods.

Research in Natural Systems

Research has been conducted on ECs in larger streams because they have been detected in rivers, though research on smaller streams is still necessary. The research conducted by Bradley et al. (2020) aimed to provide more information on ECs in headwater streams. They monitored 111 pharmaceutical compounds in 308 headwater streams in four

regions across the United States. There were 261 considered multi-sample, urban gradient sites, and 47 were single-sample, non-urban sites of the 308 streams sampled. Multiple pharmaceuticals were detected at least once in 95% of urban gradient and 68% of non-urban sites. This research speaks to the prevalence of ECs in natural systems. Non-urban sample sites were initially deemed low-impact due to their limited potential exposure to ECs. The researchers concluded that there is substantial pharmaceutical contamination within smaller headwater streams and that results were most likely underestimated due to over 4,000 pharmaceuticals in use with an unquantifiable number of metabolites.

A study conducted in the Hudson River Estuary and New York Harbor focused on sources and spatial patterns of ECs introduced to the estuary and harbor in which 16 pharmaceuticals were analyzed at 72 sites (Cantwell et al., 2017). This study found that the pharmaceutical concentrations varied widely across the sampling sites. The primary controlling factors were the proximity and magnitude of the WWTF's discharge and inputs from tributaries and tidal mixing. Therefore, this study emphasizes the variability of ECs in surface waters due to the surface water conditions and WWTF's discharge.

Additionally, studies regarding ECs in surface waters have been conducted worldwide. For example, the study conducted by Malnes et al. (2023) tested the persistence, mobility, and toxicity of 71 substances in Sweden's three largest lakes at 31 sampling sites. The risk quotient (RQ) was calculated based on the environmental results. The results displayed that 20 of the ECs tested posed a moderate risk ($0.01 < RQ < 1$), whereas eight ECs displayed a high risk ($RQ > 1$), and five ECs were found to be hazardous. This research stresses the need for increased removal of ECs because of the effects on aquatic ecosystems.

Aquatic organisms are the first to be affected by ECs in the environment. A study by Huggett et al. (2002) focused on the effects of beta-blockers on vertebrates and invertebrates. The three invertebrates tested were *H. azteca*, *C. dubia*, and *D. magna*, and the vertebrates were medaka fish. They were all tested for their sensitivity to metoprolol, nadolol, and propranolol. It was found that *H. azteca* was the most resistant, medaka was the second most resistant, *C. dubia* was the most sensitive, and *D. magna* was moderately sensitive, as shown in Table 1. Subsequent tests focused on propranolol because it was the only EC that affected medaka fish.

Table 1: 48-hour Lethal Concentration of β-Blockers for 50% of the Population (Huggett et al., 2002)

Invertebrate	Metoprolol ($LC_{50} \pm SD$)	Nadolol	Propranolol ($LC_{50} \pm SD$)
H. azteca	≥ 100	≥ 100	29.8 ± 12.4
C. dubia	8.8 ± 1.9	≥ 100	0.8 ± 0.02
D. magna	63.9 ± 6.2	≥ 100	1.6 ± 0.3
Medaka	≥ 100	≥ 100	24.3 ± 10.5

Huggett et al. (2002) determined that the invertebrates' growth and reproduction were not likely to be affected by propranolol. On the other hand, exposure to propranolol in the medaka fish significantly decreased the number of viable eggs, posing a significant threat to their reproduction. This research shows that each organism reacts differently to individual EC exposure, even though *H. azteca* invertebrate was the most resistant to the effects of the beta blockers. There is the potential that affected invertebrates in nature could cause bioaccumulation in their predators. Tracking ECs that have been proven harmful to specific species is essential to understanding ecosystem effects.

Brodin et al. (2014) studied the uptake and behavioral impact of a psychiatric drug (oxazepam) on the European perch as well as their invertebrate prey (damselfly larvae). The associated changes in behavior from exposure to oxazepam can indicate an approaching lethal dose. Behavioral changes, such as aggression, boldness, exploration, and sociality, can then affect the survival and reproduction of any species. Oxazepam uptake by damselfly was also monitored, and it was found that the drug had no significant effect on the invertebrates' behavior, while perch displayed increased activity. This research proves that effects depend on the species. Another focus of this research was bioaccumulation, which was studied by exposing the perch to oxazepam through water, live food, and a combination of food and water. Perch exposed through water and food were found to have higher concentrations than perch that were only exposed through water from the bioaccumulation analysis, though the difference was not significant. This was shown with the measured bioconcentration factor (BCF) and bioaccumulation factor (BAF). Although exposed perch that were fed exposed damselfly on average received 0.0024 μg of oxazepam, unexposed perch that consumed exposed damselfly had a concentration of 0.0011 μg, indicating that after seven days, roughly 50% of the pharmaceuticals remaining in the perch were from ingestion. Potential ecological impacts could be underestimated if only concentrations, BCFs, and BAFs were analyzed.

Research in Engineered Systems

WWTFs are primary point sources of ECs to surface waters. More studies have been conducted on the WWTFs' influent and effluent concentrations due to increased detection of ECs in the environment. A study in upstate New York monitored 89 compounds from the influent and effluent of the facility (Asimakopoulos et al., 2017). Particulate matter

was removed from the samples to prevent clogging of instrumentation in previous studies, which underestimates concentrations since it ignores the potential for ECs in the particulate phase. Particulates do not need to be removed with the rotary evaporation-based method used by Asimakopouls et al. (2017), which provides a more accurate concentration of ECs. A few of the more common ECs studied are shown below in Table 2.

Table 2: Selected Results from Asimakopoulos et al. (2017)

Compound	Influent Concentration (ng/L)	Effluent Concentration (ng/L)
Methamphetamine	14.9	3
Cocaine	225	12.9
Lidocaine	426	422
Hydrocodone	11.4	6.1
Citalopram	115	76.7
Caffeine	50,000	1,110

These results show the variation in the EC loading at the WWTF and the difference in removal rates. Methamphetamine had a low influent concentration and a percent reduction of 80%, whereas lidocaine displayed minimal reduction through the treatment facility and a much higher loading rate. Caffeine had a substantial concentration (likely due to the prevalent use of coffee and energy drinks) and the highest reduction of 98% compared to the other ECs measured. The results display the variation of ECs through WWTFs because their influent concentrations depend on the population, while the effluent concentrations do display removals from the treatment facilities processes.

A study by Vuori et al. (2014) emphasized the variation of ECs due to population. This research aimed to find more information about abused drugs in nine locations in Finland, including two large metropolitan areas, five small university cities, and two rural towns. The results in Table 3 show that high concentrations of amphetamine were detected in metropolitan areas (Helsinki and Espoo) and university cities (Turku, Tampere, Jyväskylä,

Oulu, and Vaasa). High concentrations of methamphetamine were found in the small towns (Savonlinna and Seinäjoki). The party drugs, MDMA and cocaine, showed a statistically significant difference between weekends and weekdays in Helsinki, where overall concentrations of these drugs were higher than in other locations. Metropolitan areas were responsible for more of the total abused drug burden in proportion to the total population they represent despite being continuously detected in investigated WWTFs. This research underlines the variability of ECs in WWTFs due to the population.

Table 3: Selected Results from Vuori et al. (2014)

Constituent	Espoo	Helsinki	Jyväskl	Oulu	Savolinna	Seinäjoki	Tampere	Turku	Vaasa
Amphetamin	17.2	29.5	11.6	18.0	4.41	4.16	6.09	29.6	6.22
Methamphetamine	2.48	8.51	2.32	1.34	0.87	2.95	17.2	47.5	29.5
MDMA	7.99	20.6	6.33	1.28	0	0.75	2.61	0.69	0.33
THCA	13.1	20.7	10.5	9.28	0	0	10.4	3.77	9.95
Morphine	17.3	23.6	21.5	22.7	30.7	17.2	20.1	31.5	13.8

*Cities in the red box are the metropolitan cities, cities in the blue box are the rural towns, and the cities not in a box are the smaller university cities.

A study on ECs in a lagoon WWTF was completed in Lakefield, Ontario, Canada (Hoque et al., 2014). A unique facet of this research is that they studied the temporal variations of the influent and effluent of the WWTF by sampling through the summer, fall, and winter seasons. Table 4 depicts the percent reduction for each season, showing high percent removal of sulfamethoxazole and ibuprofen during the summer, declining through fall and winter. Carbamazepine and sucralose did not share the same linear trend in that carbamazepine only showed a reduction during fall, and sucralose increased concentration throughout the sampling periods. Overall trends can still be found despite clear evidence that some ECs do not react the same seasonally.

Table 4: Percent Reduction by Season from Hoque et al. (2014)

Constituents	Summer Reduction	Fall Reduction	Winter Reduction
Carbamazepine	-9.28%	45.8%	-54.1%
Sulfamethoxazole	98.9%	78.1%	69.5%
Ibuprofen	91.5%	81.1%	78.3%
Sucralose	-103%	-330%	-83.0%

Kosma et al. (2020) also examined the temporal variation of ECs in a hospital's WWTF in Greece where selected ECs and their metabolites were studied. A cluster analysis found July, September, and October with influent detection, whereas effluent detection was found in May, December, and April. The researchers concluded that temporal use of pharmaceutically active compounds occurs, and their removal is affected by seasonal factors, primarily temperature. This research supports the variation of ECs due to their seasonal use and weather conditions.

Research on ECs typically only monitors WWTFs' influent and effluent concentrations. A study by Baker (2022) considered the effects of each treatment process by sampling after each liquid treatment process. This sampling procedure led to a much clearer understanding of how ECs were affected by WWTF processes. One of the major findings from this research was a significant drop in ECs concentrations after primary sedimentation. This study only monitored the liquid treatment processes. It could not confirm that ECs were settling with the solids, bringing to light the need for testing both the liquid and solids treatment processes to provide a more in-depth understanding of ECs.

Tran et al. (2018) noticed the need for more research regarding the solids from WWTFs and reviewed studies conducted on ECs in wastewater, sludge, and biosolids in Asia, Europe, and North America. The study stressed the importance of testing sludge and biosolids because they can serve as a major sink for ECs, and the results could affect solids

management strategies. A selection of the many referenced results of sludge and biosolids compiled by the authors is shown in Table 5. The results vary widely due to discrepancies between countries, and the authors concluded from the data that conventional activated sludge WWTFs had limited EC reductions. This research emphasizes the need for additional research regarding sludge and biosolids.

Table 5: Selected Results from Tran et al. (2018)

Constituents	Sludge (ng/g)	Biosolids (ng/g)
Sulfamethoxazole	<MQL - 84.4	1.5 - 51
Acetaminophen	<MQL - 586	<MQL - 370
Ibuprofen	<MQL - 3,988	<MQL - 490
Salicylic Acid	<MQL - 13,743	-

*MQL is the method quantification limit.

Sadutto et al. (2020) focused on extracting ECs from Albufera National Park sediments in Spain. Their proposed method used solid-phase extraction cartridges to analyze 32 constituents. One of the methods used was Oasis HLB® solid phase extraction cartridges, resulting in recovery ranges of 61-120%, excluding the recoveries for atorvastatin, codeine, paracetamol, flufenamic acid, and salicylic acid, which had recoveries of approximately 50%. With this method, 26 compounds were detected. According to Sadutto et al. (2020), this was the first study with HLB-weak cation exchanger cartridges that displayed good recoveries. This research developed a method for analyzing sediment, which is typically overlooked, the incorporation of which can lead to a complete understanding of ECs.

Literature Summary

The threat ECs pose to the environment is evident from the research studies focusing on their effects in natural environments and aquatic ecosystems. ECs are present in urban areas with large WWTF outfalls and rural areas. Due to the presence of ECs, their persistence, mobility, and toxicity are important to investigate to further understand the potential impacts. Researchers also focused on analyzing invertebrates and vertebrate responses to ECs, the extent of their effects relatively unknown, with both studies showing a variance. A study found the varying sensitivity of beta blockers on invertebrates and reproductive decline in medaka fish. Additionally, it was found that their predator-prey had

asymmetric behavioral effects and that there is the potential to underestimate ecological impacts by only focusing on BCFs and BAFs.

The literature regarding ECs in engineered systems has only scratched the surface and shows a continued need for research. From research on ECs, improved methods were developed to include the particulates to obtain a complete concentration. Multiple studies demonstrated the variation of ECs in wastewater due to population. Seasonal testing is also gaining interest since the processes can be affected by temperature. Additionally, research into each treatment step allowed for further discoveries into the transformation or reduction of ECs. Compiled research from Asia, Europe, and North America to identify EC concentrations in solids displayed the need for increased solids testing. The research on the extraction of ECs from sediment aids in providing a complete understanding of the effects of WWTFs on them.

CHAPTER 2: RESEARCH METHODOLOGY

Overview

Chapter 2 will cover all research methods used to produce the data from the LSWRF. Beginning with how the ECs were selected and how the samples were collected and prepared. This is followed by the quality assurance and quality control used to assess data accuracy.

Selection of Emerging Constituents

The ECs selected for this study aimed to provide various drug classes and personal care products to be analyzed. Three main categories of ECs were created:

- Over-the-counter drugs and personal care products
- Common prescription drugs
- Specialized prescription drugs

Three constituents were chosen to study in the over-the-counter drugs and personal care products category. These selected ECs aim to represent how common drugs and personal care products are affected by the processes in the LSWRF. Ibuprofen and acetaminophen were selected for the over-the-counter drugs category due to their prevalence as painkillers. Salicylic acid was selected because of its growing popularity as an acne treatment and is also used to treat dandruff, psoriasis, and warts (*Salicylic Acid (Topical Route)*, 2023).

Common prescription drugs are the next category of ECs selected for this study. Previous privatized research at the LSWRF revealed that albuterol and theophylline

were present in the facility's effluent and were selected for further analysis. Albuterol and theophylline are both bronchodilators prescribed to treat asthma (*Ipratropium and Albuterol (Inhalation Route)*, 2023; *Theophylline (Oral Route)*, 2023). Theophylline is of particular interest because it has fallen out of favor as an asthma treatment and is still present in wastewater (Barnes, 2013). Another drug included in this category is cimetidine, an acid reducer used to treat ulcers and acid reflux (*Cimetidine: MedlinePlus Drug Information*, n.d.). Lastly, methylphenidate is the active ingredient in several drugs used to treat attention-deficit/hyperactivity disorder (*Methylphenidate (Oral Route)*, 2023). This drug is also a continuation of research conducted by Baker (2022) studying methylphenidate in the LSWRF.

The last category of ECs is specialized prescription drugs, which include citalopram, primidone, sulfamethoxazole, and warfarin. Citalopram is part of a subcategory of medications known as selective serotonin reuptake inhibitors (SSRIs) and is commonly known as Celexa and is used to treat depression (*Citalopram (Oral Route)*, 2023). SSRIs are the most prescribed drugs for depression (*Selective Serotonin Reuptake Inhibitors (SSRIs)*, 2019). Thus, citalopram was selected for its specialized use and detectability in wastewater. The following drugs were selected because they were present in the previous privatized testing at LSWRF. Primidone is an anticonvulsant medication, whereas sulfamethoxazole is an antibiotic (*Primidone (Oral Route)*, 2023; *Sulfamethoxazole and Trimethoprim (Oral Route)*, 2023). Both medications showed little change in concentrations from the influent to the effluent in previous testing at LSWRF, making it beneficial to collect further information on the drug as it passes through the treatment processes. Warfarin, commonly used as a blood thinner, is also found in rat poison and was not detectable in the LSWRF influent despite a

low concentration (5 ng/L) detected in the effluent. The result was unexpected, and this research aimed to better understand warfarin's behavior throughout the treatment processes. Table 6 contains a summary table of the constituents selected and analyzed, along with their drug classification and daily dosage.

Table 6: Summary of ECs

Constituent	Drug Class	Typical Dosing
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	Anti-inflammatory	3,250 mg/day
Ibuprofen	Anti-inflammatory	3,200 mg/day
Salicylic Acid	Topical treatment	NA
Common Prescription Drugs		
Albuterol	Bronchodilator	10 mg/day
Cimetidine	H_2 Blocker	800 mg/day
Methylphenidate	Central Nervous System Stimulants	30mg/day
Theophylline	Bronchodilator	NA
Specialized Prescription Drugs		
Citalopram	Serotonin Reuptake Inhibitor	40 mg/day
Primidone	Anticonvulsant	2,00 mg/day
Sulfamethoxazole	Antibiotic	NA
Warfarin	Blood Thinner	5 mg/day

Sample Collection

Liquid grab samples were collected once per month from February to July 2022 at each treatment process and the Boise River, upstream and downstream of the LSWRF discharge. Sample locations are shown in Figure 2, with treatment processes in blue and liquid sample locations in orange. A separate grab sampler was used for each location (except the influent manhole) and consisted of a plastic container attached to a long pole. The sampler was plunged well below the water surface, and the sample was transferred to a glass jar and transported to the laboratory for preparation and analysis.

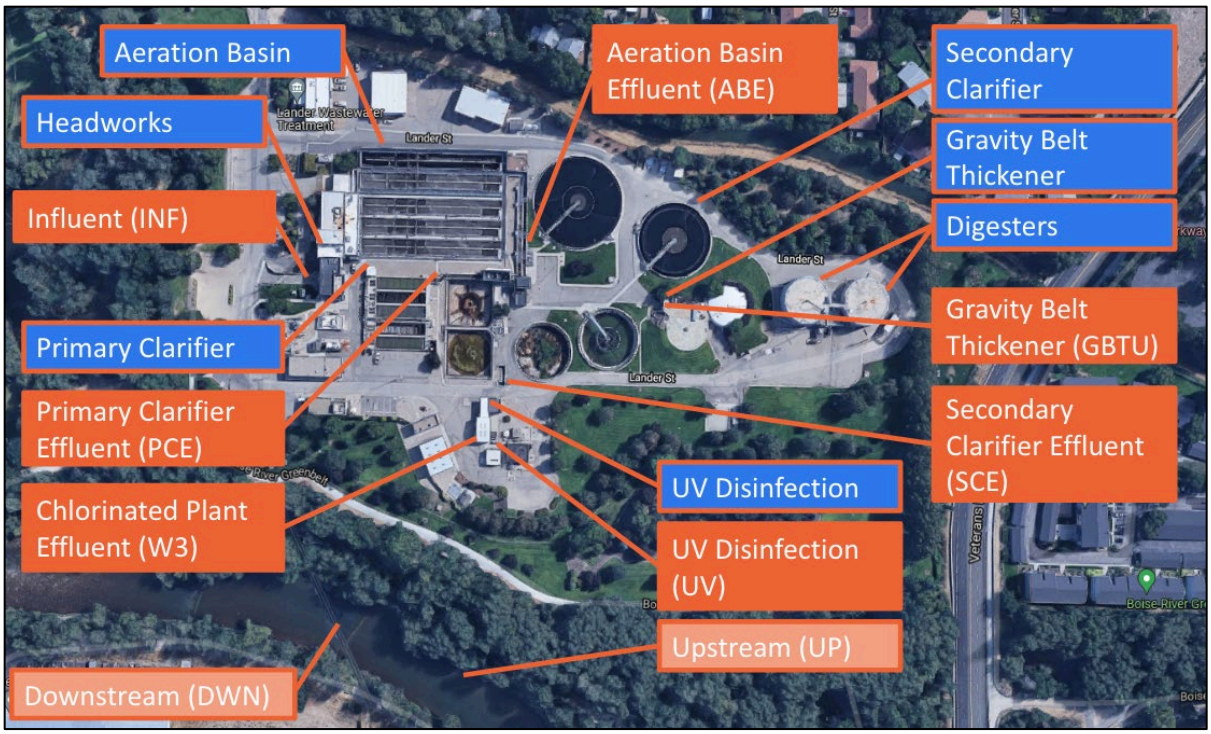

Figure 2: Liquid Sample Locations

Influent wastewater (INF) samples were collected from the main influent manhole in a glass jar using a HACH autosampler installed for routine regulatory compliance sampling at the LSWRF. A duplicate influent sample was collected each month for the quality control assessment. Primary clarifier effluent (PCE) samples were collected in the effluent channel prior to biological treatment. Aeration basin effluent (ABE) samples were collected in the channel immediately prior to the inlet of the secondary clarifier. Secondary clarifier effluent (SCE) samples were collected in the effluent launder of secondary clarifier number one. Post-UV disinfection (UV) samples were collected from February through April using the HACH autosampler installed for routine regulatory compliance sampling. A new UV disinfection system was installed in May as part of upgrades at the LSWRF, and the HACH autosampler was unavailable. UV samples were collected in May through July using the same grab sampler described. Chlorinated utility water (W3) samples were collected in glass jars using

the sampling port immediately following sodium hypochlorite dosing. Gravity belt thickener underflow (GBTU) samples were collected with a glass jar beneath the belt where the liquid drains from solids. Two samples were collected from the Boise River (1) at the Willow Lane Athletic Complex Boise River access (DWN) located approximately 0.25 miles downstream of the LSWRF discharge and (2) at the Ann Morrison Park Boise River access (UP) located approximately 2.5 miles upstream of the LSWRF discharge. Boise River samples were collected using glass jars submerged just below the water's surface.

Solids grab samples were collected once per month from February to July 2022 at each treatment process. Sample locations are shown in Figure 3, with treatment processes in blue and solids sample locations in orange. Solids samples were collected using separate glass jars and transported to the laboratory for preparation and analysis.

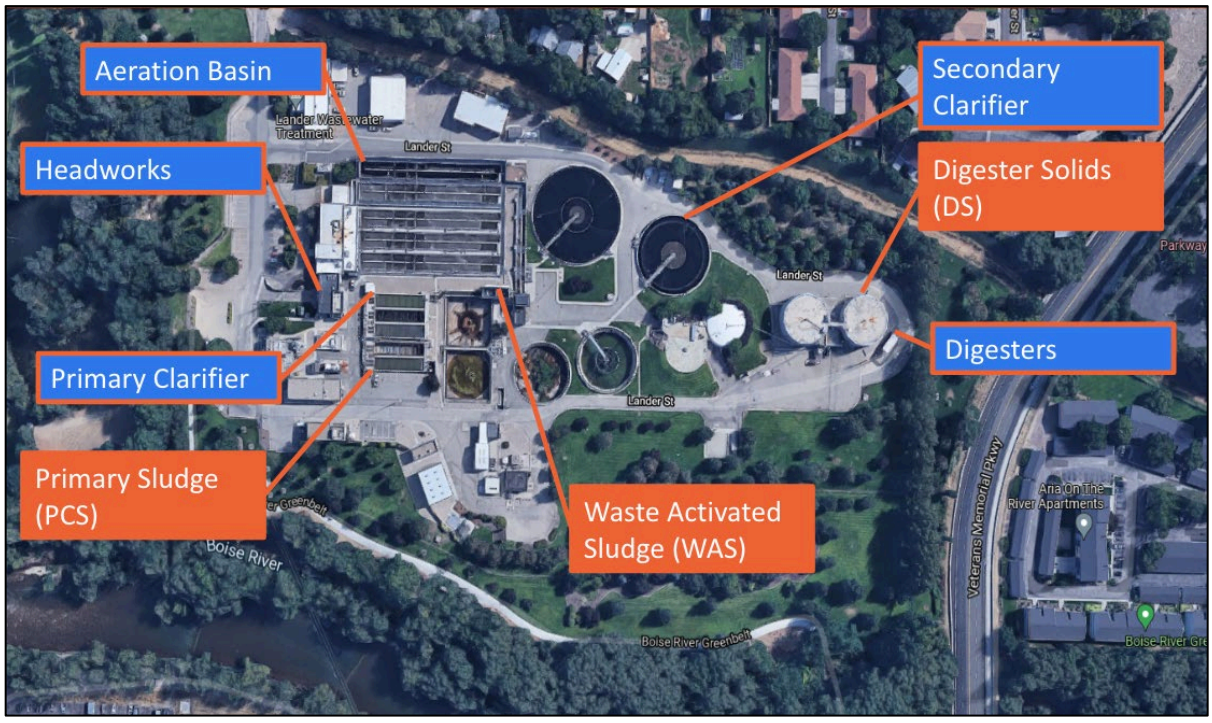

Figure 3: Solids Sample Locations

Primary sludge (PS) samples were collected in glass jars at the primary solids collection and pumping system sampling port. Waste-activated sludge (WAS) was collected at a sampling port in the pumping system that returns a portion of solids to the aeration basin to support microbial growth. Digested solids (DS) were collected at a sampling port within the digesters.

Sample Preparation

The pH of liquid samples was immediately lowered to 2.5 ± 0.1 with hydrochloric acid to stop any biological activity. The samples were refrigerated at 4℃ until the sample preparation was completed. Solids samples were refrigerated at -20°C to stop the biological activity and stored until sample preparation was completed.

Liquid Sample Preparation

An 80 ng/L mixture of dihydro carbamazepine and methanol was used as a surrogate (a quality control measure). First, 25 mL of the sample was diluted with 25 mL of the surrogate and placed in a round bottom flask. Next, a rotary evaporator with a bath temperature of 55°C was used to reduce the mixture volume to between 2 and 5 mL ± 0.1 mL. The solution was then transferred to a 15 mL graduated centrifuge tube with 10 mL ethyl acetate and evaporated to 0.5 mL under a gentle stream of N_2 gas at 50°C. Finally, the sample was reconstituted to 1 mL with methanol and transferred to a vial for instrument analysis. The sample was then analyzed by liquid chromatography and triple quadrupole mass spectrometry (LC/MS), the procedure is outlined in Appendix A. Testing was performed by Dr. Xinzhu Pu at the Boise State University Biochemical Research Center.

Solids Sample Preparation

The methods that Sadutto et al. (2020) developed were modified for solids samples from LSWRF. Solids samples were first thawed and homogenized by agitation on a shaker table for 10 minutes at 190 rpm. Homogenized solids were centrifuged for 10 minutes at 3,000 rpm to separate solids from excess liquids, which are referred to as aqueous samples. Aqueous samples were prepared according to the liquid sample preparation method previously described. Solids were then lyophilized (freeze-dried), sealed, and stored at -20°C. A 1.00 ± 0.05 g of the lyophilized solids was mixed with 15 mL of equal parts deionized water, methanol, and McIlvaine-EDTA buffer. The surrogate dihydro carbamazepine (100 µL of 100 µg/L) was added to the solids mixture during this step. The mixture was then homogenized by agitation on a shaker table for 15 minutes at 250 rpm and centrifuged for 10 minutes at 3,000 rpm. This step extracted constituents from the solids into a supernatant, which was then separated from the solids and diluted with 15 mL of deionized water in preparation for solid phase extraction.

Solid phase extraction was completed using Oasis HLB® sorbent cartridges (WAT106202, 6 cc vac cartridge, 200 mg sorbent per cartridge, 30 µm, Waters Corporation, Milford, MA). Oasis HLB® cartridges use a reverse phase solid phase extraction method, which can be activated, equilibrated, loaded, washed, and eluted. Cartridges were activated with 6 mL of methanol and equilibrated with 6 mL of deionized water, each at 6 mL/min. The supernatant was loaded onto the cartridge and eluted at 6 mL/min. Eluant was collected and evaporated to 0.5 mL under a gentle stream of N_2 gas at 50°C and reconstituted to 1 mL with a mixture of deionized water and methanol (70:30 v/v). The sample was then transferred to a vial for instrument analysis. Samples were analyzed by liquid chromatography with

triple quadrupole mass spectrometry (LC/MS), the procedure is outlined in Appendix A. Testing was performed by Dr. Xinzhu Pu at the Boise State University Biochemical Research Center.

One major modification from the Sudutto et al. (2020) method was the cartridge used for solid phase extraction. They used Phenomenex Strata-X (33 µm, polymetric reversed phase, 200 mg/6 mL, Phenomenex, Torrance, CA), whereas Oasis HLB® was used for this research. The manufacturer recommends using a standard five-step process (condition, equilibrate, load, wash, and elute) for Oasis HLB® cartridges. Errors were introduced into the samples because the washing and elution steps were not completed for this research, which may have introduced errors in underrepresenting constituent concentrations in solids samples. Non-polar constituent concentrations would be most affected by missing the elution step with a non-polar solvent solution. The surrogate is considered non-polar and could not be detected with the LC/MS. Therefore, the constituents were quantified from the pure constituent standards. All constituents in this research are considered polar, and the results are likely unaffected. Aqueous samples were prepared using the procedure for the liquids and were not affected.

Quality Control

Several measures were taken to maintain a high level of quality control throughout the sample collection, preparation, and analysis processes. Quality control measures taken during the sample preparation included surrogate additions, pure EC standards, blank samples, and duplicate field samples. Surrogates were added to each sample prior to sample preparation and analysis to account for losses through methods. EC standards were used for calibrating the Agilent 1290 LC/MS to account for instrument drift. Liquid blank (BLK)

samples of DI water were prepared according to the liquid preparation methods. Each sampling period, BLK samples were compared to each sample location (INF, PCE, ABE, SCE, UV, W3, GBTU, UP, and DWN) as an additional quality control measure. A duplicate sample of the INF for each sampling period was the last quality control measure, which indicates method reproducibility. A summary of these quality control measures is found in Chapter 3.

CHAPTER 3: RESULTS AND DISCUSSION

Overview

This chapter will present the data analysis performed and discuss the quantification of BLK samples. Results and potential causes for the ECs' reductions and temporal behaviors will also be presented. Certain aspects of the LSWRF process will be discussed to aid in understanding EC behaviors.

Results

Analysis techniques performed were specifically chosen to address the following research questions:

1. What are the EC concentrations entering the LSWRF?
2. How are the EC concentrations being affected by the LSWRF's system?
3. Are EC concentrations varying temporally within the LSWRF?

A quartile analysis was selected to identify and remove any outliers within the dataset to address the first question and accurately depict average concentrations. The corrected mean concentrations at each sample location are shown in Table 7.

Table 7: Corrected Mean Concentration Summary

Mean Concentration (ng/L)				
Over-The-Counter Drugs and Personal Care Products				
Sample Location	**Acetaminophen**	**Ibuprofen**	**Salicylic Acid**	
INF	412	122	1132	
PCE	215	91.1	1071	
ABE	0.24	2.50	14.7	
SCE	0.01	2.50	25.0	
UV	1.00	2.50	17.4	
W3	0.04	2.50	10.0	
GBTU	0.01	2.50	12.3	
UP	1.48	2.50	1.47	
DWN	0.17	2.50	2.31	
BLK	0.03	2.50	5.52	
PCS	16.8	818	5294	
WAS	0.10	12.0	281	
DS	0.29	975	466	
Common Prescripion Drugs				
Sample Location	**Albuterol**	**Cimetidine**	**Methylphenidate**	**Theophylline**
INF	0.56	2.26	0.46	639
PCE	0.43	2.47	0.59	459
ABE	0.21	1.21	0.99	1.80
SCE	0.49	1.41	1.17	2.38
UV	0.34	0.92	0.72	2.64
W3	0.31	0.48	0.52	2.79
GBTU	0.23	0.53	0.65	1.31
UP	0.03	0.17	0.12	0.47
DWN	0.03	0.27	0.13	0.32
BLK	0.03	0.17	0.12	0.15
PCS	0.07	0.32	1.04	38.9
WAS	0.05	0.50	0.54	0.05
DS	0.23	1.33	0.14	8.45
Specialized Prescripion Drugs				
Sample Location	**Citalopram**	**Primidone**	**Sulfamethoxazole**	**Warfarin**
INF	7.36	1.13	23.2	1.25
PCE	5.76	1.22	21.4	1.25
ABE	23.6	0.61	9.55	1.25
SCE	7.27	1.12	0.79	1.25
UV	6.44	1.11	0.56	1.25
W3	5.47	1.23	1.27	1.25
GBTU	7.93	1.11	0.95	1.25
UP	0.23	0.01	0.02	1.25
DWN	0.51	0.10	0.15	1.25
BLK	0.15	0.01	0.01	1.25
PCS	13.6	0.65	12.5	2.50
WAS	8.69	0.55	11.7	2.50
DS	3.96	1.23	0.18	2.50

The quartile analysis process was completed in MATLAB using the boxplot function. This function plotted quartiles for each sample location. Outliers from these plots were identified and removed from the averages. There were very few outliers, most of which were from sample locations with ND values and one detection. Box plots for each constituent are included in Appendix D.

Non-Detect Analysis Approach

A non-detect (ND) value occurs when a measured value is below the equipment's detection limit, and they are a widespread occurrence in studies. There are several approaches to addressing NDs within a dataset. One option is to ignore the ND values, which would cause an overestimation of a dataset (Giskeødegård & Lydersen, 2022). A standard method to address NDs is the imputation of a value, the most common being one-half the detection limit and zero (Wendelberg & Campbell, 1994; Giskeødegård & Lydersen, 2022). Simulation studies have demonstrated that imputing zero creates more bias in a dataset. This study used an imputation of half the detection limit for all the NDs. This method of addressing NDs was also implemented in research conducted by Vuori et al. (2014).

The detection limit for all ECs in liquid samples collected for this research was 0.40 ng/L except for ibuprofen (200 ng/L) and warfarin (100 ng/L). The detection limit of all ECs in solids samples collected for this research was 0.020 ng/g except for ibuprofen (5.00 ng/g) and warfarin (10.0 ng/g).

Blank Samples

Concentrations in BLK samples were all ND for acetaminophen, ibuprofen, sulfamethoxazole, and theophylline. This is a good indication of minimal contamination and detection interference from the LC/MS process. Concentrations in BLK samples were

detected for albuterol, cimetidine, citalopram, methylphenidate, primidone, and salicylic acid. This could be due to quality control issues such as instrument contamination in combination with detection interference. All quality control measures (Chapter 2, Quality Control) were followed for each sample. The percent difference between INF and BLK samples are all less than 7.5%, indicating valid detections of each constituent in the facility.

The student's t-test was performed between BLK samples and each liquid sample location (Table 8), and a table of p-values can be found in Appendix A. This analysis illustrated when constituent concentrations differed significantly from BLK samples. There were more non-significant differences for constituent concentrations comparable to the BLK concentration. Constituent concentrations that decreased gradually across the treatment process differed more significantly from BLK samples.

Initial concentration results from LC/MS analysis can be found in Appendix C. It is important to note that the aqueous and solids sample results were combined to achieve total solids concentrations. The following results are the corrected values from the quartile analysis. The following sections will discuss each EC's liquid and total solids results.

Table 8: Blank Samples T-Test Results

Student's T-Test Blank Liquid Results				
Over-The-Counter Drugs and Personal Care Products				
Sample Location	**Acetaminophen**	**Ibuprofen**	**Salicylic Acid**	
INF vs BLK	S	S	S	
PCE vs BLK	S	S	S	
ABE vs BLK	NS	NA	NS	
SCE vs BLK	NS	NA	NS	
UV vs BLK	NS	NA	NS	
W3 vs BLK	NS	NA	NS	
GBTU vs BLK	NS	NA	NS	
UP vs BLK	NS	NA	NS	
DWN vs BLK	NS	NA	NS	
Common Prescription Drugs				
Sample Location	**Albuterol**	**Cimetidine**	**Methylphenidate**	**Theophylline**
INF vs BLK	S	S	NS	S
PCE vs BLK	S	S	NS	S
ABE vs BLK	S	S	S	S
SCE vs BLK	S	S	S	NS
UV vs BLK	S	S	S	NS
W3 vs BLK	S	NS	S	S
GBTU vs BLK	S	S	S	S
UP vs BLK	NS	NS	NS	NS
DWN vs BLK	NS	NS	NS	NS
Specialized Prescription Drugs				
Sample Location	**Citalopram**	**Primidone**	**Sulfamethoxazole**	**Warfarin**
INF vs BLK	S	S	S	NA
PCE vs BLK	S	S	S	NA
ABE vs BLK	S	S	S	NA
SCE vs BLK	S	S	NS	NA
UV vs BLK	S	S	NS	NA
W3 vs BLK	S	S	NS	NA
GBTU vs BLK	S	S	NS	NA
UP vs BLK	NS	NS	NS	NA
DWN vs BLK	S	NS	NS	NA

S = Significant, NS = Not Significant, and NA = Not Applicable

A student's t-test was selected to address the second research question. The t-test was completed for liquid samples to (1) determine if the mean influent concentration differed significantly from the mean effluent concentration and (2) if mean concentrations significantly differed between treatment steps. A student's t-test was also completed for

solids samples. Student's t-tests were completed in MATLAB using the t-test function with a sensitivity of 5%, the code for which can be found in Appendix E. Outputs determined if there was a significant change in concentration and the corresponding p-value. Results are discussed for each EC in the following sections.

Liquid t-test results are presented in Table 9. Constituents with significant changes in the beginning treatment processes exhibited significant reductions in concentration. Several constituents exhibited differences between UV and river (UP and DWN) concentrations and between river concentrations. Conclusions about this data cannot be made because the concentration standard error was below zero. Concentration values were similar enough to zero that values cannot be compared. The student's t-test analysis and corresponding p-values are included in Appendix E.

Table 9: Liquid Removal T-Test Results

Over-The-Counter Drugs and Personal Care Products			
Sample Location	**Acetaminophen**	**Ibuprofen**	**Salicylic Acid**
INF vs PCE	S	S	NS
PCE vs ABE	S	S	S
ABE vs SCE	NS	NS	NS
SCE vs UV	NS	NS	NS
UV vs W3	NS	NS	NS
UV vs UP	NS	NS	S
UV vs DWN	NS	NS	S
UP vs DWN	NS	NS	S
INF vs UV	S	S	S

Common Prescription Drugs				
Sample Location	**Albuterol**	**Cimetidine**	**Methylphenidate**	**Theophylline**
INF vs PCE	NS	NS	NS	S
PCE vs ABE	NS	NS	NS	S
ABE vs SCE	NS	NS	NS	NS
SCE vs UV	NS	NS	NS	NS
UV vs W3	NS	NS	NS	NS
UV vs UP	S	S	S	NS
UV vs DWN	S	S	S	NS
UP vs DWN	S	S	S	NS
INF vs UV	NS	S	NS	S

Specialized Prescription Drugs				
Sample Location	**Citalopram**	**Primidone**	**Sulfamethoxazole**	**Warfarin**
INF vs PCE	NS	NS	NS	NA
PCE vs ABE	S	NS	NS	NA
ABE vs SCE	NS	NS	S	NA
SCE vs UV	NS	NS	NS	NA
UV vs W3	NS	NS	NS	NA
UV vs UP	S	S	NS	NA
UV vs DWN	S	S	NS	NA
UP vs DWN	S	S	NS	NA
INF vs UV	NS	NS	S	NA

S = Significant, NS = Not Significant, and NA = Not Applicable

Solids t-test results are presented in Table 10. There are many constituents with significantly different, though not all differences correspond with decreasing concentrations. These significant differences will be discussed further discussed in the following sections. The student's t-test analysis and corresponding p-values are included in Appendix E.

Table 10: Solids Removal T-Test Results

Constituent	PCS vs WAS	WAS vs DS	PCS vs DS
Over-The-Counter Drugs and Personal Care Products			
Acetaminophen	S	NS	S
Ibuprofen	S	S	NS
Salicylic Acid	S	S	S
Common Prescription Drugs			
Albuterol	NS	S	NS
Cimetidine	NS	NS	NS
Methylphenidate	S	S	S
Theophylline	S	S	S
Specialized Prescription Drugs			
Citalopram	NS	S	S
Primidone	S	S	S
Sulfamethoxazole	NS	S	S
Warfarin	NA	NA	NA

S = Significant, NS = Not Significant, and NA = Not Applicable

A student's t-test was also performed to address the third research question. Samples were grouped by season: winter (February and March), spring (April and May), and summer (June and July). Seasonal mean concentrations were compared for liquid samples (Table 11) and solid samples (Table 12) to determine significant differences. Five constituents displayed significantly different seasonal averages. Acetaminophen was the only constituent that displayed significantly different solids results. The results will be discussed in the temporal analysis section. The student's t-test analysis and corresponding p-values are included in Appendix E.

Table 11: Temporal Liquid Variation T-Test Results

Constituent	Winter vs Spring	Spring vs Summer
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	NS	NS
Ibuprofen	NS	NS
Salicylic Acid	NS	NS
Common Prescription Drugs		
Albuterol	S	S
Cimetidine	S	S
Methylphenidate	NS	NS
Theophylline	NS	NS
Specialized Prescrription Drugs		
Citalopram	S	NS
Primidone	S	S
Sulfamethoxazole	NS	NS
Warfarin	NA	NA

Where S = Significant, NS = Not Significant, and NA = Not Applicable

Table 12: Temporal Solids Variation T-Test Results

Constituent	Winter vs Spring	Spring vs Summer
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	NS	S
Ibuprofen	NS	NS
Salicylic Acid	NS	NS
Common Prescription Drugs		
Albuterol	NS	NS
Cimetidine	NS	NS
Methylphenidate	NS	NS
Theophylline	NS	NS
Specialized Prescription Drugs		
Citalopram	NS	NS
Primidone	NS	NS
Sulfamethoxazole	NS	NS
Warfarin	NA	NA

Where S = Significant, NS = Not Significant, and NA = Not Applicable

Over-the-Counter Drugs and Personal Care Product Results

Acetaminophen

Seven outliers were detected from the liquid quartile analysis of acetaminophen, one from each of the following sample locations: PCE, ABE, SCE, UV, W3, UP, and BLK (Figure D.1). The PCE sample outlier had a concentration three times the

median value. The remaining outliers were caused by low detection values, where the data mostly consisted of ND values. No outliers were detected in the solids quartile analysis (Figure D.12). All acetaminophen outliers were removed from the dataset.

Acetaminophen concentrations in liquids and solids generally decreased as they progressed through the LSWRF treatment processes (Figure 4). Concentrations are nearly zero at ABE and WAS. Liquid acetaminophen concentrations sharply decrease from INF (16,473 ng/L) to PCE (8,614 ng/L) and from PCE to ABE (1.44 ng/L). These reductions were statistically significant, and there is a significant decrease across the treatment facility from INF to UV (Table 8). There is a significant decrease in solids concentrations from PCS (16.8 ng/g) to WAS (0.10 ng/g), a slight increase from WAS to DS (0.29 ng/g), though not significantly different, and a significant decrease from PCS to DS (Table 10).

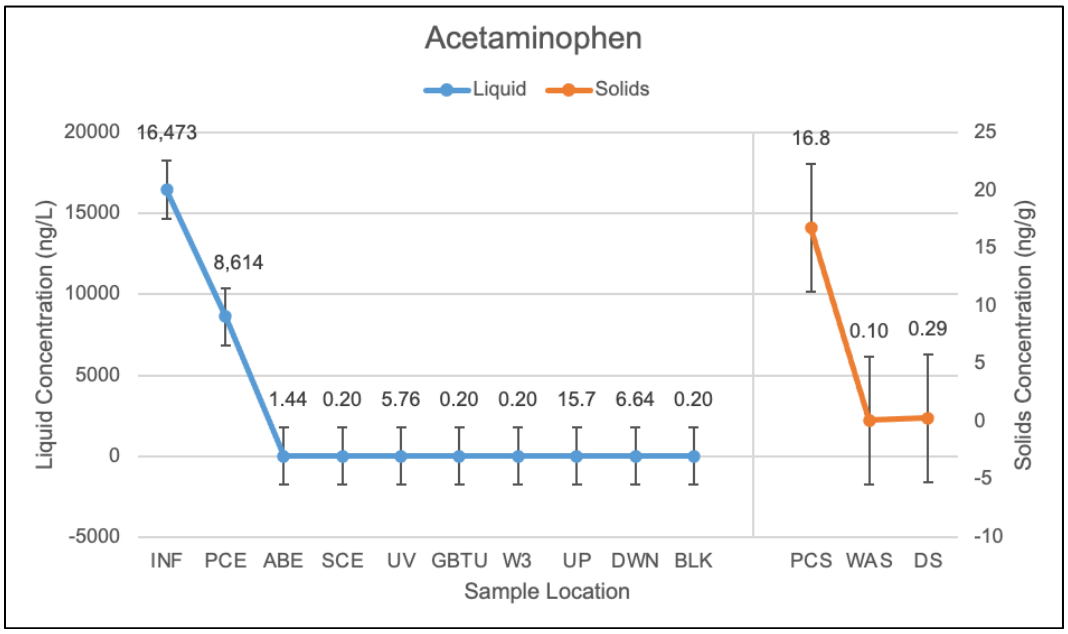

Figure 4: Mean Acetaminophen Concentrations

Ibuprofen

Four outliers were detected from the liquid quartile analysis of ibuprofen, one in the INF sample, two in the duplicate INF sample (INF 2), and one in the ABE sample (Figure D.2). The INF outlier concentration was an order magnitude greater than the median. The INF 2 outlier concentrations consisted of one above the upper fence and one below the lower fence. The ABE outlier concentration was approximately four orders of magnitude higher than the median concentration. One outlier was detected in the solids quartile analysis from the WAS sample location (Figure D.13). The outlier was caused by low detection values, where the data mostly consisted of ND values. All ibuprofen outliers were removed from the datasets.

Ibuprofen concentrations in liquid generally decrease as they progress through the LSWRF treatment processes (Figure 5), like acetaminophen. Samples resulting in an ND value were substituted with the equivalent of half the detection limit, or 100 ng/L. Liquid ibuprofen concentrations decrease sharply from INF (4,893 ng/L) to PCE (3,643 ng/L) and decrease sharply from PCE to ABE (100 ng/L). There is no significant difference in liquid concentrations within the LSWRF (Table 8). There is a significant decrease across the treatment facility from INF to UV (100 ng/L). There is a significant decrease in solids ibuprofen concentrations from PCS (818 ng/g) to WAS (12.0 ng/g), a significant increase from WAS to DS (975 ng/L), and a slight, though not significantly different, increase from PCS to DS (Table 10).

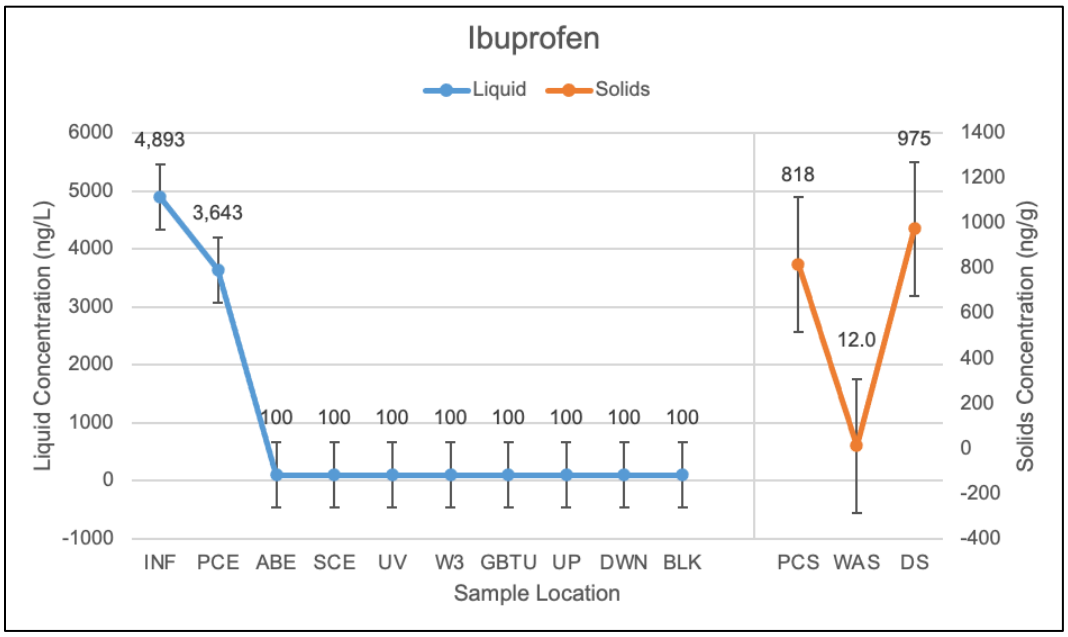

Figure 5: Mean Ibuprofen Concentrations

Salicylic Acid

Eight outliers were detected from the liquid quartile analysis of salicylic acid, one from each of the following sample locations: INF, ABE, SCE, UV, W3, UP, DWN, and BLK (Figure D.3). The INF outlier concentration was above the upper fence. The remaining outliers were caused by low detection values, where the data mostly consisted of ND values. No outliers were detected in the solids quartile analysis (Figure D.14). All salicylic acid outliers were removed from the dataset.

Salicylic acid concentrations in liquid generally decrease as they progress through the LSWRF treatment processes (Figure 6), like acetaminophen and ibuprofen. Liquid salicylic acid concentrations decrease slightly from INF (45,263 ng/L) to PCE (42,857 ng/L) and decrease sharply from PCE to ABE (589 ng/L). There is no significant difference in liquid concentrations within the LSWRF except between PCE and ABE (Table 8). There is a significant decrease across the treatment facility from INF to UV (440 ng/L). There is a

significant difference between UV and river samples (UP and DWN) and between the two river samples (Table 10). Solids salicylic acid concentration sharply decreased from PCS (5,294 ng/g) to WAS (281 ng/g) and slightly increased from WAS to DS sample (466 ng/g), all significantly different (Table 10).

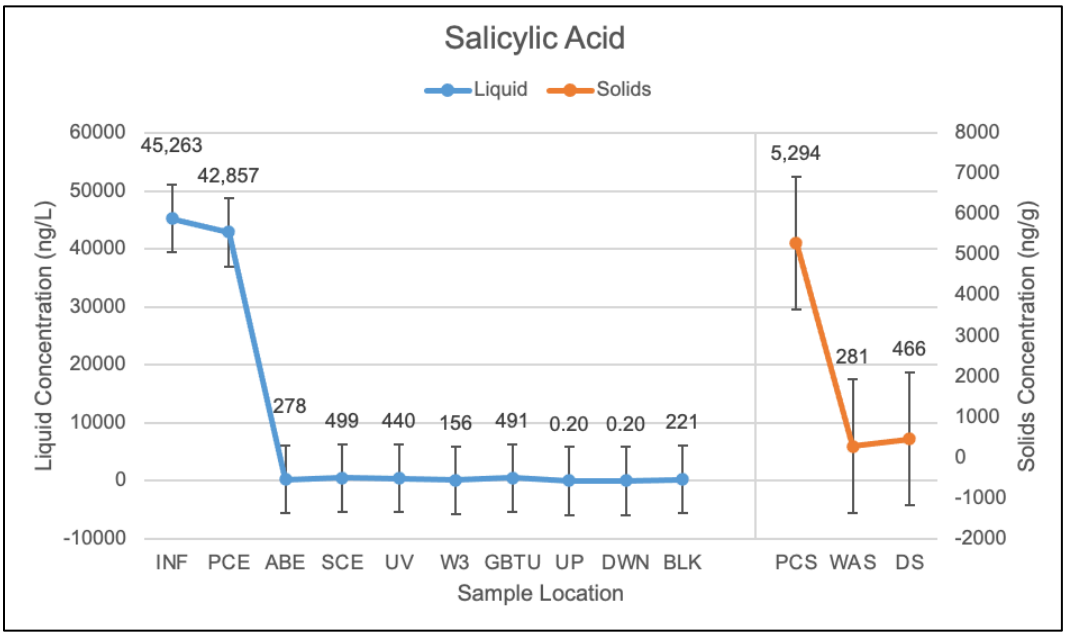

Figure 6: Mean Salicylic Acid Concentrations

Common Prescription Drugs

Albuterol

No outliers were detected from the quartile analysis of albuterol (Figure D.4 and Figure D.15). Albuterol concentrations in liquids and solids vary widely as they progress through the LSWRF treatment processes (Figure 7). Liquid albuterol concentrations decrease from INF (22.3 ng/L) to ABE (8.20 ng/L), increase from ABE to SCE (19.5 ng/L), and generally decrease to GBTU (9.07 ng/L). There is no significant difference in liquid samples within the LSWRF (Table 8). There is a significant difference between UV and river samples

(UP and DWN), and between the two river samples. There is no significant difference across the treatment facility from INF to UV (13.7 ng/L). Solids sample results mirror those for liquid samples. There is a slight, though not significantly different, decrease in solids albuterol concentrations from PCS (0.07 ng/g) to WAS (0.05 ng/g), a significant increase from WAS to DS (0.23 ng/g), and a sharp, though not significantly different, increase from PCS to DS (Table 10).

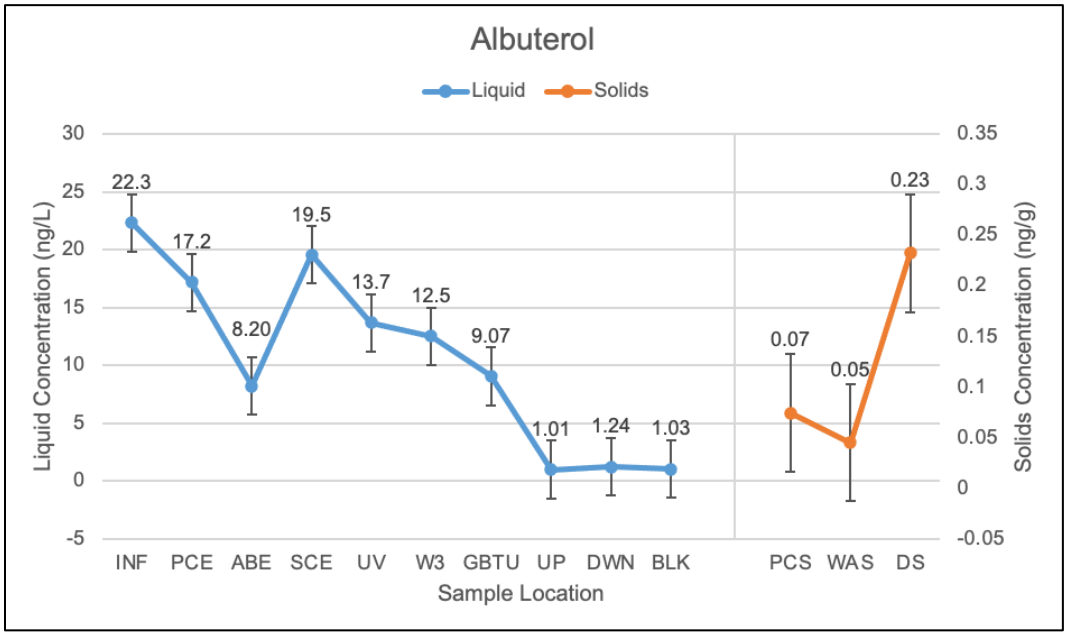

Figure 7: Mean Albuterol Concentrations

Cimetidine

Two outliers were detected from the liquid quartile analysis of cimetidine, both from the W3 sample location (Figure D.5). The W3 outliers had one concentration above the upper fence and one below the lower fence. One outlier was detected from the solids quartile analysis from the PS (Figure D.16). The outlier was caused by low detection

values, where the data mostly consisted of ND values. All cimetidine outliers were removed from the datasets.

Cimetidine concentrations in liquid generally decrease while increasing in the solids as they progress through the LSWRF treatment processes (Figure 8). Liquid cimetidine concentrations slightly increase from INF (90.4 ng/L) to PCE (98.7 ng/L), decrease sharply from PCE to ABE (48.4 ng/L), increase slightly from ABE to SCE (56.3 ng/L), generally decrease from SCE to W3 (19.1 ng/L), and slightly increase from W3 to GBTU (21.0 ng/L). There is a significant difference between UV and river samples (UP and DWN) and between the two river samples (Table 8). There is a significant decrease across the treatment facility from INF to UV (36.9 ng/L). Solids cimetidine concentrations consistently increase from PCS (0.32 ng/g) to DS (1.33 ng/g), though not significantly different (Table 10).

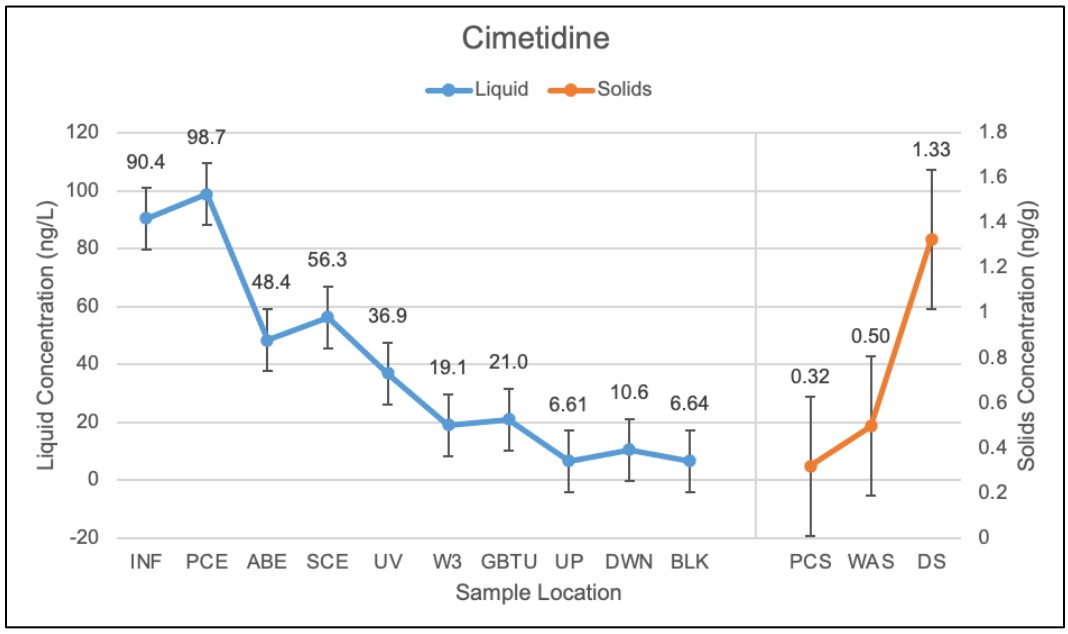

Figure 8: Mean Cimetidine Concentrations

Methylphenidate

Three outliers were detected from the liquid quartile analysis of methylphenidate, one from each of the following sample locations: UV, W3, and GBTU (Figure D.6). Median concentrations ranged from 20 to 40 ng/L, while outlier concentrations ranged from approximately 60 to 80 ng/L, above the upper fence. No outliers were detected from the solids quartile analysis (Figure D.17). All methylphenidate outliers were removed from the dataset.

Methylphenidate concentrations in liquid presented an unexpected result: they increased through the LSWRF treatment processes (Figure 9). Liquid methylphenidate concentrations consistently increase from INF (18.2 ng/L) to SCE (46.9 ng/L), then consistently decrease from SCE to W3 (20.9 ng/L). Liquid methylphenidate concentrations at GBTU (26.2 ng/L) are notable because they are higher than INF. There is no significant difference in liquid concentrations within the LSWRF (Table 8). There is no significant difference across the treatment facility from INF to UV (28.6 ng/L). There is a significant difference between UV and river samples (UP and DWN) and between the two river samples. Solids methylphenidate concentrations consistently decrease from (1.04 ng/g) to DS (0.14 ng/g), all significantly different (Table 10).

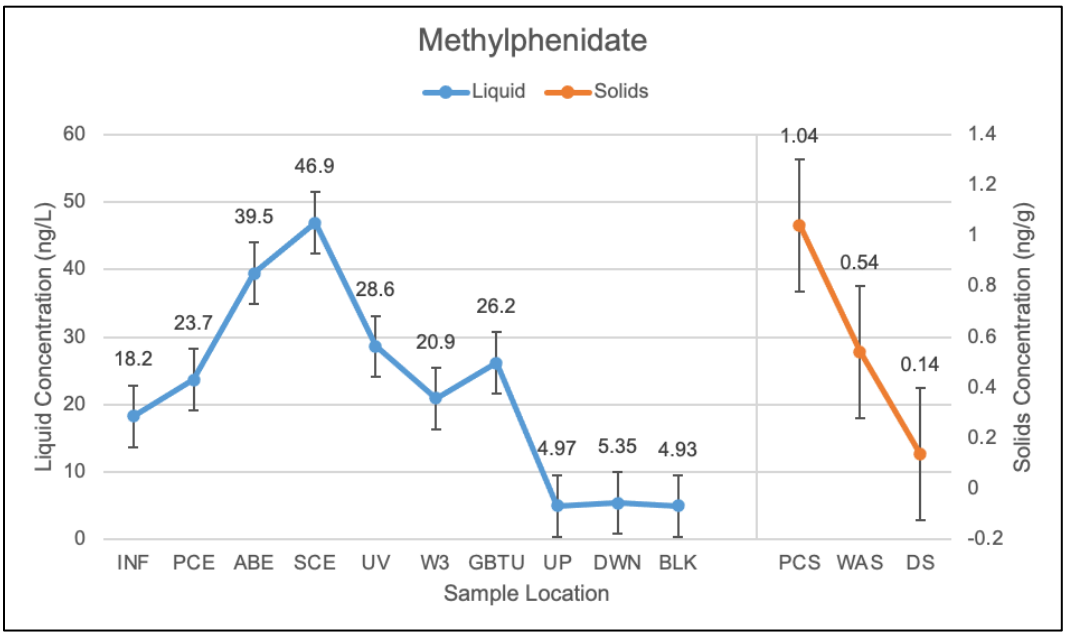

Figure 5: Mean Methylphenidate Concentrations

Theophylline

Five outliers were detected from the liquid quartile analysis of theophylline, one from each of the following sample locations: SCE, UV, W3, DWN, and BLK (Figure D.7). These outliers were caused by low detection values, where the data mostly consisted of ND values. One outlier was detected from the solids quartile analysis from the PS (Figure D.18). The outlier was an order of magnitude higher than the median. All theophylline outliers were removed from the datasets.

Theophylline concentrations in liquid generally decrease as they progress through the LSWRF treatment process (Figure 10), like acetaminophen, ibuprofen, and salicylic acid. Liquid theophylline concentrations decrease slightly from INF (25,554 ng/L) to PCE (18,348 ng/L) and decrease sharply from PCE to ABE (72.0 ng/L). There is no significant difference in liquid concentrations within the LSWRF except from INF to PCE and PCE to ABE (Table 8). There is a significant decrease across the treatment facility from INF to UV (54.6 ng/L). Solids theophylline concentrations decrease sharply from PCS (38.9 ng/g) to WAS (0.050

ng/g), sharply increase from WAS to DS (8.45 ng/g), and sharply decrease from PCS to DS, all significantly different (Table 10).

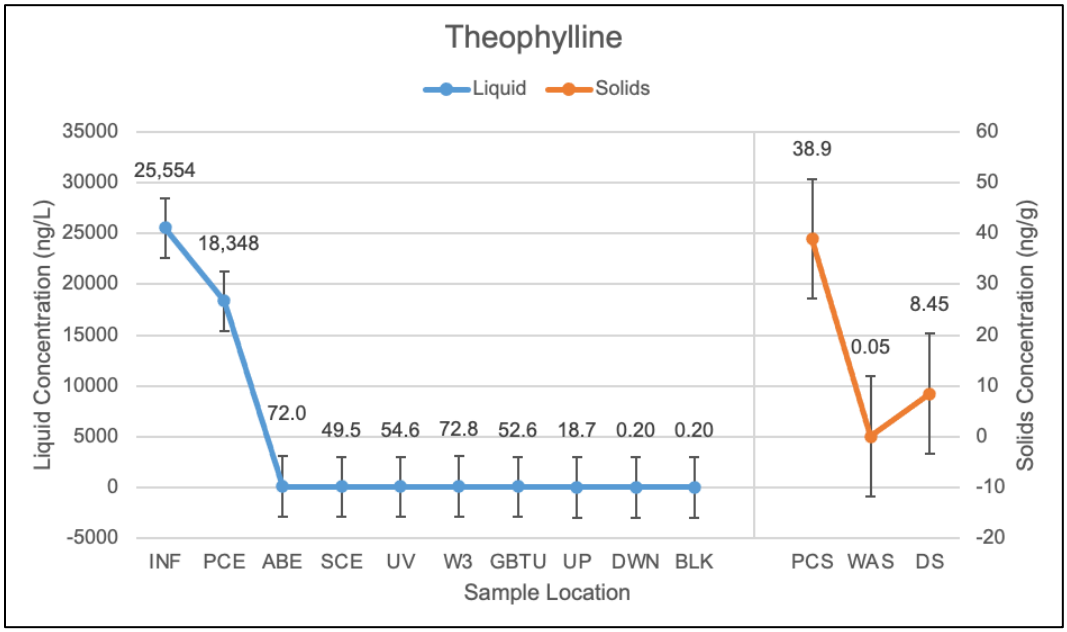

Figure 10: Mean Theophylline Concentrations

Specialized Prescription Drugs

Citalopram

Four outliers were detected from the liquid quartile analysis of citalopram, one from each of the following sample locations: ABE, SCE, GBTU, and BLK (Figure D.8). The ABE outlier concentration was above the upper fence. The remaining outliers were caused by low detection values, where the data mostly consisted of ND values. One outlier was detected from the solids quartile analysis from the WAS sample location (Figure D.19). The outlier was below the lower fence. All citalopram outliers were removed from the datasets.

Citalopram concentrations in liquid and solid generally decrease as they progress through the LSWRF treatment processes (Figure 11). Liquid citalopram concentrations

decrease slightly from INF (295 ng/L) to PCE (230 ng/L), increase sharply from PCE to ABE (945 ng/L), decrease sharply from ABE to SCE (291 ng/L), generally decrease to W3 (219 ng/L), and increase at GBTU (317 ng/L). There is a significant difference between PCE and ABE, between ABE and SCE, between the UV and river samples (UP and DWN), and between the two river samples (Table 8). There is no significant difference across the treatment facility from INF to UV (258 ng/L). There is a sharp decrease in solids citalopram concentrations from PCS (13.6 ng/g) to WAS (8.69 ng/g), though not significantly different, a significant decrease from WAS to DS (3.96 ng/g), and a significant decrease from PCS to DS (Table 10).

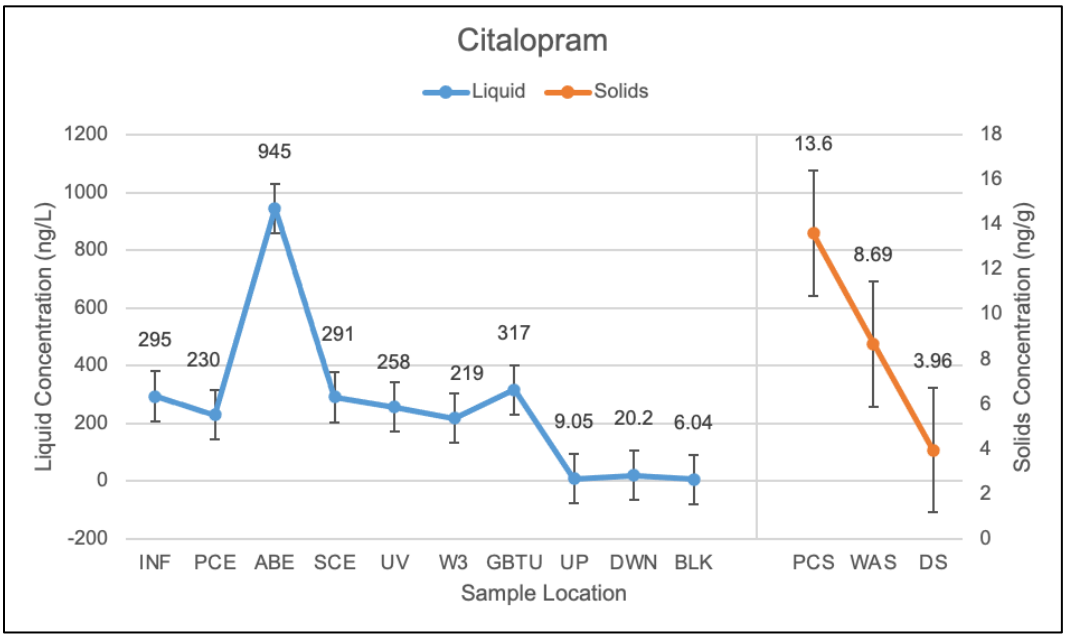

Figure 11: Mean Citalopram Concentrations

Primidone

Three outliers were detected from the liquid quartile analysis of primidone, one from each of the following sample locations: INF 2, SCE, and UP (Figure D.9). The INF 2 and SCE outlier concentrations were both above the upper fence. The UP outlier was caused by

low detection values, where the data mainly consisted of ND values. Two outliers were detected from the solids analysis, one from PS and DS (Figure D.20). The PS outlier was above the upper fence, and the DS was below the lower fence. All primidone outliers were removed from the datasets.

Primidone concentrations in liquid also present an unexpected result: they remain generally unchanged (average 46.1 ng/L) as they progress through the LSWRF processes (Figure 12). Liquid primidone concentrations decrease sharply from PCE (48.6 ng/L) to ABE (24.5 ng/L). There is no significant difference in liquid concentrations within the LSWRF (Table 8). There is no significant difference across the treatment facility from INF to UV (44.5 ng/L). There is a significant difference between UV and river samples (UP and DWN) and between the two river samples. Solids primidone concentrations slightly decreased from PCS (0.65 ng/g) to WAS (0.55 ng/g) and sharply increased from WAS to DS (1.23 ng/g), all significantly different (Table 10).

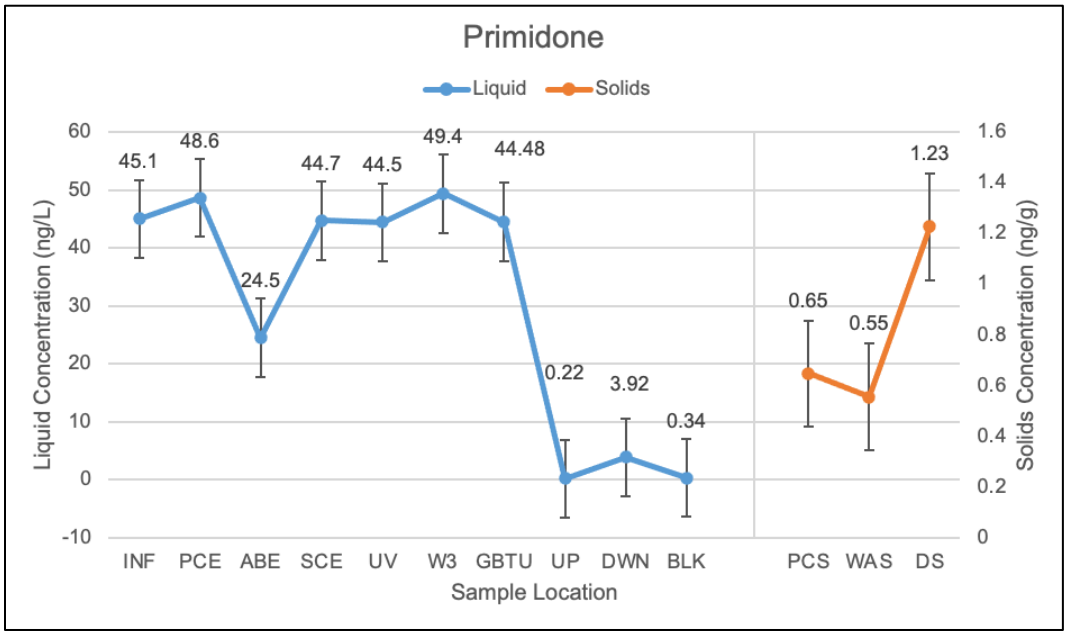

Figure 12: Mean Primidone Concentrations

Sulfamethoxazole

Four outliers were detected from the liquid quartile analysis of sulfamethoxazole, one from each of the following sample locations: ABE, SCE, GBTU, and BLK (Figure D.10). The ABE outlier had a concentration above the upper fence. The remaining outliers were caused by low detection values, where the data mostly consisted of ND values. No outliers were detected from the solids quartile analysis (Figure D.21). All sulfamethoxazole outliers were removed from the dataset.

Sulfamethoxazole concentrations in liquid generally decrease as they progress through the LSWRF treatment processes (Figure 13). Liquid sulfamethoxazole concentrations consistently decrease from INF (930 ng/L) to SCE (31.6 ng/L). There is no significant difference in liquid concentrations within the LSWRF except from ABE (382 ng/L) to SCE (Table 8). There is a significant decrease across the treatment facility from INF to UV (22.3 ng/L). There is a slight, though not significantly different, decrease in solids sulfamethoxazole concentrations from PCE (12.5 ng/g) to WAS (11.7 ng/g), a significant decrease from WAS to the DS (0.18 ng/g), and significant difference from PCS to DS (Table 10).

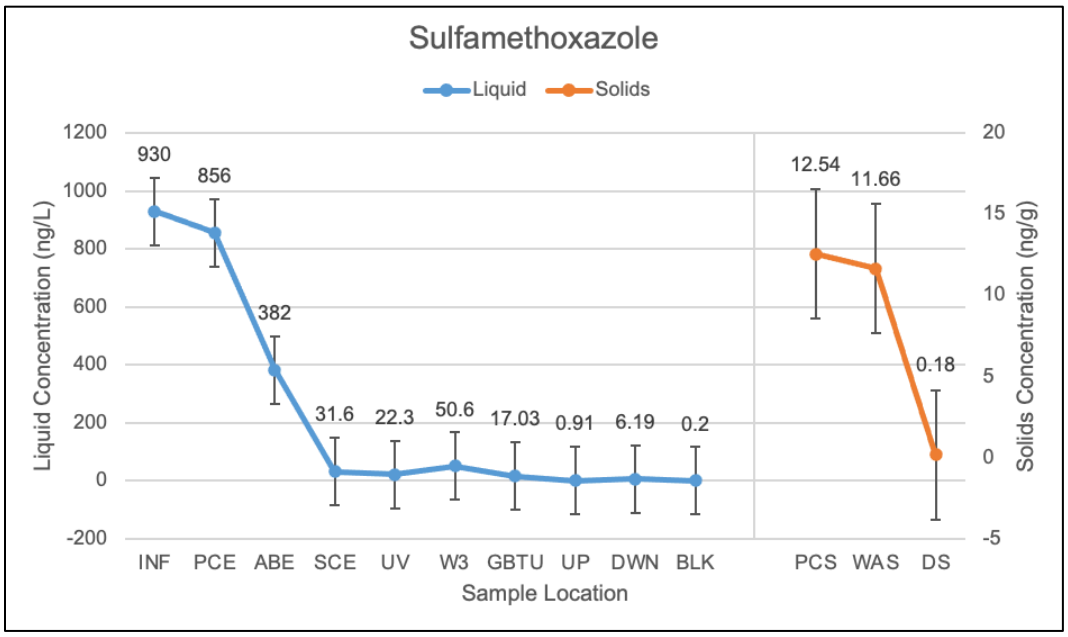

Figure 13: Mean Sulfamethoxazole Concentrations

Warfarin

All the warfarin results were, unfortunately, ND values. It is hypothesized that the LC/MS was not sensitive enough to detect warfarin in either liquid or solids samples. Warfarin was one of the two ECs with a higher detection limit of 100 ng/L for liquid samples and 10.0 ng/g for solids samples. Previous research conducted by the City of Boise reported a very low warfarin concentration and a low detection frequency. It was initially hypothesized that monthly testing over six months with this research would have shown an increased detection frequency, though that was not the case, and no conclusions about warfarin in the LSWRF can be made at this time. Methods optimization specifically for warfarin could allow for detection in future liquid and solids samples from the LSWRF.

Temporal Results

Student's t-test results generally show no significant differences for liquid and solids seasonal average concentrations. Seasonal averages were defined as winter: February and March; spring: April and May; and summer: June and July. There are significant differences

in some liquid seasonal average concentrations of albuterol, cimetidine, citalopram, and primidone (Table 11). Only acetaminophen solids seasonal average concentrations exhibit a significant difference (Table 12). It was difficult to determine if these differences were due to temporal variation or the general variability of wastewater due to the dataset's limited size.

Acetaminophen

There is no significant difference in liquid seasonal average acetaminophen concentrations (Table 11). The solids seasonal average acetaminophen concentrations are only significantly different between spring and summer, meaning the p-value was less than 5% (Table 12). Seasonal and total average solids acetaminophen concentrations are shown in Figure 14 and follow the same trend. Acetaminophen concentrations are highest in winter, which coincides with cold and flu season.

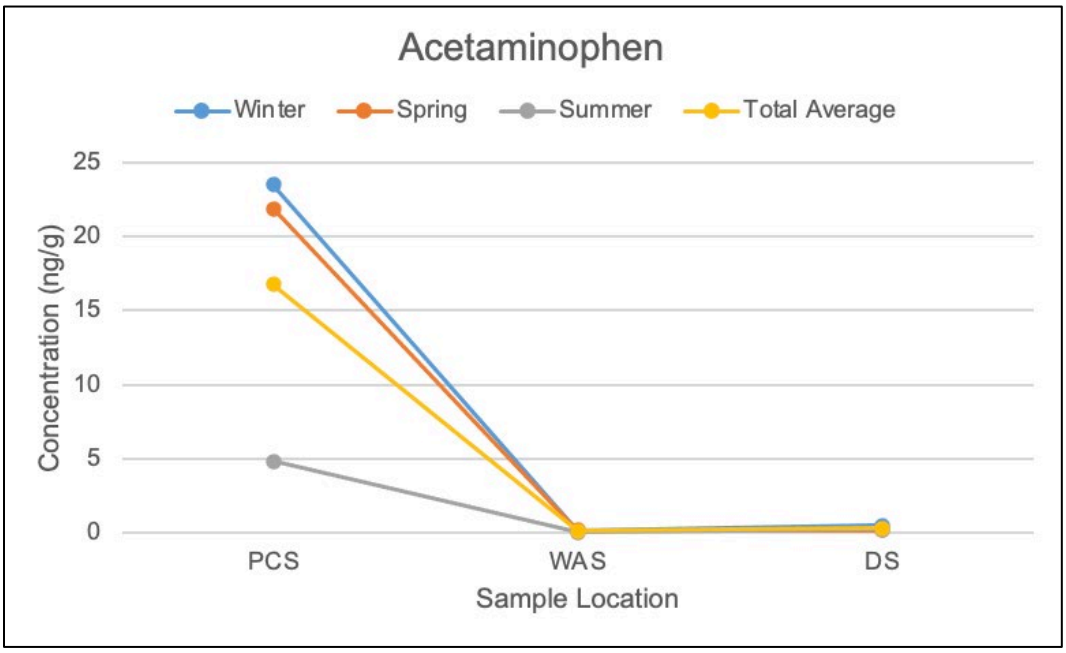

Figure 6: Temporal Solids Acetaminophen Concentrations

Albuterol

There are significant differences (p-value < 5%) between the liquid seasonal averages of albuterol (Table 11). This is no significant difference in solids seasonal average albuterol concentrations (Table 12). Seasonal and total average liquid albuterol concentrations are shown in Figure 14 and appear to not follow the same trend. Winter liquid albuterol concentrations exhibited the most extreme changes. Liquid albuterol concentrations are highest in spring, which coincides with allergy season.

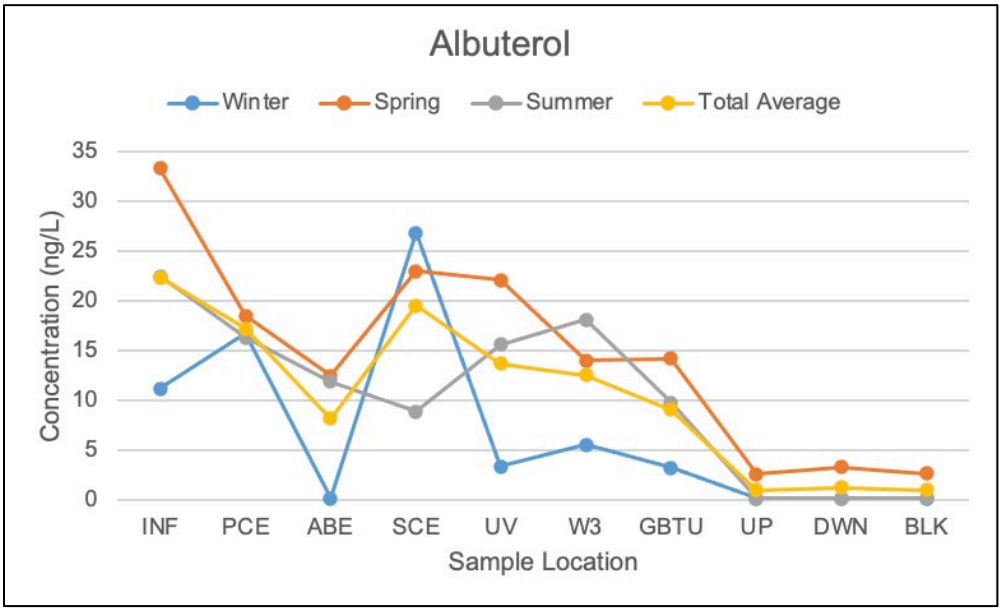

Figure 7: Temporal Liquid Albuterol Concentrations

Cimetidine

There are significant differences (p-value < 5%) between the liquid seasonal averages of cimetidine (Table 11). There is no significant difference in solids seasonal average cimetidine concentrations (Table 12). Seasonal and total average liquid cimetidine concentrations are shown in Figure 15 and appear to follow the same trend. Liquid cimetidine concentrations are generally highest in spring.

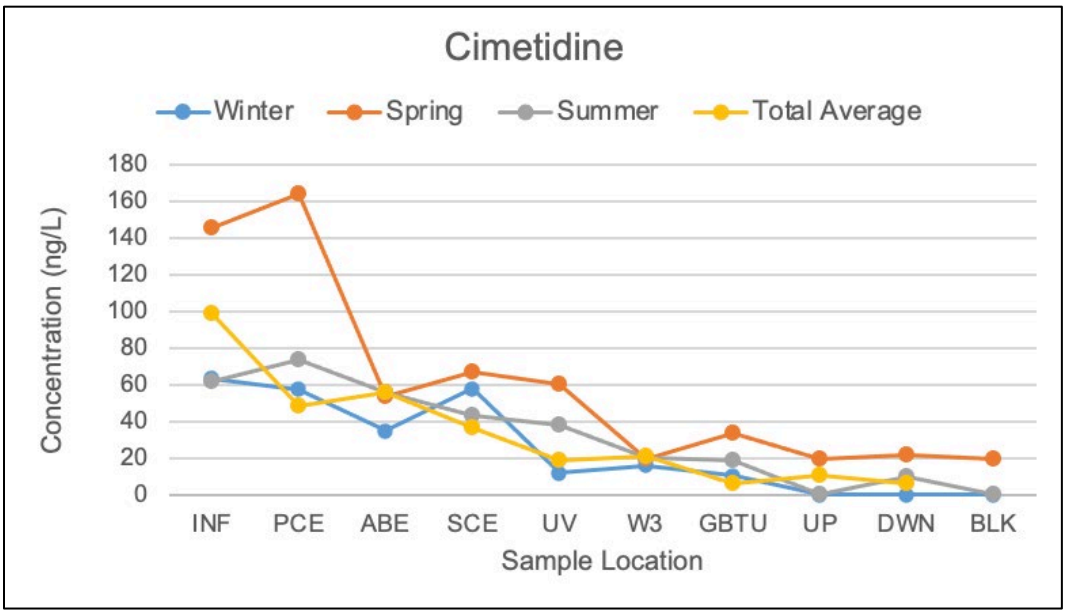

Figure 8: Temporal Liquid Cimetidine Concentrations

Citalopram

Liquid seasonal average citalopram concentrations are only significantly different (p-value < 5%) between winter and spring (Table 11). There is no significant difference in solids seasonal average citalopram concentrations (Table 12). Seasonal and total average liquid citalopram concentrations are shown in Figure 17 and follow the same trend. Liquid citalopram concentrations are generally highest in spring.

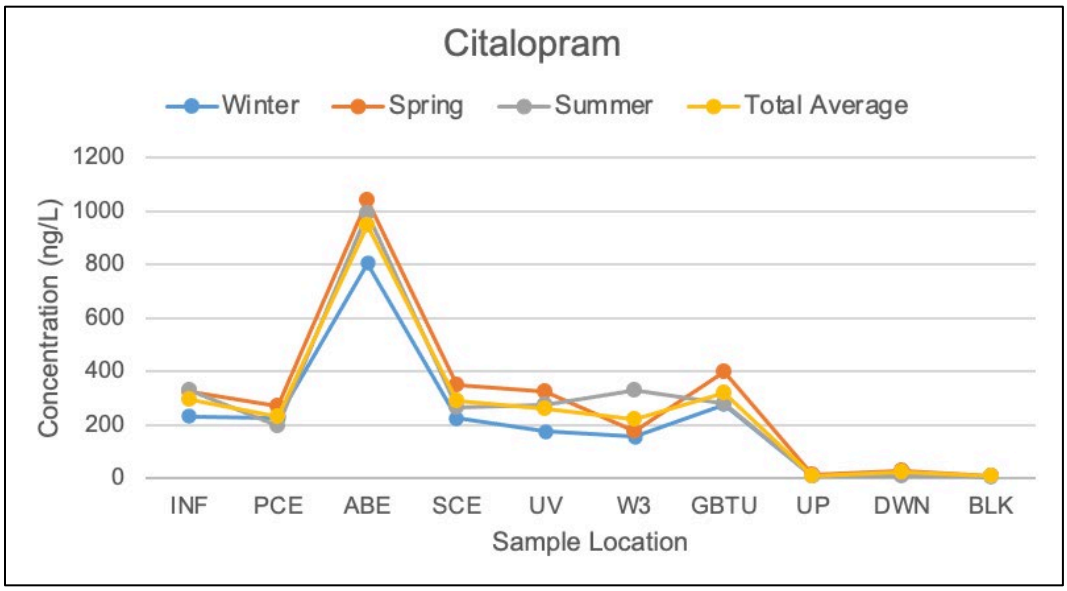

Figure 9: Temporal Liquid Citalopram Concentrations

Primidone

There are significant differences (p-value < 5%) between the liquid seasonal averages of primidone (Table 11). There is no significant difference in solids seasonal average primidone concentrations (Table 12). Seasonal and total average liquid primidone concentrations are shown in Figure 18 and follow the same trend. Liquid primidone concentrations are generally highest in winter.

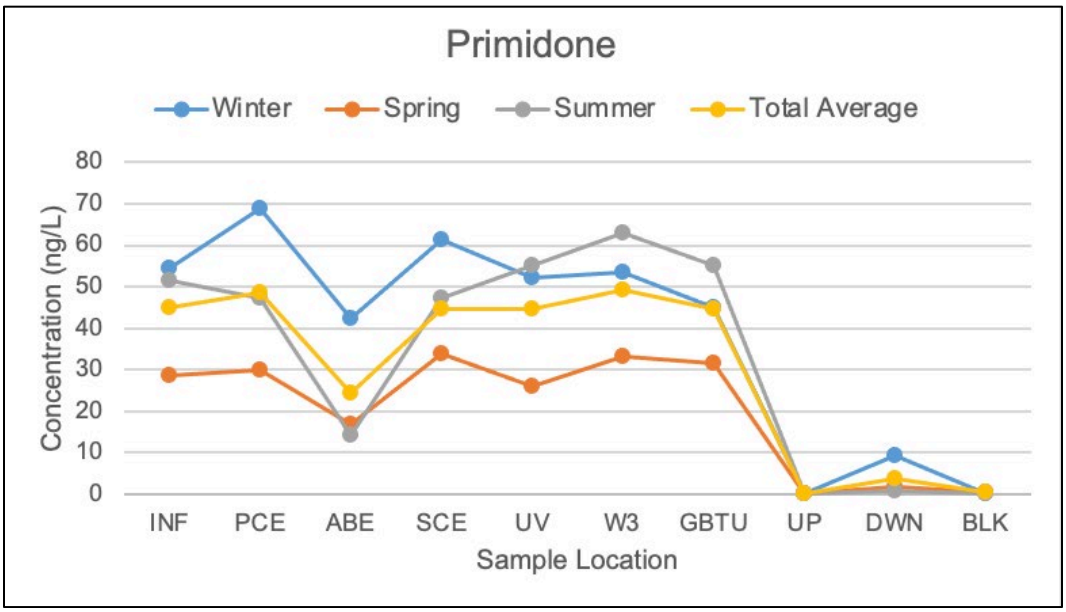

Figure 10: Temporal Liquid Primidone Concentrations

Summary

Statistical analyses were completed to answer the original research questions: (1) what are the concentrations entering the LSWRF; (2) how are the EC concentrations being affected by the LSWRF's system; and (2) are EC concentrations varying temporally within the LSWRF system? Seven trends resulted from these analyses, four relative to the liquid concentrations and three relative to solids concentrations.

First, liquid concentrations of acetaminophen, ibuprofen, salicylic acid, sulfamethoxazole, and theophylline exhibit various levels of reduction in PCE, and concentration sharply decreased from ABE to SCE. This first trend results in the highest overall liquid percent reductions, ranging from 97.6% to 99.8% (Table 13). Second, liquid concentrations of albuterol, cimetidine, and primidone exhibit a sharp decrease in ABE, followed by an increase in SCE. There is more variation in the overall percent removal of these constituents due to their individual behavior, ranging from 1.30% to 59.1% (Table 13).

Table 13: Liquid Percent Removal

Constituent	Percent Removal
Over-The-Counter Drugs and Personal Care Products	
Acetaminophen	99.8%
Ibuprofen	98.0%
Salicylic Acid	98.5%
Common Prescription Drugs	
Albuterol	38.7%
Cimetidine	59.1%
Methylphenidate	-56.7%
Theophylline	99.6%
Specialized Prescription Drugs	
Citalopram	12.5%
Primidone	1.30%
Sulfamethoxazole	97.6%

Third, liquid concentrations of citalopram and methylphenidate are highest in ABE. Liquid citalopram concentrations in ABE then sharply decreased in SCE and were similar to INF concentrations. The percent reduction of citalopram was only 12.5% (Table 13) due to the concentration increase. Liquid methylphenidate concentrations consistently increased from INF to SCE. It appears that the LSWRF liquid processes serve as a source of methylphenidate in that percent reduction is -56.7%, indicating a concentration increase. Fourth, liquid concentrations of cimetidine, methylphenidate, and primidone increased slightly from INF to PCE. These liquid trends will be discussed further in the discussion section.

Fifth, solids concentrations of acetaminophen, citalopram, methylphenidate, salicylic acid, and sulfamethoxazole are either gradually removed through the treatment processes or there is a sharp decrease from PCS to WAS, while there is no additional reduction in DS. This fifth trend results in percent reductions ranging from 70.9% to 98.5% (Table 14). Sixth, solids concentrations of albuterol, cimetidine, and primidone generally increase from PCS to

DS. The LSWRF solids processes increase the concentration because these constituents had negative percent reductions. Seventh, solids concentrations of ibuprofen and theophylline sharply decrease from PCS to WAS and sharply increase from WAS to DS. There is an immediate increase in concentration at the DS. For theophylline, this resulted in moderate removal of theophylline (78.2%). However, for ibuprofen, this resulted in a negative percent removal (-19.2%), indicating a concentration increase. All the solids trends will be discussed further in the discussion section.

Table 14: Solids Percent Removal

Constituent	Percent Removal
Over-The-Counter Drugs and Personal Care Products	
Acetaminophen	98.3%
Ibuprofen	-19.2%
Salicylic Acid	91.2%
Common Prescription Drugs	
Albuterol	-213%
Cimetidine	-315%
Methylphenidate	86.9%
Theophylline	78.3%
Specialized Prescription Drugs	
Citalopram	70.9%
Primidone	-89.4%
Sulfamethoxazole	98.5%

Lastly, only acetaminophen, albuterol, cimetidine, citalopram, and primidone appear to exhibit temporal variation. There otherwise appears to be no temporal variation of the constituents in this study. Liquid concentrations of four constituents (albuterol, cimetidine, citalopram, and primidone) exhibit significantly different seasonal averages. Only acetaminophen solids exhibit a significant difference in the seasonal average concentrations. Temporal variations are highly dependent on individual constituents, which will be discussed further in the discussion section.

Reduction Discussion

Overall, this research gained more information on the ECs in the LSWRF's wastewater and successfully studied the removals in the liquid and solids treatment processes. Table 15 displays the loading rate of the ECs from their initial to final loading rate. These conversions were based on 11 MGD flow for the liquid samples, 39,000 lbs/day for the PCS solids samples, and 55,000 lbs/day for the DS solids samples (Baker, 2023).

Table 15: Annual Loading Rates

Constituent	In Flow (lbs/yr)	Out Flow (lbs/yr)	Net Change (lbs/yr)	Net Change (%)
Over-The-Counter Drugs and Personal Care Products				
Acetaminophen	552	1.35	551	99.8%
Ibuprofen	176	17.2	158	90.2%
Salicylic Acid	1592	29.9	1562	98.1%
Common Prescription Drugs				
Albuterol	0.75	0.46	0.29	38.3%
Cimetidine	3.03	1.26	1.78	58.6%
Methylphenidate	0.63	0.96	-0.33	-53.3%
Theophylline	857	3.66	853	99.6%
Specialized Prescription Drugs				
Citalopram	10.1	8.69	1.37	13.6%
Primidone	1.52	1.51	0.011	0.75%
Sulfamethoxazole	31.3	0.75	30.6	97.6%

Four trends were observed in liquid results, and three trends in solids results. Percent changes were graphed to clearly illustrate trends rather than absolute concentrations. All percent changes were calculated by dividing individual sample location concentrations by the highest concentration of each EC.

The first was a large liquid concentration reduction from PCE to ABE (acetaminophen, ibuprofen, salicylic acid, sulfamethoxazole, and theophylline). The solids concentrations are generally reduced by two orders of magnitude. Trends such as percent concentration change are shown for liquid (Figure 19) and solids (Figure 20).

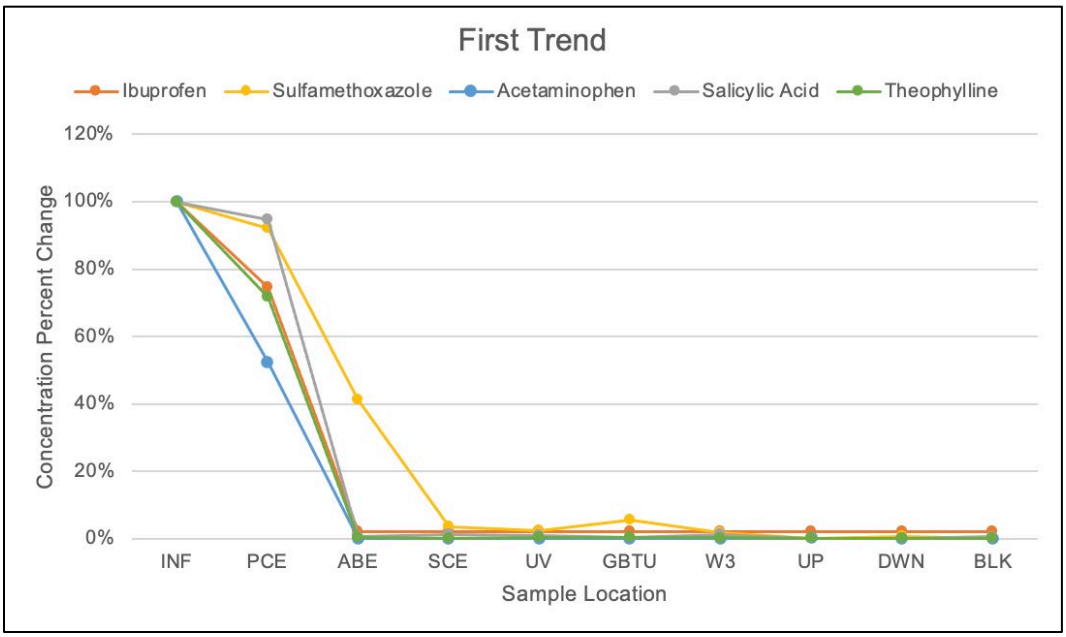

Figure 19: Liquid Results: Significant Reduction Trend

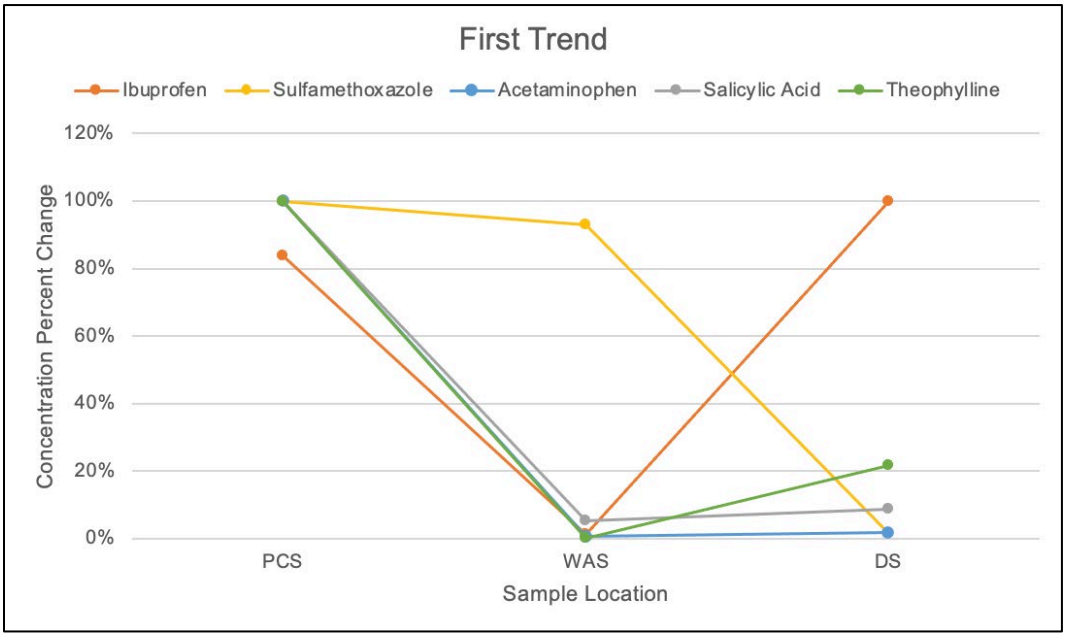

Figure 20: Solids Results: Significant Reduction Trend

The second trend consisted of liquid concentration reduction from INF to ABE, followed by a general increase from ABE to SCE and consistent reductions from SCE to

GBTU (albuterol, cimetidine, and primidone). Solids concentrations generally increase by an order of magnitude. Trends such as percent concentration change are shown for liquid (Figure 21) and solids (Figure 22).

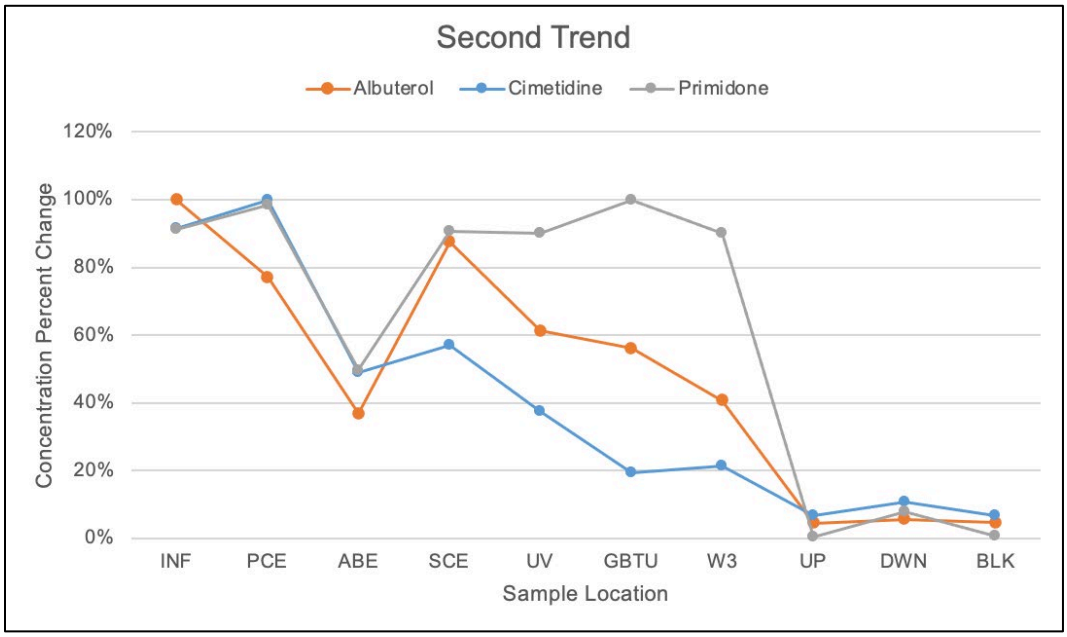

Figure 21: Liquid Results: Sequestering and Release Trend

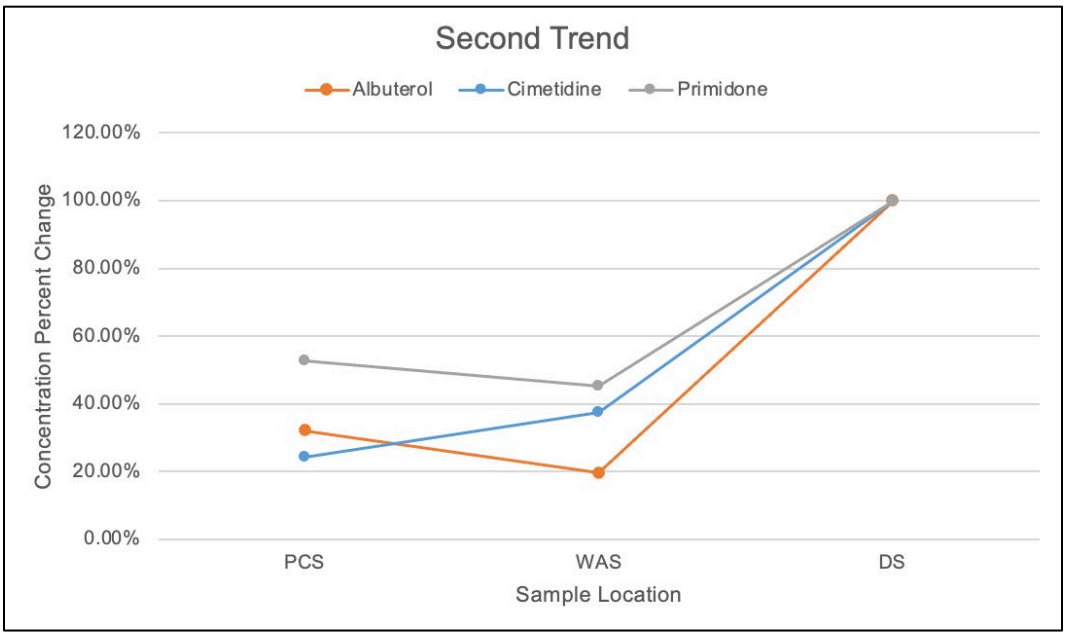

Figure 22: Solids Results: Sequestering and Release Trend

The third trend consisted of increased concentrations in liquid samples from INF to ABE, followed by reductions to INF levels, and no net change through GBTU (citalopram and methylphenidate). Solids concentrations are generally reduced by an order of magnitude. Liquid concentrations (cimetidine, methylphenidate, and primidone) increased slightly from INF to PCE. Trends such as percent concentration change are shown for liquid (Figure 23) and solids (Figure 24).

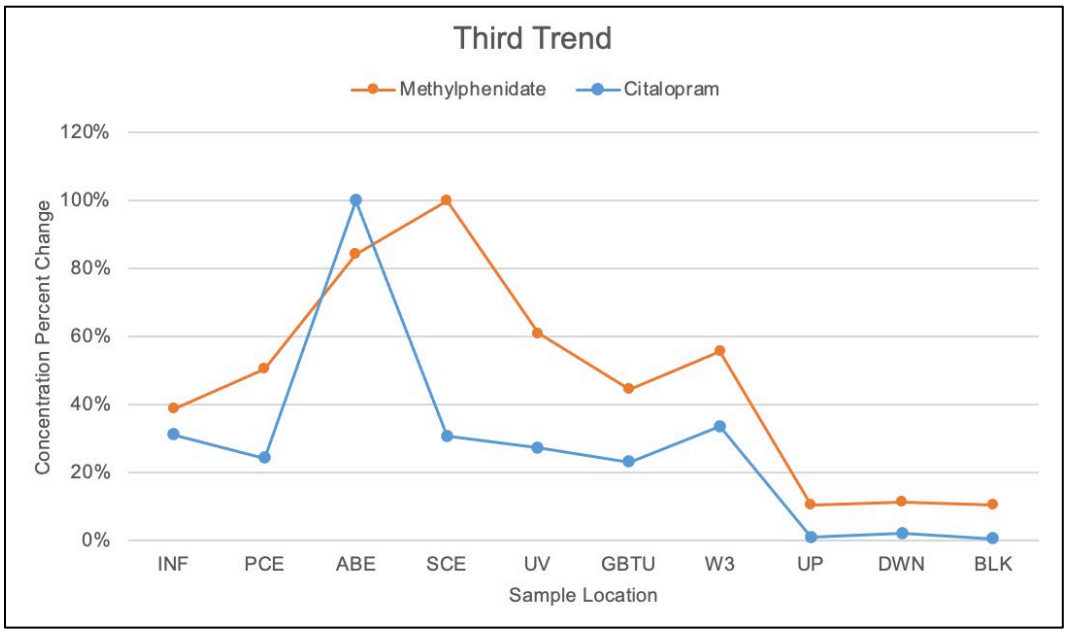

Figure 23: Liquid Results: Continual Release

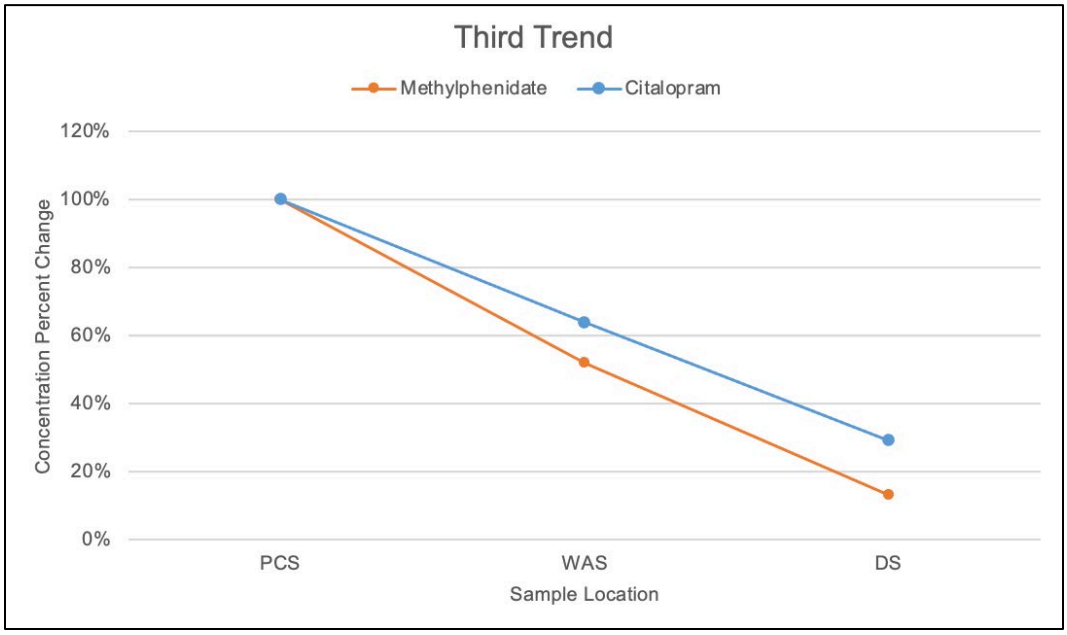

Figure 24: Solid Results: Continual Decrease

Significant Reduction Trend

The concentration reductions in the first trend are generally small for liquid acetaminophen from INF to PCE (Figure 19). Larger reductions occur following biological

treatment with lower ABE and SCE concentrations, representing a fourth order of magnitude decrease following biological treatment. This large reduction was mirrored in the solids results (Figure 19). Although there is a significant reduction in concentration, it cannot be concluded that acetaminophen was transformed from the parent constituent to its metabolites because it could have been biologically transformed (Susa, 2023). It can be concluded that there is a significant net reduction of acetaminophen (99.8%) from the LSWRF because metabolites were not an aim of this study (Table 15).

Ibuprofen concentration reductions follow the same trend as acetaminophen in the liquid results (Figure 19). The ibuprofen concentrations in the solids generally remained the same despite a significant reduction from PCS to WAS (Figure 20). This reduction was believed to be caused by combining the primary and secondary solids. Ibuprofen that is separated from the liquid treatment process with the primary solids is not biologically treated and does not experience a reduction in concentration. Thus, causing the increase once the p solids are combined. There is a net reduction of ibuprofen (90.2%) from the LSWRF (Table 15).

Salicylic acid and sulfamethoxazole liquid concentrations follow trends like those of acetaminophen and ibuprofen, with significant reductions following biological treatment in ABE and SCE. This trend is also apparent in the results of the solids. There is a significant net reduction of salicylic acid (98.1%) and sulfamethoxazole (97.6%) from the LSWRF. Theophylline liquid concentrations also follow a trend like the previous four constituents. The concentration of theophylline in the solids results generally reduced despite increasing from WAS to DS, though not as extreme as ibuprofen. There is a significant net reduction of theophylline (99.6%) from the LSWRF.

Sequestering and Release Trend

Albuterol liquid concentrations are consistently reduced from INF to ABE and increase in SCE, followed by consistent reductions (Figure 21). It is believed that albuterol is biologically sequestered in the aeration basin and released in the secondary clarifier. This phenomenon occurs with phosphorus when the aeration basins are not operating under ideal conditions (Baker, 2023). The release of the albuterol in the liquid resulted in an increase in concentration in the solids. There is a slight reduction of albuterol (38.7%) from the LSWRF (Table 15).

Cimetidine liquid concentrations follow a similar trend to albuterol, with two key differences. First, the concentration increased slightly from INF to PCE (Figure 21) due to the reintegration of the GBTU liquid. The gravity belt thickener removes excess liquid from the secondary clarifier solids before digestion. Excess GBTU liquid is redirected to the LSWRF headworks downstream of the INF sampling location. Second, liquid cimetidine concentrations increased slightly from ABE to SCE in contrast to albuterol. Cimetidine is believed to be biologically sequestered in the aeration basin and released in the secondary clarifier. Cimetidine solids concentrations consistently increase more than fourfold from PCS to DS (Figure 22). There is a moderate net reduction of cimetidine (58.4%) from the LSWRF (Table 15).

Primidone liquid concentrations follow a trend like that of cimetidine. The concentration increased from INF to PCE due to the reintegration of GBTU liquid. The concentration then decreased by half in ABE, then nearly doubled in SCE (Figure 21). There is essentially no reduction of liquid primidone. Primidone solids concentration doubled

despite a slight decrease from PCS to WAS (Figure 22). There is an insignificant net reduction of primidone (0.75%) from the LSWRF (Table 15).

Continuous Release Trend

Reductions are generally small for liquid citalopram concentrations from INF to PCE. Citalopram generally increased following biological treatment with fourfold higher ABE concentrations. The concentration then decreased in SCE and remained nearly unchanged (Figure 23). There is essentially no reduction of liquid citalopram. The sharp increase of liquid citalopram in ABE may be due to biological activity. Phosphorus enters the LSWRF in two forms (Baker, 2023): (1) first as orthophosphate, which is easy to identify and treat through primary sedimentation, and (2) second as polyphosphates, which are difficult to treat due to their chemical properties. Polyphosphates require biological transformation to orthophosphate and removal through sedimentation (Burton et al., 2013). It is believed that citalopram also requires biological transformation, as evidenced in the fourfold concentration increase in the aeration basin that is then broken down in the secondary clarifier. This theory is supported because other pharmaceuticals used to treat depression contain similar structures as citalopram. These compounds include escitalopram and didemethylcitalopram. Citalopram solids concentrations consistently decrease more than fourfold from PCS to DS (Figure 24). Notably, citalopram in WAS decreased, which could be a result either of (1) biological transformation to a metabolite, or (2) biologically mediated transfer from solids into liquid (Figure 24). Both processes would cause decreased concentration in the solids citalopram and be consistent with liquid concentration trends. There is an insignificant net reduction of citalopram (14.0%) from the LSWRF (Table 15).

Methylphenidate liquid concentrations consistently increased more than twofold from INF to SCE, then decreased in GBTU and remained nearly unchanged (Figure 23). There is an increase in liquid methylphenidate. Concentrations increased from INF to PCE due to the redirection of GBTU liquid. It is believed that methylphenidate also requires biological transformation through aeration basin and continuing in the secondary clarifier to treat the methylphenidate. This theory is supported because other pharmaceuticals used to treat ADHD contain similar structures as methylphenidate. These compounds include dichloro-methylphenidate, chlorobenzyl-methylphenidate, and serdexmethylphenidate. Concentrations are reduced by over half from SCE to UV. Methylphenidate solids concentrations consistently decreased by an order of magnitude (Figure 24). It is believed that methylphenidate follows the same biological processes as citalopram. There is a moderate net increase of methylphenidate (52.4%) from the LSWRF (Table 15).

Temporal Analysis Discussion

Temporal variations did not present overarching trends like treatment variations and are dependent on individual constituents. Five constituents exhibited statistically significant temporal variations: acetaminophen, albuterol, cimetidine, citalopram, and primidone. Acetaminophen solids concentrations in PCS were nearly five times higher in winter and spring compared to summer. This is likely due to the increased use of medications, whose common ingredient is acetaminophen, used during the winter-spring cold and flu season (FDA, 2018).

Albuterol liquid concentrations exhibited the most extreme changes in winter. Concentrations are highest in spring, given that albuterol is a standard medication used to treat asthma. There are three types of asthma: (1) sports-induced, (2) seasonal allergy-

induced, and (3) occupational, which is triggered by workplace irritants (*Asthma - Symptoms and Causes - Mayo Clinic*, 2022). Increased albuterol concentrations are likely due to its seasonal use for treating all three types of asthma associated with outdoor spring sports, spring allergies, and spring agricultural activities. Summer liquid albuterol concentrations follow a trend like that of spring results, though significantly lower. It is believed that temperature affects biological treatment, causing a rapid release of albuterol in the secondary clarifier. Biological treatment efficiency is influenced significantly by the temperature dependence of the reaction rate (Alisawi, 2020).

Cimetidine liquid concentrations in spring INF and PCE samples are an order of magnitude higher than in winter and summer (Figure 16). Though not significantly different, the remaining spring concentrations were higher than winter and spring. Significant differences in INF and PCE are believed to be from wastewater variability rather than temporal variations.

Citalopram liquid concentrations followed the same trend very closely across the seasons. However, the winter concentrations are nearly half the concentration of the spring INF samples and approximately one-quarter the concentration of the spring ABE samples (Figure 17). Significant differences are believed to be due to wastewater variability rather than temporal variations.

Primidone liquid concentrations are like citalopram in that all the samples closely follow the same trend across the seasons. Significant differences are believed to be due to wastewater variability rather than temporal variations due to lack clear indication of temporal influence.

Overall, the temporal analysis is challenging to accomplish since there is high variability in wastewater, and the size of the dataset causes limitations. The current dataset is unable to definitively conclude the reasoning posed to explain the seasonal differences. Temporal variability could be due to the seasonal usage of an individual constituent, which adds another layer of complexity to this analysis. Increased sampling and extended sampling period would be required to obtain enough data to properly assess the temporal variation.

CHAPTER 4: CONCLUSIONS AND FUTURE WORK

This chapter will conclude the analysis and discussion of the analyzed ECs. In addition, the potential future pathways to expand this research will be discussed.

Conclusions

The presence of ECs at detectable levels in the environment and LSWRF is cause for further investigation. Several studies found the effects of ECs on aquatic organisms detrimental (Brodin et al., 2014; Huggett et al., 2002). Increased constituent concentrations in the environment pose the threat of bioaccumulation and contamination (water, soil, and crops) that could affect humans. A better understanding of individual constituents' behavior is ultimately required for limiting discharge to surface waters and land applications affecting groundwater. This research was conducted by analyzing liquid and solids from the LSWRF.

This research was able to provide new insights into the behavior of the ten ECs that were detected within the LSWRF. Six ECs studied (acetaminophen, cimetidine, ibuprofen, salicylic acid, sulfamethoxazole, and theophylline) exhibited significant liquid reductions, whereas five ECs studied (acetaminophen, citalopram, methylphenidate, sulfamethoxazole, and theophylline) exhibited significant solids reductions. All ECs studied, except methylphenidate and primidone, exhibited net reduction from the LSWRF.

An analysis of temporal variation exhibited significant differences in acetaminophen solids concentrations and albuterol, cimetidine, citalopram, and primidone liquid concentrations. Temporal acetaminophen and albuterol concentration variations are likely due to their seasonal usage, whereas cimetidine, citalopram, and primidone differences may

be due to wastewater variability rather than temporal variability. Increased sampling frequency over a longer study period would provide better insights into this behavior. This study answered the research questions, yet much remains to learn about the behavior of ECs in an operational activated sludge WWTF.

Future Work

Discoveries remain to be made relative to ECs behavior in engineered and natural systems despite this research answering the questions posed. One potential pathway for expanding this research is to study solids at the West Boise Water Renewal Facility (West Boise). Solids from the LSWRF are combined with those at West Boise, necessitating studying liquid concentrations at that WWTF. Another potential research expansion pathway is further evaluation of the solids methods. Solids and associated aqueous concentrations were analyzed separately, and the results were combined for the total solids concentrations as previously discussed. Future studies could analyze samples individually to assess if the ECs are adsorbed to the surfaces of the solids or absorbed into the solids.

There are currently several unanswered questions about the fate of the ECs studied. One potential outcome is that the ECs are broken down into metabolite forms. There is the potential that some metabolites could be more harmful to the environment in that form. Emphasizing the need for increased research on ECs and their metabolites. Another potential expansion of this research would be monitoring metabolite compounds with their parent constituent. Studying its metabolites would give a better understanding of an EC's fate. Finally, increased sampling frequency over a longer duration is recommended for future projects to provide a more robust temporal variation analysis. Better correlations can be made, and errors reduced with larger datasets.

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APPENDIX A: LC/MS METHODS AND QUALITY CONTROL

Methods

LC-MS/MS analysis was performed using an Agilent 1290 Infinity II LC consisting of an Agilent 1290 Infinity II multi-sampler (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 made using Agilent MassHunter workstation software. Dihydro-carbamazepine was used as an internal standard for quantification.

Table A.1: Chromatographic Conditions

Parameter	Setting
Guard Column	Agilent Eclipse Plus C18, 2.1x1 mm, 1.8 µm
Analytical Column	Agilent Eclipse Plus C18, 2.1 x 150 mm, 1.8 µm
Column Oven	40 ±2 °C
Injection Volume	5 µL
Run Time	15 minutes
Autosampler Tempe	12 ±2 °C
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile

Table A.2: Liquid Chromatography Grade Settings

Time (min)	Flow (mL/min)	%A	%B
0	0.3	90	10
10	0.3	10	90
15	0.3	10	90

Table A.3: Mass Spectrometry Parameters

Parameter	Setting
MS Acquisition	Multiple reaction monitoring (MRM)
Ion Source Type	Agilent Jet Stream electrospray ionization
Drying Gas Temperature	250 °C
Drying Gas Flow	11 L/min
Nebulizer	40 psi
Sheath Gas Heater	400 °C
Sheath Gas Flow	12 L/min
Capillary	Positive: 4,000 V; Negative: 3000V
Nozzle Voltage	0 V
Precursor Ion and Production Ion Resolution	Unit
Compound-Specific Conditions	See **Table 4**

Table A.4: Compound-Specific Conditions: Precursor-to-Product Ion Transitions, Fragmentor, Collision Energies (CE), Cell Accelerator Voltage (CAV), and Retention Times (RT)

Compound Name	Precursor Ion	Product Ion	Dwell (ms)	Fragmentor (V)	CE[a] (V)	CAV[b] (V)	Polarity
Acetaminophen	152.1	110.1	20	84	16	4	Positive
Acetaminophen	152.1	65.1	20	86	36	4	Positive
Albuterol	240.2	222.1	20	94	8	4	Positive
Albuterol	240.2	148	20	94	20	4	Positive
Cimetidine	253.1	159	20	94	16	4	Positive
Cimetidine	253.1	95.1	20	94	36	4	Positive
Citalopram	325.2	109	20	131	32	4	Positive
Citalopram	325.2	262.1	20	131	20	4	Positive
Dihydrocarbamazepine	239.1	194	20	126	28	4	Positive
Dihydrocarbamazepine	239.1	180	20	126	48	4	Positive
Ibuprofen	205.1	161.1	20	67	4	4	Negative
Methylphenidate	234.1	84.1	20	109	32	4	Positive
Methylphenidate	234.1	56.1	20	109	60	4	Positive
Primidone	219.1	162.1	20	82	12	4	Positive
Primidone	219.1	91.1	20	82	28	4	Positive
Salicylic Acid	137	93.1	20	82	20	4	Negative
Salicylic Acid	137	65.1	20	82	36	4	Negative
Sulfamethoxazole	254.1	156	20	104	16	4	Positive
Sulfamethoxazole	254.1	92.1	20	104	32	4	Positive
Theophylline	181.1	124	20	108	20	4	Positive
Theophylline	181.1	69.1	20	108	32	4	Positive
Warfarin	309.1	251	20	118	20	4	Positive
Warfarin	309.1	163	20	118	12	4	Positive

[a] CE: Collision Energy

[b] CAV: Cell Accelerator Voltage

Quality Control

The following figures are the calibration curves of each EC researched in this study.

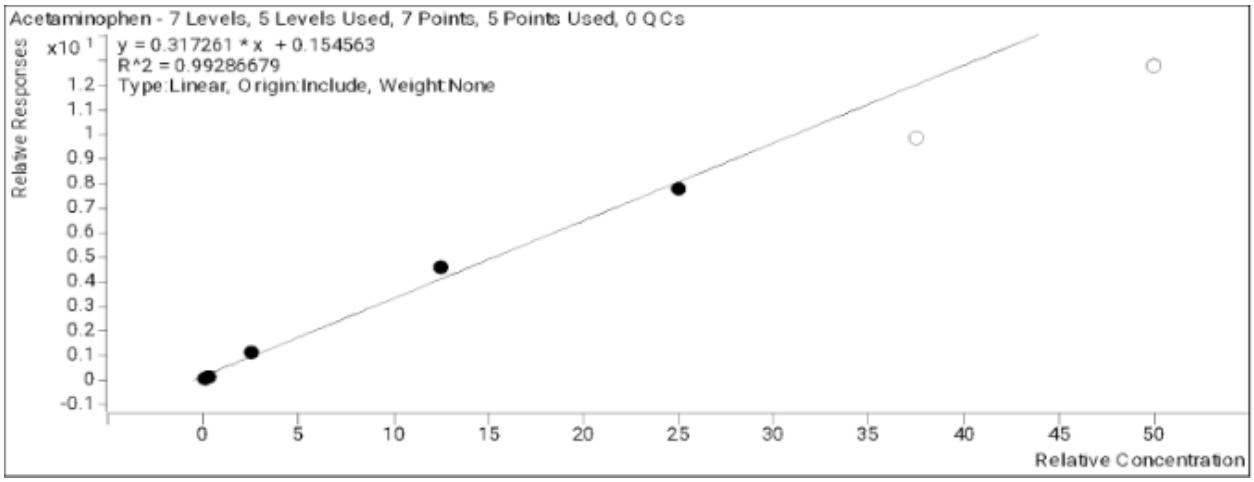

Figure A.1: Acetaminophen Calibration Curve

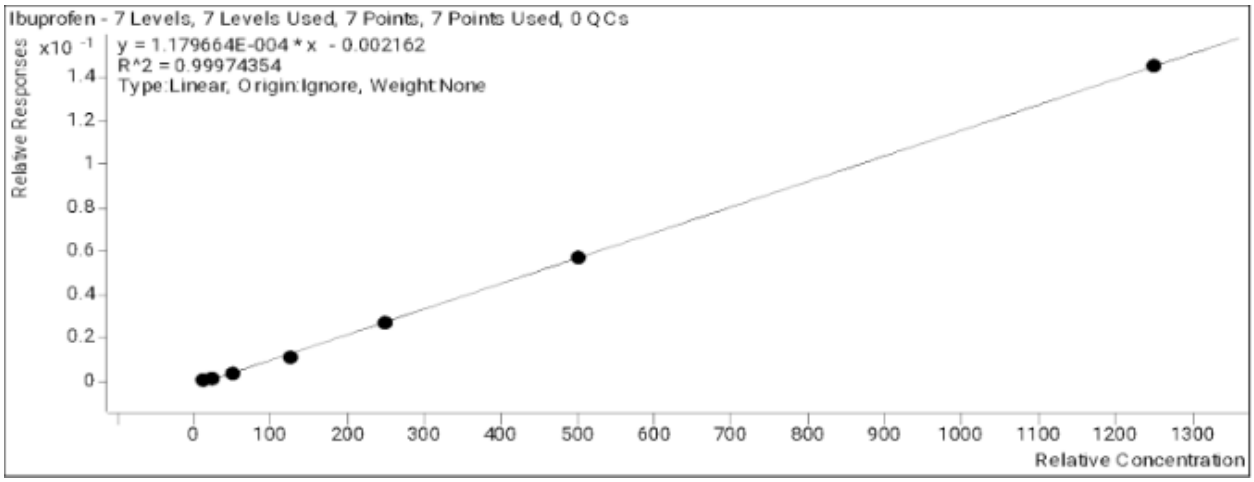

Figure A.2: Ibuprofen Calibration Curve

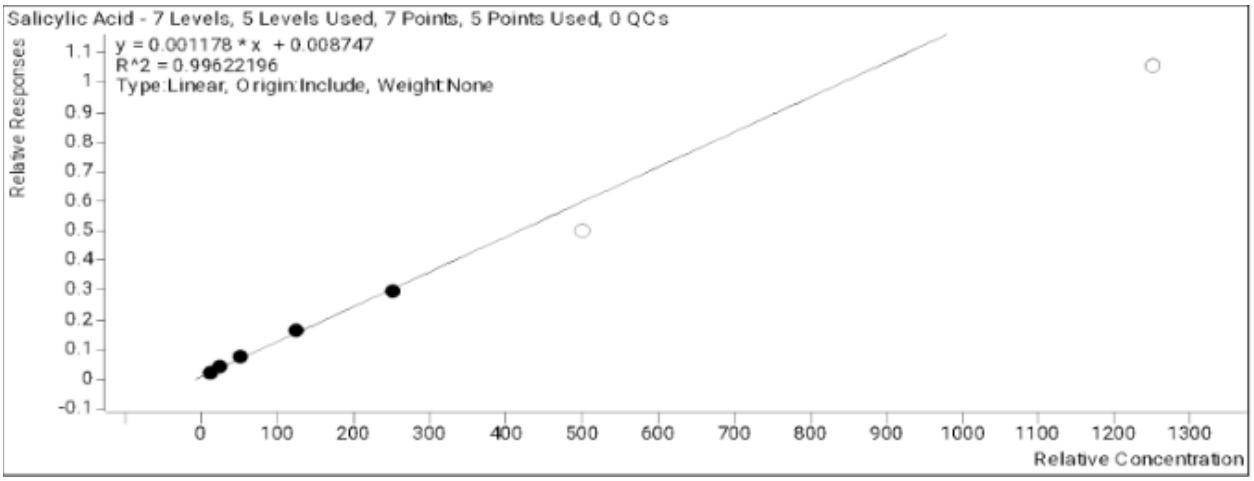

Figure A.3: Salicylic Acid Calibration Curve

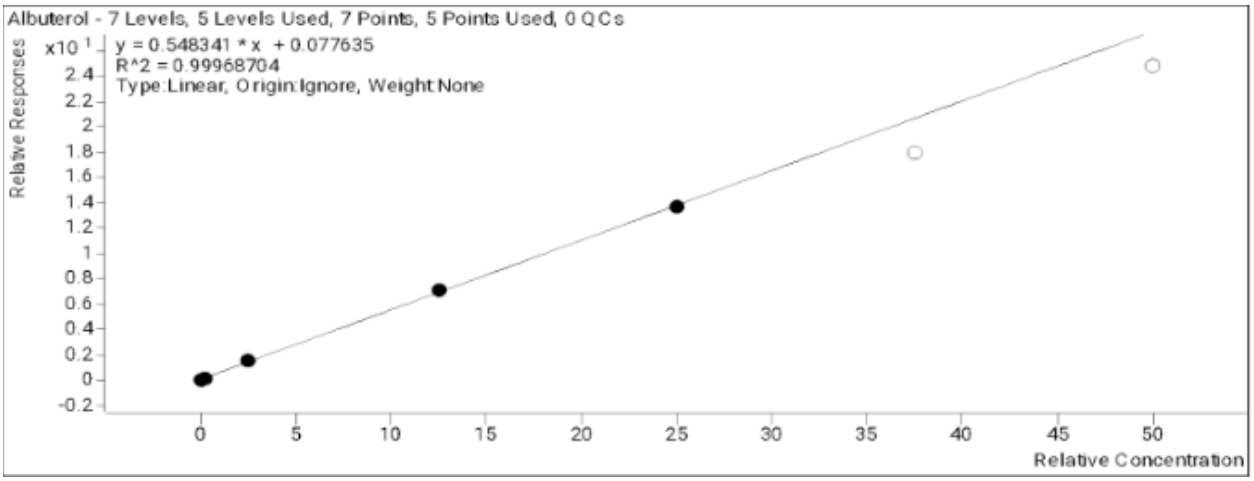

Figure A.4: Albuterol Calibration Curve

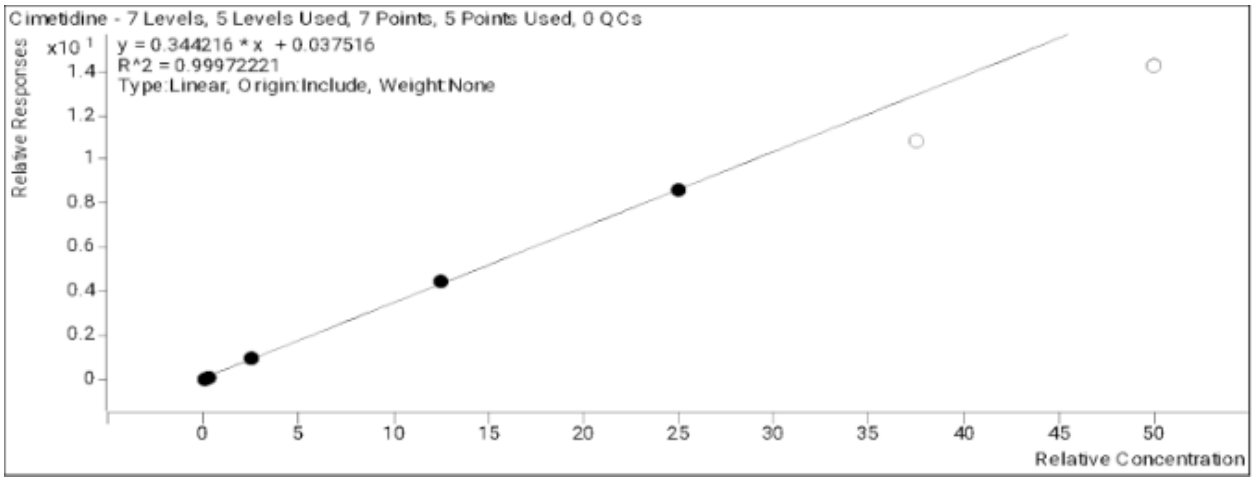

Figure A.5: Cimetidine Calibration Curve

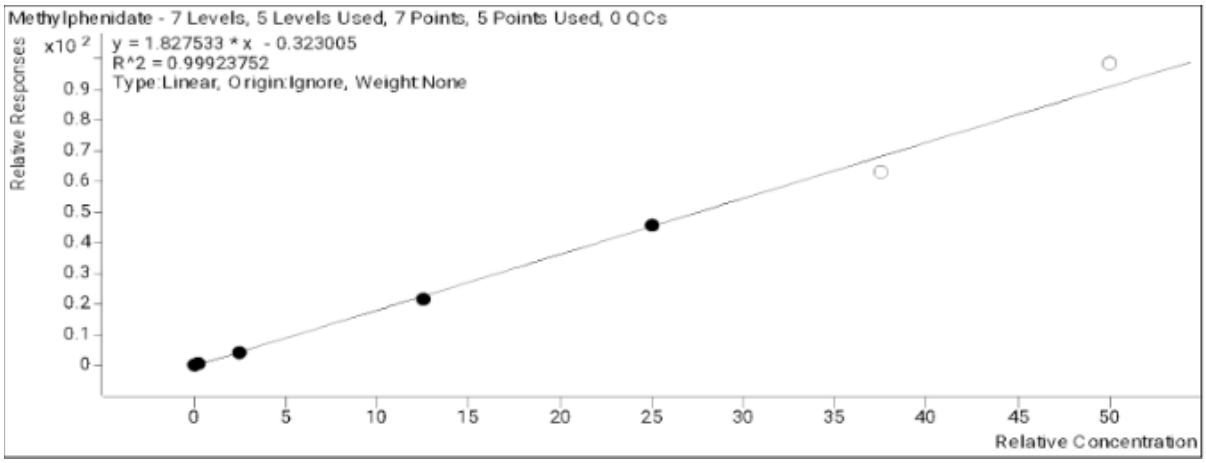

Figure A.6: Methylphenidate Calibration Curve

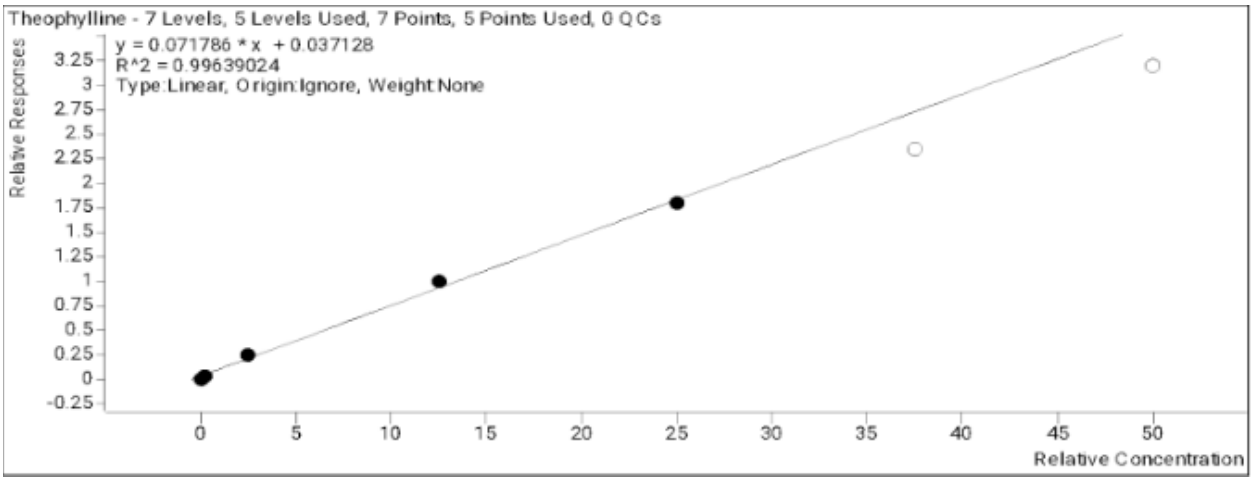

Figure A.7: Theophylline Calibration Curve

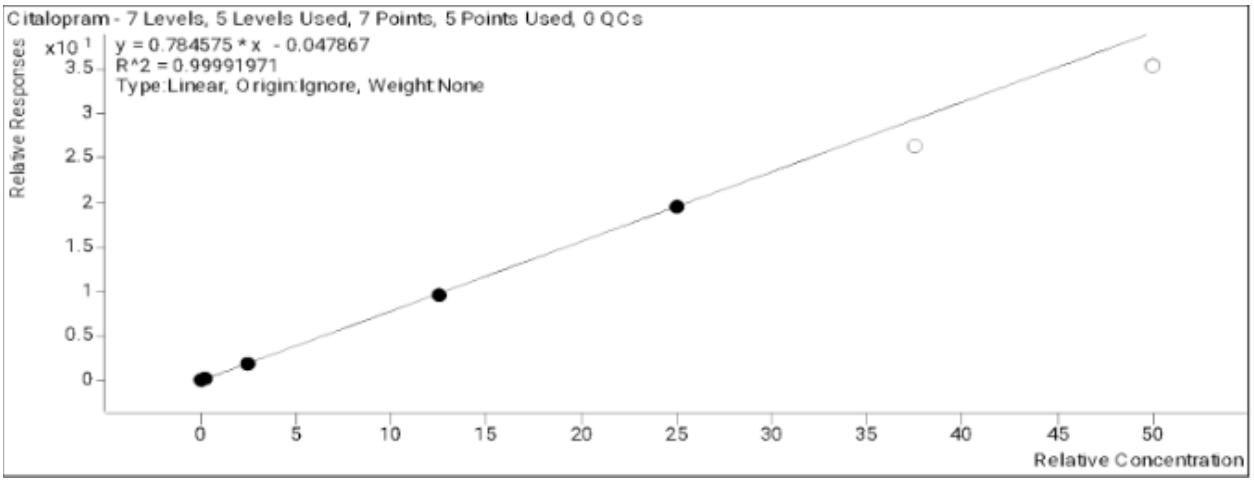

Figure A.8: Citalopram Calibration Curve

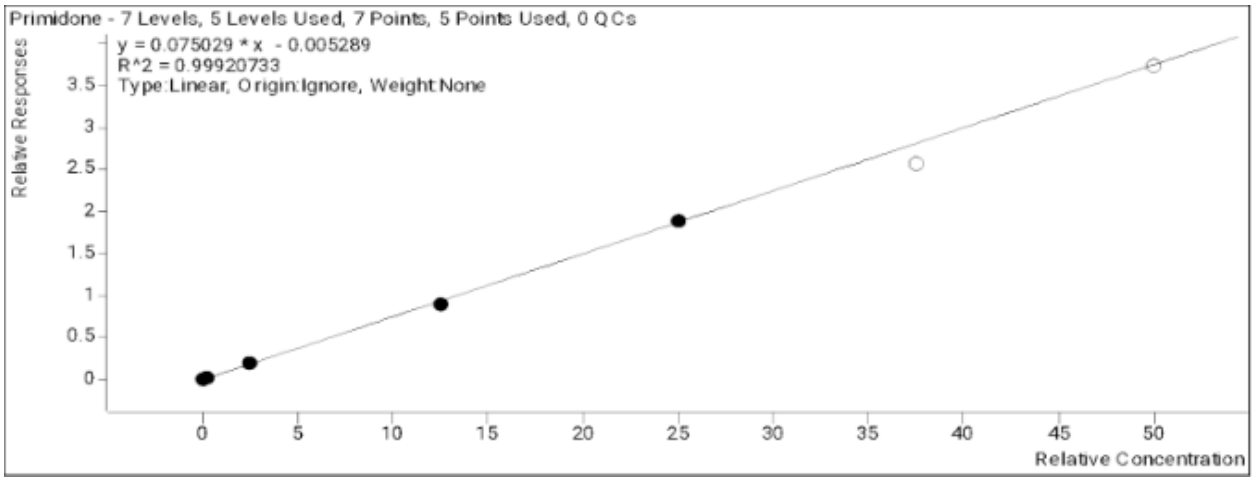

Figure A.9: Primidone Calibration Curve

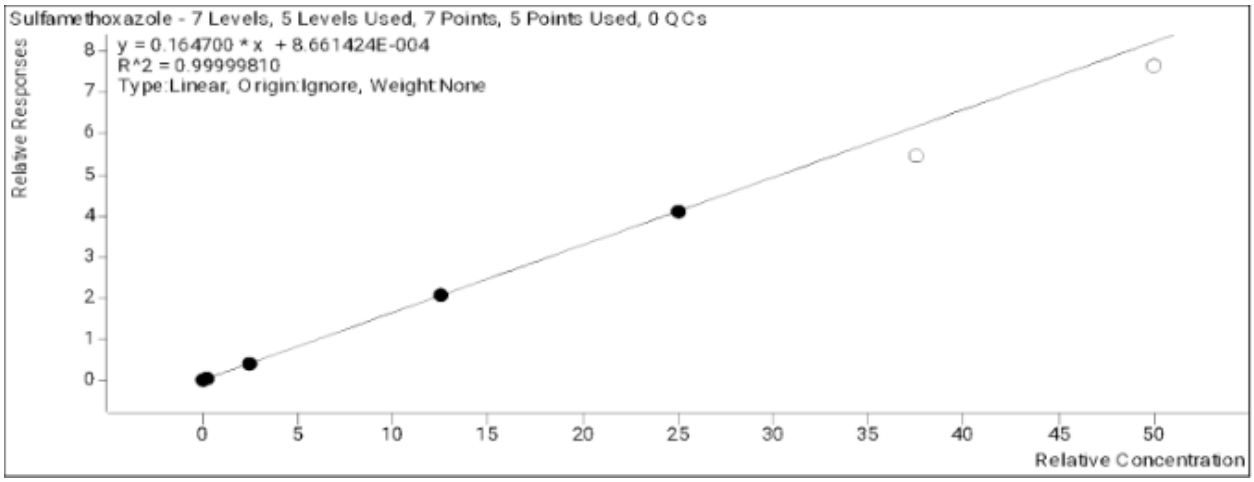

Figure A.10: Sulfamethoxazole Calibration Curve

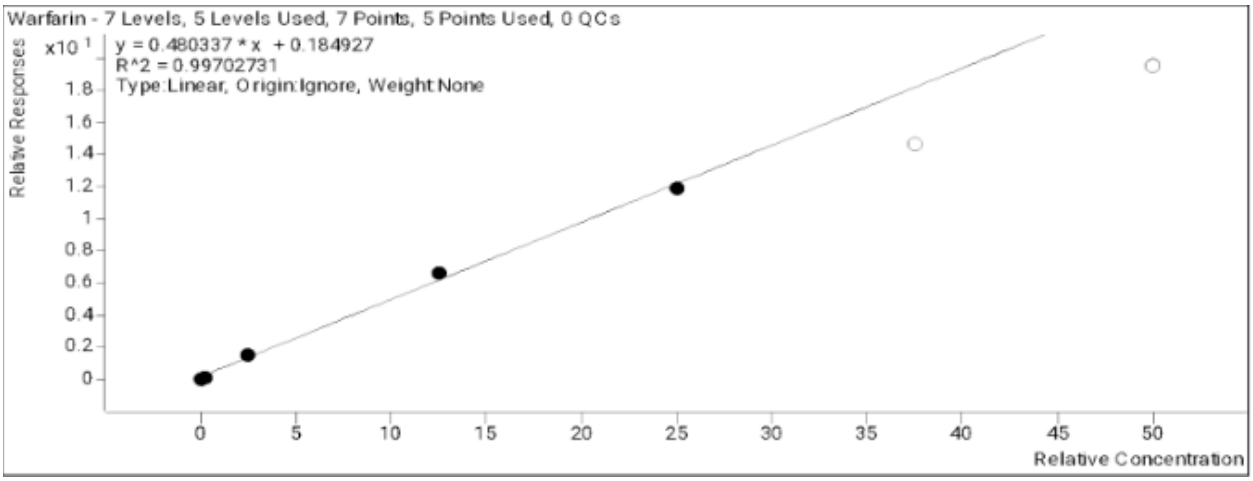

Figure A.11: Warfarin Calibration Curve

The following are the standard chromatograms of each EC researched in this study. Pure forms of the ECs were procured and provided to Dr. Pu. The units were calibrated to the standards. Blank solvent samples were also periodically run through the LC/MS device to clean the sensor and reduce instrument drift.

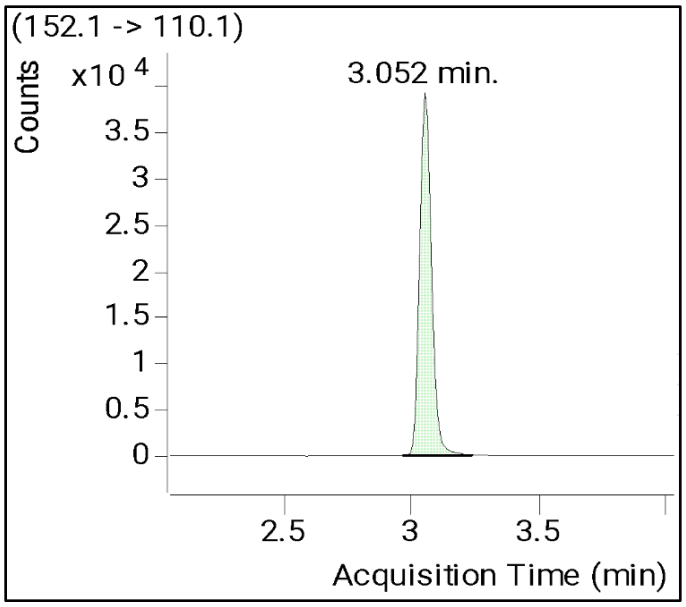

Figure A.12: Acetaminophen Standard Chromatogram

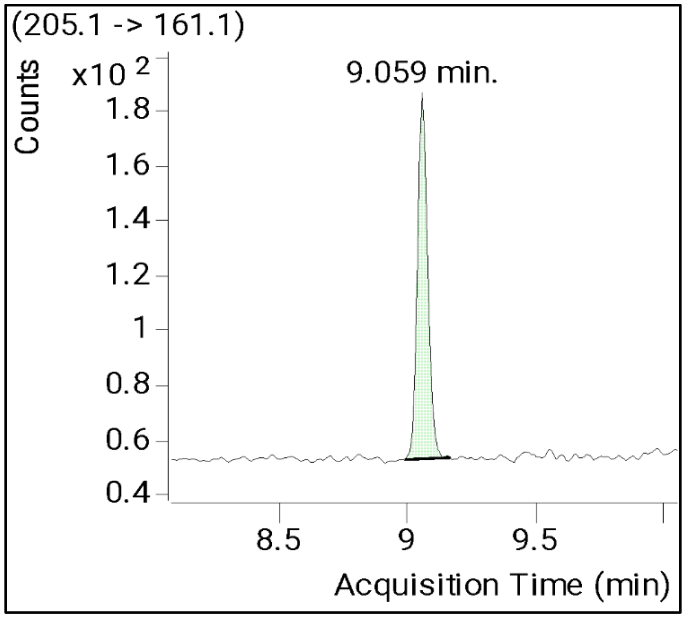

Figure A.13: Ibuprofen Standard Chromatogram

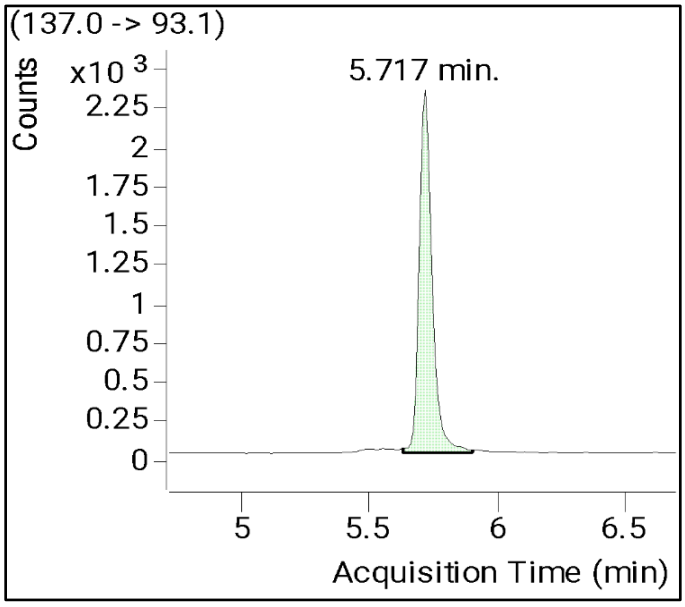

Figure A.14: Salicylic Acid Standard Chromatogram

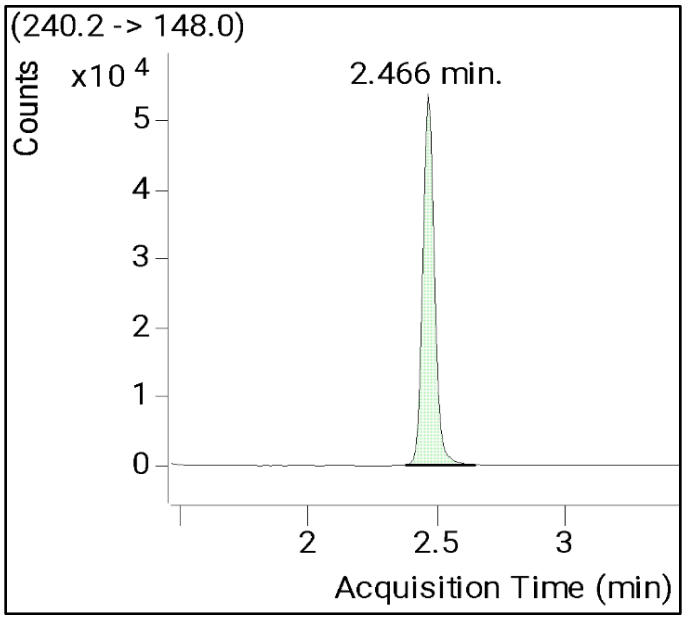

Figure A.15: Albuterol Standard Chromatogram

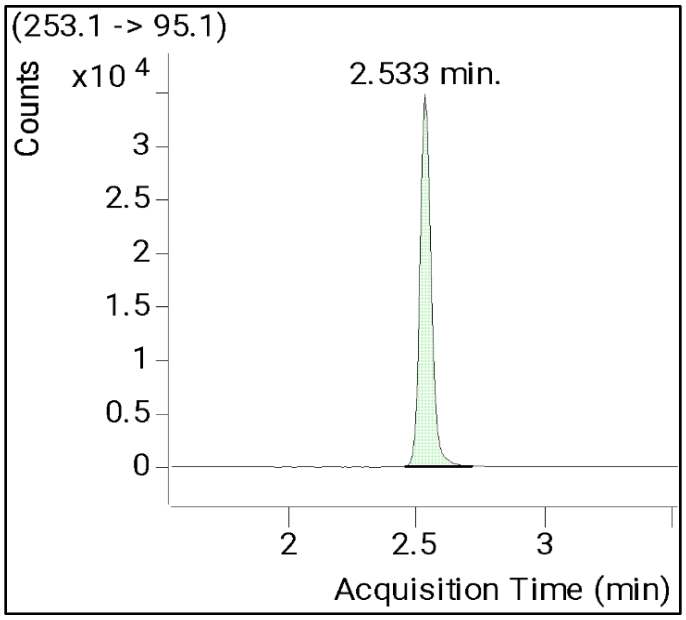

Figure A.16: Cimetidine Standard Chromatogram

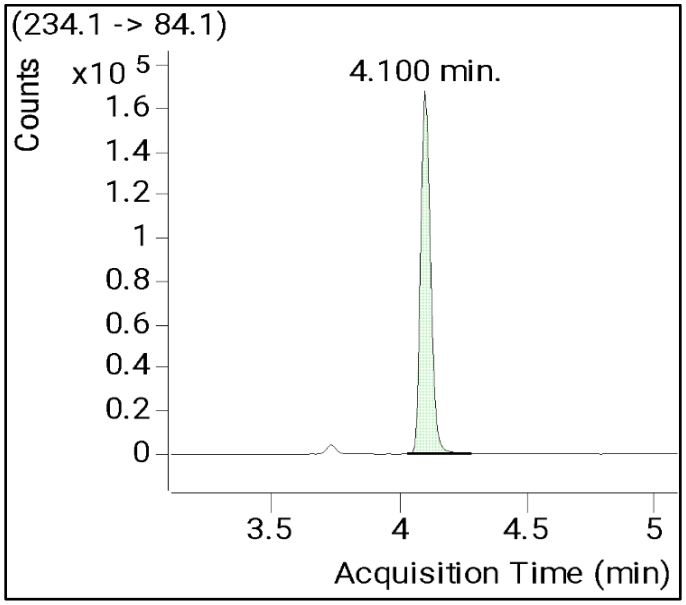

Figure A.17: Methylphenidate HCl Standard Chromatogram

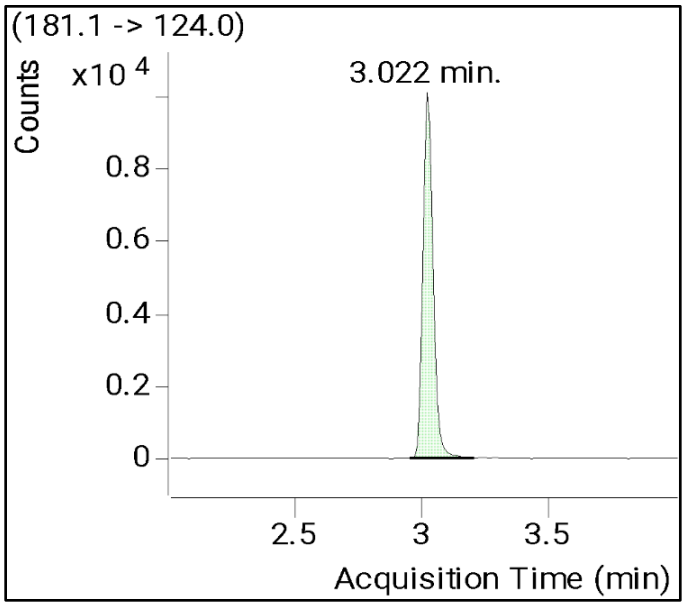

Figure A.18: Theophylline Standard Chromatogram

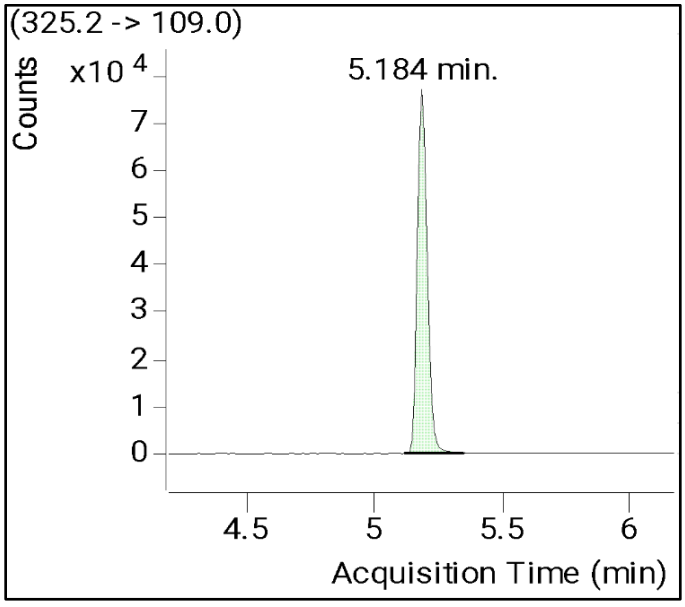

Figure A.19: Citalopram Standard Chromatogram

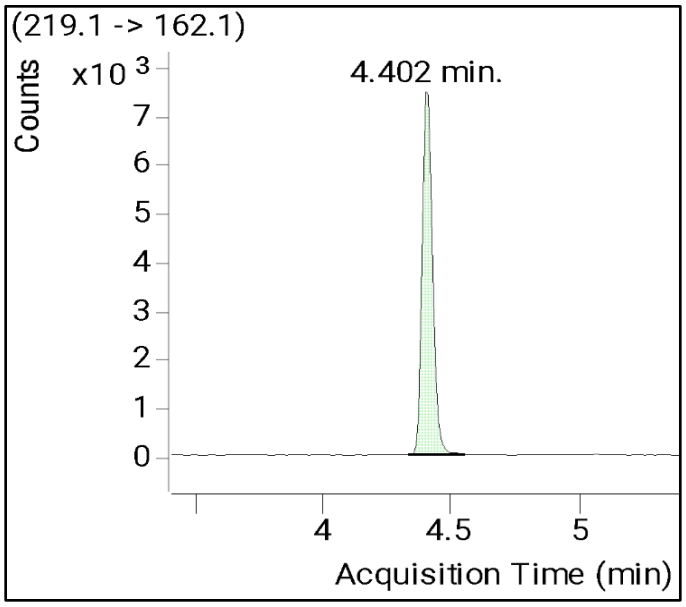

Figure A.20: Primidone Standard Chromatogram

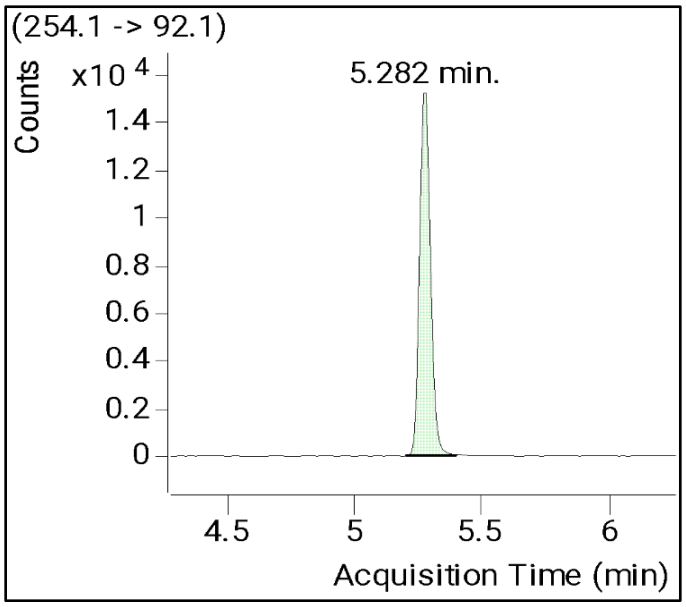

Figure A.21: Sulfamethoxazole Standard Chromatogram

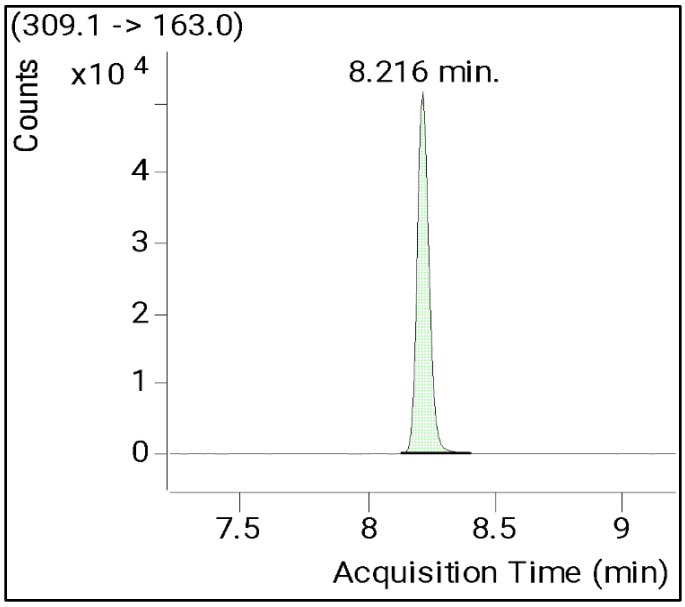

Figure A.22: Warfarin Standard Chromatogram

MATLAB Code

The following MATLAB code compared each constituent's mean sample location concentration versus the mean BLK concentration.

```
%Student's T-Test Code for the Liquid Samples versus the Blanks

clear; clc;

%Reading in data
format long g
fid = fopen('liquid_datafile_no_outliers.m','r');
x = fscanf(fid,'%f',[11 66]);
A = zeros(66,11);
for j = 1:11
    for n = 1:6
        i = 1+(n-1)*11;
        k = n+(j-1)*6;
        A(k,:) = x(j,i:i+10)';
    end
end


%T-test of constituents (Sample Location)
names = ["Albuterol" "Cimetidine" "Theophylline" "Acetaminophen"
"Methylphenidate" ...
    "Primidone" "Citalopram" "Sulfamethoxazole" "Salicylic Acid"
"Warfarin" "Ibuprofin"];
```

```
      for i = 1:10
          [h1(i),p1(i)] = ttest(A(1:6,i),A(1:6,11),'Alpha',.05);
          [h2(i),p2(i)] = ttest(A(7:12,i),A(7:12,11),'Alpha',.05);
          [h3(i),p3(i)] = ttest(A(13:18,i),A(13:18,11),'Alpha',.05);
          [h4(i),p4(i)] = ttest(A(19:24,i),A(19:24,11),'Alpha',.05);
          [h5(i),p5(i)] = ttest(A(25:30,i),A(25:30,11),'Alpha',.05);
          [h6(i),p6(i)] = ttest(A(31:36,i),A(31:36,11),'Alpha',.05);
          [h7(i),p7(i)] = ttest(A(37:42,i),A(37:42,11),'Alpha',.05);
          [h8(i),p8(i)] = ttest(A(43:48,i),A(43:48,11),'Alpha',.05);
          [h9(i),p9(i)] = ttest(A(49:54,i),A(49:54,11),'Alpha',.05);
          [h10(i),p10(i)] = ttest(A(55:60,i),A(55:60,11),'Alpha',.05);
          [h11(i),p11(i)] = ttest(A(61:66,i),A(61:66,11),'Alpha',.05);
      end

      varNames =
{'Constituent','INF1_BLK','INF2_BLK','PE_BLK','ABE_BLK','SCE_BLK','UV_BLK'
,...
          'W3_BLK','GBTU_BLK','UP_BLK','DWN_BLK'};
      H = [h1;h2;h3;h4;h5;h6;h7;h8;h9;h10;h11];
      disp(table(names',H(:,1),H(:,2),H(:,3),H(:,4),H(:,5),H(:,6),H(:,7),.
..
          H(:,8),H(:,9),H(:,10),'VariableNames',varNames));

      P = [p1;p2;p3;p4;p5;p6;p7;p8;p9;p10;p11];
      disp(table(names',
P(:,1),P(:,2),P(:,3),P(:,4),P(:,5),P(:,6),P(:,7),...
          P(:,8),P(:,9),P(:,10),'VariableNames',varNames));
```

Table A.5: Student's T-Test Results Blank P-Values

Student's T-Test Blank Liquid Results				
Over-The-Counter Drugs and Personal Care Products				
Sample Location	**Acetaminophen**	**Ibuprofen**	**Salicylic Acid**	
INF vs BLK	0.005	0.002	0.015	
PCE vs BLK	0.002	0.0003	0.010	
ABE vs BLK	0.359	$9.32*10^{-5}$	0.831	
SCE vs BLK	0.363	NA	0.342	
UV vs BLK	0.3	NA	0.466	
W3 vs BLK	0.363	NA	0.810	
GBTU vs BLK	0.363	NA	0.799	
UP vs BLK	0.250	NA	0.374	
DWN vs BLK	0.190	NA	0.374	
Common Prescription Drugs				
Sample Location	**Albuterol**	**Cimetidine**	**Methylphenidate**	**Theophylline**
INF vs BLK	0.026	0.0149	0.139	0.007
PCE vs BLK	0.008	0.008	0.124	0.013
ABE vs BLK	0.031	0.003	0.032	0.038
SCE vs BLK	0.016	0.011	0.014	0.135
UV vs BLK	0.011	0.004	0.005	0.094
W3 vs BLK	0.014	0.173	0.0002	0.029
GBTU vs BLK	0.014	0.021	0.017	0.049
UP vs BLK	0.576	0.391	0.299	0.182
DWN vs BLK	0.299	0.254	0.113	0.363
Specialized Prescription Drugs				
Sample Location	**Citalopram**	**Primidone**	**Sulfamethoxazole**	**Warfarin**
INF vs BLK	0.003	0.020	0.006	NA
PCE vs BLK	0.003	0.006	0.011	NA
ABE vs BLK	0.0004	0.025	0.004	NA
SCE vs BLK	0.001	0.009	0.074	NA
UV vs BLK	0.001	0.006	0.121	NA
W3 vs BLK	0.008	0.006	0.085	NA
GBTU vs BLK	0.0004	0.008	0.141	NA
UP vs BLK	0.086	0.363	0.175	NA
DWN vs BLK	0.033	0.102	0.108	NA

APPENDIX B: SAMPLE PREPARATION TABLES

Table B.1 Liquid Sample Preparation

Sample Number	Sample Type	Collection Date	Preparation Date	Sample Location	Sample pH	Sample Volume (mL)	QC Volume (mL)	Pre-Rotovap Volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate Volume (mL)	Pre N2 Volume (mL)	Post N2 Volume (mL)	Reconstituted Volume (mL)
WW1001	Influent 1	2/26/22	4/14/22	INF	2.42	25	25	50	2.34	10	12.34	0.49	1
WW1002	Influent 2	2/26/22	4/14/22	INF	2.54	25	25	50	1.89	10	11.89	0.45	1
WW1003	Primary Clarifier Effluent	2/26/22	4/14/22	PE	2.55	25	25	50	5.58	10	15.58	0.55	1
WW1004	Aeration Basin Effluent	2/26/22	4/14/22	ABE	2.43	25	25	50	5.15	10	15.15	0.6	1.5
WW1005	Secondary Clarifier Effluent	2/26/22	4/14/22	SCE	2.57	25	25	50	3.99	10	13.99	0.49	1
WW1006	UV Disinfection	2/26/22	4/18/22	UV	2.59	25	25	50	5.2	10	15.2	0.59	1
WW1007	Chlorinated Utility Water	2/26/22	4/18/22	W3	2.47	25	25	50	2.99	10	12.99	0.58	1
WW1008	Gravity Belt Thickener Underflow	2/26/22	4/18/22	GBTU	2.55	25	25	50	3.05	10	13.05	0.42	1
WW1009	Upstream	2/26/22	4/19/22	UP	2.57	25	25	50	4.58	10	14.58	0.58	1
WW1010	Downstream	2/26/22	4/19/22	DWN	2.54	25	25	50	4.61	10	14.61	0.5	1
WW1011	Lab Blank	2/26/22	4/19/22	BLNK	2.57	25	25	50	4.49	10	14.49	0.51	1
WW2012	Influent 1	4/1/22	5/25/22	INF 1	2.52	25	25	50	3.8	10	13.8	0.42	1
WW2013	Influent 2	4/1/22	5/25/22	INF 2	2.57	25	25	50	4.98	10	14.98	0.48	1
WW2014	Primary Clarifier Effluent	4/1/22	5/25/22	PE	2.4	25	25	50	4.15	10	14.15	0.39	1
WW2015	Aeration Basin Effluent	4/1/22	5/25/22	ABE	2.43	25	25	50	5.2	10	15.2	0.51	1
WW2016	Secondary Clarifier Effluent	4/1/22	5/25/22	SCE	2.54	25	25	50	2.88	10	12.88	0.32	1
WW2017	UV Disinfection	4/1/22	5/26/22	UV	2.5	25	25	50	4.68	10	14.68	0.48	1

Sample Number	Sample Type	Collection Date	Preparation Date	Sample Location	Sample pH	Sample Volume (mL)	QC Volume (mL)	Pre-Rotovap Volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate Volume (mL)	Pre N2 Volume (mL)	Post N2 Volume (mL)	Reconstituted Volume (mL)
WW2018	Chlorinated Utility Water	4/1/22	5/26/22	W3	2.54	25	25	50	3.42	10	13.42	0.33	1
WW2019	Gravity Belt Thickener Underflow	4/1/22	5/26/22	GBTU	2.59	25	25	50	3	10	13	0.41	1
WW2020	Upstream	4/1/22	5/26/22	UP	2.48	25	25	50	5.09	10	15.09	0.29	1
WW2021	Downstream	4/1/22	5/26/22	DWN	2.57	25	25	50	5.13	10	15.13	0.28	1
WW2022	Lab Blank	4/1/22	5/25/22	BLNK	2.6	25	25	50	2.89	10	12.89	0.48	1
WW3033	Blank	4/29/22	6/2/22	BLNK	2.41	25	25	50	3.82	10	13.82	0.31	1
WW3023	Influent 1	4/29/22	6/2/22	INF 1	2.56	25	25	50	3.88	10	13.88	0.48	1
WW3024	Influent 2	4/29/22	6/2/22	INF 2	2.44	25	25	50	3.1	10	13.1	0.5	1
WW3025	Primary Clarifier Effluent	4/29/22	6/2/22	PE	2.51	25	25	50	3.78	10	13.78	0.51	1
WW3026	Aeration Basin Effluent	4/29/22	6/2/22	ABE	2.41	25	25	50	4.18	10	14.18	0.5	1
WW3027	Secondary Clarifier Effluent	4/29/22	6/2/22	SCE	2.4	25	25	50	3.15	10	13.15	0.4	1
WW3028	UV Disinfection	4/29/22	6/2/22	UV	2.58	25	25	50	4.62	10	14.62	0.49	1
WW3029	Chlorinated Utility Water	4/29/22	6/2/22	W3	2.41	25	25	50	3.18	10	13.18	0.25	1
WW3030	Gravity Belt Thickener Underflow	4/29/22	6/2/22	GBTU	2.59	25	25	50	4.72	10	14.72	0.32	1
WW3031	Upstream	4/29/22	6/2/22	UP	2.43	25	25	50	5.08	10	15.08	0.36	1
WW3032	Downstream	4/29/22	6/2/22	DWN	2.51	25	25	50	4.9	10	14.9	0.49	1
WW4034	Influent 1	5/31/22	6/26/22	INF 1	2.45	25	25	50	4.38	10	14.38	0.59	1

Sample Number	Sample Type	Collection Date	Preparation Date	Sample Location	Sample pH	Sample Volume (mL)	QC Volume (mL)	Pre-Rotovap Volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate Volume (mL)	Pre N2 Volume (mL)	Post N2 Volume (mL)	Reconstituted Volume (mL)
WW4035	Influent 2	5/31/22	6/26/22	INF 2	2.47	25	25	50	3.29	10	13.29	0.4	1
WW4036	Primary Clarifier Effluent	5/31/22	6/26/22	PE	2.41	25	25	50	3.71	10	13.71	0.35	1
WW4037	Aeration Basin Effluent	5/31/22	6/26/22	ABE	2.42	25	25	50	2.99	10	12.99	0.52	1
WW4038	Secondary Clarifier Effluent	5/31/22	6/26/22	SCE	2.48	25	25	50	3.1	10	13.1	0.58	1
WW4039	UV Disinfection	5/31/22	6/26/22	UV	2.52	25	25	50	4.69	10	14.69	0.48	1
WW4040	Chlorinated Utility Water	5/31/22	6/26/22	W3	2.58	25	25	50	3.71	10	13.71	0.41	1
WW4041	Gravity Belt Thickener Underflow	5/31/22	6/26/22	GBTU	2.52	25	25	50	4.4	10	14.4	0.38	1
WW4042	Upstream	5/31/22	6/26/22	UP	2.55	25	25	50	4.89	10	14.89	0.4	1
WW4043	Downstream	5/31/22	6/26/22	DWN	2.58	25	25	50	3.21	10	13.21	0.55	1
WW4044	Blank	5/31/22	6/26/22	BLNK	2.41	25	25	50	3.69	10	13.69	0.5	1
WW5045	Influent 1	6/27/22	6/28/22	INF 1	2.4	25	25	50	3.19	10	13.19	0.58	1
WW5046	Influent 2	6/27/22	6/28/22	INF 2	2.41	25	25	50	3.5	10	13.5	0.51	1
WW5047	Primary Clarifier Effluent	6/27/22	6/28/22	PE	2.51	25	25	50	3.38	10	13.38	0.35	1
WW5048	Aeration Basin Effluent	6/27/22	6/28/22	ABE	2.4	25	25	50	3.75	10	13.75	0.52	1
WW5049	Secondary Clarifier Effluent	6/27/22	6/28/22	SCE	2.41	25	25	50	3.01	10	13.01	0.5	1
WW5050	UV Disinfection	6/27/22	6/28/22	UV	2.57	25	25	50	3.62	10	13.62	0.27	1
WW5051	Chlorinated Utility Water	6/27/22	6/28/22	W3	2.43	25	25	50	4.02	10	14.02	0.38	1

Sample Number	Sample Type	Collection Date	Preparation Date	Sample Location	Sample pH	Sample Volume (mL)	QC Volume (mL)	Pre-Rotovap Volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate Volume (mL)	Pre N2 Volume (mL)	Post N2 Volume (mL)	Reconstituted Volume (mL)
WW5052	Gravity Belt Thickener Underflow	6/27/22	6/28/22	GBTU	2.46	25	25	50	4.8	10	14.8	0.41	1
WW5053	Upstream	6/27/22	6/28/22	UP	2.42	25	25	50	2.95	10	12.95	0.5	1
WW5054	Downstream	6/27/22	6/28/22	DWN	2.47	25	25	50	4	10	14	0.41	1
WW5055	Blank	6/27/22	6/28/22	BLNK	2.53	25	25	50	4.89	10	14.89	0.38	1
WW6056	Influent 1	7/25/22	8/27/22	INF 1	2.46	25	25	50	4.98	10	14.98	0.46	1
WW6057	Influent 2	7/25/22	8/27/22	INF 2	2.5	25	25	50	3.54	10	13.54	0.51	1
WW6058	Primary Clarifier Effluent	7/25/22	8/30/22	PE	2.55	25	25	50	3.1	10	13.1	0.4	1
WW6059	Aeration Basin Effluent	7/25/22	8/30/22	ABE	2.43	25	25	50	4.99	10	14.99	0.45	1
WW6060	Secondary Clarifier Effluent	7/25/22	8/30/22	SCE	2.59	25	25	50	2.5	10	12.5	0.49	1
WW6061	UV Disinfection	7/25/22	8/27/22	UV	2.52	25	25	50	4.39	10	14.39	0.27	1
WW6062	Chlorinated Utility Water	7/25/22	8/27/22	W3	2.46	25	25	50	4.43	10	14.43	0.28	1
WW6063	Gravity Belt Thickener Underflow	7/25/22	8/30/22	GBTU	2.56	25	25	50	5.58	10	15.58	0.1	1
WW6064	Upstream	7/25/22	8/27/22	UP	2.44	25	25	50	4.27	10	14.27	0.38	1
WW6065	Downstream	7/25/22	8/27/22	DWN	2.42	25	25	50	5.08	10	15.08	0.31	1
WW6066	Blank	7/25/22	8/27/22	BLNK	2.53	25	25	50	4.31	10	14.31	0.49	1
WW1101	Primary Solids Liquid	2/25/22	9/8/22	PSL	2.26	25	25	50	3.7	10	13.7	0.3	1
WW1102	Activated Sludge Liquid	2/25/22	9/8/22	ASL	2.44	25	25	50	2.9	10	12.9	0.48	1

Sample Number	Sample Type	Collection Date	Preparation Date	Sample Location	Sample pH	Sample Volume (mL)	QC Volume (mL)	Pre-Rotovap Volume (mL)	Post Rotovap Volume (mL)	Ethyl Acetate Volume (mL)	Pre N2 Volume (mL)	Post N2 Volume (mL)	Reconstituted Volume (mL)
WW1103	Digested Solids Liquid	2/25/22	9/8/22	DSL	2.58	25	25	50	5.05	10	15.05	0.5	1
WW2104	Primary Solids Liquid	4/1/22	9/8/22	PSL	2.69	25	25	50	4.3	10	14.3	0.3	1
WW2105	Activated Sludge Liquid	4/1/22	9/8/22	ASL	2.68	25	25	50	2.98	10	12.98	0.4	1
WW2106	Digested Solids Liquid	4/1/22	9/8/22	DSL	2.38	25	25	50	3.6	10	13.6	0.49	1
WW3107	Primary Solids Liquid	4/29/22	9/10/22	PSL	2.53	25	25	50	3.4	10	13.4	0.6	1
WW3108	Activated Sludge Liquid	4/29/22	9/10/22	ASL	2.57	25	25	50	3.1	10	13.1	0.59	1
WW3109	Digested Solids Liquid	4/29/22	9/10/22	DSL	2.58	25	25	50	4.3	10	14.3	0.4	1
WW4110	Primary Solids Liquid	5/31/22	9/10/22	PSL	2.47	25	25	50	4.1	10	14.1	0.55	1
WW4111	Activated Sludge Liquid	5/31/22	9/13/22	ASL	2.46	25	25	50	4.62	10	14.62	0.5	1
WW4112	Digested Solids Liquid	5/31/22	9/10/22	DSL	2.52	25	25	50	3.5	10	13.5	0.49	1
WW5113	Primary Solids Liquid	6/27/22	9/13/22	PSL	2.41	25	25	50	4.5	10	14.5	0.49	1
WW5114	Activated Sludge Liquid	6/27/22	9/13/22	ASL	2.53	25	25	50	3.7	10	13.7	0.5	1
WW5115	Digested Solids Liquid	6/27/22	9/13/22	DSL	2.51	25	25	50	3.3	10	13.3	0.56	1
WW6116	Primary Solids Liquid	7/25/22	9/15/22	PSL	2.41	25	25	50	3.2	10	13.2	0.56	1
WW6117	Activated Sludge Liquid	7/25/22	9/13/22	ASL	2.25	25	25	50	3.5	10	13.5	0.58	1
WW6118	Digested Solids Liquid	7/25/22	9/15/22	DSL	2.27	25	25	50	5.4	10	15.4	0.5	1

Table B.2 Solid Sample Preparation

Sample Number	Sample Type	Sample Location	Collection Date	Preparation Date	Sample Weight (g)	Methanol Volume (mL)	DI Water Volume (mL)	McIlvaine-EDTA Buffer Volume (mL)	Sample Volume (mL)	Reconstituted Post Mixing (mL)
WS1002a	Activated Sludge Solids	WAS	2/25/22	9/27/22	1.005	5	5	5	7.6	15
WS1002b	Activated Sludge Solids	WAS	2/25/22	9/27/22	1.008	5	5	5	7.3	15
WS1001	Primary Clarifier	PCS	2/25/22	11/2/22	0.997	5	5	5	8.2	15
WS1003	Digested Solids	DS	2/25/22	11/2/22	1.005	5	5	5	7.4	15
WS2004	Primary Clarifier	PCS	4/1/22	11/2/22	0.999	5	5	5	6.0	15
WS2005	Activated Sludge Solids	WAS	4/1/22	11/2/22	1.006	5	5	5	6.5	15
WS2006	Digested Solids	DS	4/1/22	11/2/22	1.001	5	5	5	7.9	15
WS3007	Primary Clarifier	PCS	4/29/22	11/3/22	0.995	5	5	5	6.4	15
WS3008	Activated Sludge Solids	WAS	4/29/22	11/3/22	0.953	5	5	5	6.9	15
WS3009	Digested Solids	DS	4/29/22	11/3/22	1.001	5	5	5	6.5	15
WS4010	Primary Clarifier	PCS	5/31/22	11/3/22	0.995	5	5	5	6.0	15
WS4011	Activated Sludge Solids	WAS	5/31/22	11/3/22	1.010	5	5	5	5.9	15
WS4012	Digested Solids	DS	5/31/22	11/3/22	1.011	5	5	5	5.4	15
WS5013	Primary Clarifier	PCS	6/27/22	11/6/22	1.042	5	5	5	6.5	15
WS5014	Activated Sludge Solids	WAS	6/27/22	11/6/22	0.994	5	5	5	6.5	15
WS5015	Digested Solids	DS	6/27/22	11/6/22	1.004	5	5	5	6.0	15
WS6016	Primary Clarifier	PCS	7/25/22	11/6/22	1.002	5	5	5	6.9	15
WS6017	Activated Sludge Solids	WAS	7/25/22	11/6/22	1.004	5	5	5	6.1	15
WS6018	Digested Solids	DS	7/25/22	11/6/22	1.013	5	5	5	7.9	15

Sample Number	Methanol Cardridge Prep Volume (mL)	DI Water Cartridge Prep Volume (mL)	Sample Volume Post SPE (mL)	Sample Volume Post N2 (mL)	Reconstituted Volume (mL)
WS1002a	6	6	5.90	0.41	1
WS1002b	6	6	5.85	0.58	1
WS1001	6	6	5.95	0.59	1
WS1003	6	6	5.96	0.65	1
WS2004	6	6	5.97	0.49	1
WS2005	6	6	5.80	0.65	1
WS2006	6	6	5.83	0.60	1
WS3007	6	6	5.89	0.40	1
WS3008	6	6	5.90	0.50	1
WS3009	6	6	5.87	0.50	1
WS4010	6	6	5.94	0.39	1
WS4011	6	6	5.87	0.58	1
WS4012	6	6	5.87	0.45	1
WS5013	6	6	5.93	0.10	1
WS5014	6	6	5.96	0.31	1
WS5015	6	6	5.87	0.50	1
WS6016	6	6	5.90	0.47	1
WS6017	6	6	5.85	0.55	1
WS6018	6	6	5.83	0.45	1

APPENDIX C: DATA RESULTS

The following tables contain the data prior to statistical analysis.

Table C.1: Liquid Data Results

Original Liquid Data (ug/L)														
Sample/Data file	Sample Location	Albuterol	Cimetidine	Theophylline	Acetaminophen	Methylphenidate	Primidone	Citalopram	Sulfamethoxazole	Salicylic Acid	Warfarin	Ibuprofen
WW1001.d	INF 1	7.52	5.39	15116.27	15552.13	0.20	40.32	123.68	516.15	24146.53	50.00	2443.07
WW1002.d	INF 2	1.91	0.20	12360.67	11515.23	0.20	37.83	134.74	990.71	20171.83	50.00	2509.26
WW1003.d	PE	3.78	38.81	9156.73	6862.43	0.20	46.96	100.36	233.61	28645.86	50.00	2356.09
WW1004.d	ABE	0.20	18.34	84.75	0.20	29.85	33.87	494.91	196.88	0.00	50.00	100.00
WW1005.d	SCE	12.29	11.38	102.03	0.20	44.61	61.38	224.55	46.34	887.86	50.00	100.00
WW1006.d	UV	5.39	11.49	137.03	27.99	38.29	53.67	150.69	6.02	605.29	50.00	100.00
WW1007.d	W3	7.82	0.49	112.52	0.20	27.97	58.30	114.65	9.29	318.69	50.00	100.00
WW1008.d	GBTU	5.53	0.20	76.64	0.20	38.26	52.36	242.08	10.06	936.23	50.00	100.00
WW1009.d	UP	0.20	0.20	0.20	0.20	14.32	0.20	6.17	0.20	0.20	50.00	100.00
WW1010.d	DWN	0.20	0.20	0.20	12.67	15.14	7.98	17.58	13.01	0.20	50.00	100.00
WW1011.d	BLNK	0.20	0.20	0.20	0.20	14.29	0.20	5.62	0.20	0.20	50.00	100.00
WW2012.d	INF 1	0.20	88.94	31210.52	25897.22	51.19	102.20	367.57	764.03	29197.83	50.00	4123.34
WW2013.d	INF 2	35.27	160.21	41671.47	31905.05	48.40	164.75	302.05	771.33	13379.34	50.00	5918.09
WW2014.d	PE	29.73	76.91	41057.41	36967.56	59.36	90.54	352.62	1412.83	73710.54	50.00	3679.93
WW2015.d	ABE	0.20	51.72	137.31	49.38	34.37	50.83	1114.35	285.87	585.80	50.00	100.00
WW2016.d	SCE	41.32	104.56	323.44	0.20	95.08	197.24	784.95	174.70	3500.44	50.00	100.00
WW2017.d	UV	1.34	12.67	47.60	0.20	32.47	50.65	196.12	36.84	285.35	50.00	100.00
WW2018.d	W3	3.23	16.02	66.96	0.20	31.61	48.68	195.48	34.51	363.02	50.00	100.00
WW2019.d	GBTU	0.97	20.89	19.08	0.20	27.02	37.81	308.01	33.30	383.55	50.00	100.00
WW2020.d	UP	0.20	0.20	0.20	11.17	14.49	0.20	9.97	1.85	0.20	50.00	100.00
WW2021.d	DWN	0.20	0.20	0.20	0.20	15.55	10.47	31.55	16.74	0.20	50.00	100.00
WW2022.d	BLNK	0.20	0.20	0.20	0.20	14.27	0.20	0.20	0.20	0.20	50.00	100.00
WW3023.d	INF 1	21.77	126.23	13954.10	8988.24	16.09	15.03	177.33	251.05	46924.77	50.00	4780.97
WW3024.d	INF 2	22.00	115.95	16880.90	9926.44	19.98	18.09	193.35	307.50	54601.18	50.00	5131.20
WW3025.d	PE	14.75	134.46	8706.11	5283.45	47.35	19.92	203.74	522.08	24667.47	50.00	4020.80
WW3026.d	ABE	9.28	44.97	29.88	0.20	83.50	32.25	1116.05	501.82	237.64	50.00	100.00
WW3027.d	SCE	24.63	68.00	22.61	0.20	71.33	38.16	380.33	0.00	482.32	50.00	100.00
WW3028.d	UV	22.88	61.01	22.55	0.20	66.86	31.82	362.03	15.98	415.53	50.00	100.00
WW3029.d	W3	20.32	20.24	93.87	0.20	76.10	35.05	277.98	80.42	67.32	50.00	100.00
WW3030.d	GBTU	16.23	40.67	15.42	0.20	76.39	42.67	483.33	143.48	32.47	50.00	100.00
WW3031.d	UP	2.30	19.43	0.20	58.44	0.20	0.30	12.65	0.20	0.20	50.00	100.00
WW3032.d	DWN	2.38	22.74	0.20	0.20	0.20	3.00	35.82	0.20	0.20	50.00	100.00
WW3033.d	BLNK	2.23	19.42	0.20	0.20	0.20	0.67	9.29	0.20	1103.17	50.00	100.00

Original Liquid Data (ng/L)												
Sample/Data file	Sample Location	Albuterol	Cimetidine	Theophylline	Acetaminophen	Methylphenidate	Primidone	Citalopram	Sulfamethoxazole	Salicylic Acid	Warfarin	Ibuprofen
WW4034.d	INF 1	47.73	172.49	21640.87	12779.68	9.11	25.41	440.77	566.83	61445.47	50.00	5884.18
WW4035.d	INF 2	41.71	168.67	24113.69	14948.08	9.55	55.98	475.35	1068.93	63024.39	50.00	5771.48
WW4036.d	PE	22.26	194.38	15436.07	10645.25	5.17	39.71	335.93	580.07	41785.35	50.00	4210.50
WW4037.d	ABE	15.73	62.98	19.63	0.20	17.95	1.60	964.86	536.43	377.58	50.00	100.00
WW4038.d	SCE	21.29	66.82	0.26	0.20	13.21	29.51	317.00	0.20	120.49	50.00	100.00
WW4039.d	UV	21.31	60.15	26.44	0.20	11.32	20.49	286.38	0.20	892.02	50.00	100.00
WW4040.d	W3	7.66	19.57	28.67	0.20	11.42	28.11	68.80	1.79	0.20	50.00	100.00
WW4041.d	GBTU	12.12	26.55	0.20	0.20	8.73	23.62	314.90	27.24	177.58	50.00	100.00
WW4042.d	UP	2.95	19.43	44.30	276.73	0.20	0.60	12.70	0.20	0.20	50.00	100.00
WW4043.d	DWN	4.26	20.37	0.20	0.20	0.20	0.61	17.35	0.20	0.20	50.00	100.00
WW4044.d	BLNK	3.16	19.63	0.20	0.20	0.20	0.60	10.78	0.20	0.20	50.00	100.00
WW5045.d	INF 1	26.17	74.63	17776.22	10231.84	2.90	29.11	423.06	1366.20	48464.58	50.00	4770.50
WW5046.d	INF 2	23.65	77.18	17238.64	9809.10	2.17	17.10	436.56	1484.64	50785.35	50.00	4717.99
WW5047.d	PE	22.27	109.78	11913.29	8089.21	2.85	29.41	251.99	784.10	33980.99	50.00	4412.18
WW5048.d	ABE	11.00	41.17	0.20	0.20	14.76	23.78	762.38	389.85	188.03	50.00	2902.98
WW5049.d	SCE	3.36	55.85	1.73	0.20	21.26	23.27	319.20	59.99	0.20	50.00	100.00
WW5050.d	UV	17.62	45.42	39.13	0.20	24.54	26.22	340.08	0.20	0.20	50.00	100.00
WW5051.d	W3	22.18	20.39	61.83	0.20	16.99	29.70	407.94	23.77	28.99	50.00	100.00
WW5052.d	GBTU	5.17	28.23	51.86	0.20	18.36	20.02	347.03	2.96	21.94	50.00	100.00
WW5053.d	UP	0.20	0.20	0.20	0.20	0.20	0.20	10.80	0.20	0.20	50.00	100.00
WW5054.d	DWN	0.20	19.51	0.20	0.20	0.20	1.28	16.71	0.20	0.20	50.00	100.00
WW5055.d	BLNK	0.20	0.20	0.20	0.20	0.20	0.20	9.96	0.20	0.20	50.00	100.00
WW6056.d	INF 1	21.20	41.60	50753.60	24731.20	29.20	88.40	238.80	1148.40	111657.20	50.00	11368.00
WW6057.d	INF 2	18.80	53.20	43932.40	21393.20	30.00	71.20	221.60	1920.40	88974.40	50.00	8444.00
WW6058.d	PE	10.40	38.00	23820.80	12187.60	27.20	65.20	136.80	1604.40	54353.60	50.00	3180.00
WW6059.d	ABE	12.80	71.20	160.00	6.40	56.40	4.80	1218.00	919.60	2147.20	50.00	100.00
WW6060.d	SCE	14.40	31.20	120.80	1.60	36.00	71.20	213.60	51.60	1003.60	50.00	100.00
WW6061.d	UV	13.60	30.80	360.80	212.40	36.40	84.00	210.80	74.80	1974.80	50.00	100.00
WW6062.d	W3	14.00	41.60	304.80	9.60	16.40	96.40	248.00	154.00	1624.40	50.00	100.00
WW6063.d	GBTU	14.40	9.60	152.40	0.20	38.40	90.40	208.40	11.60	1393.60	50.00	100.00
WW6064.d	UP	0.20	0.20	67.20	8.40	0.40	0.20	2.00	2.80	351.60	50.00	100.00
WW6065.d	DWN	0.20	0.80	74.80	26.40	0.80	0.20	2.40	6.80	552.40	50.00	100.00
WW6066.d	BLNK	0.20	0.20	36.00	6.00	0.40	0.20	0.40	1.20	6538.40	50.00	100.00

Table C.2: Solid Data Results

Original Solids Data														
Sample/Data file	Sample Location	Albuterol	Cimetidine	Theophylline	Acetaminophen	Methylphenidate	Primidone	Citalopram	Sulfamethoxazole	Salicylic Acid	Warfarin	Ibuprofen
WS1001.d	PCS	0.01	0.04	159.30	8.51	1.64	0.75	18.27	21.86	8940.57	2.50	1419.47
WS1002.d	WAS	0.07	0.69	0.01	0.05	0.44	0.06	8.87	7.56	415.25	2.50	42.75
WS1003.d	DS	0.01	1.64	5.82	0.58	0.06	0.13	4.29	0.01	645.04	2.50	993.74
WS2004.d	PCS	0.16	0.30	60.70	38.55	1.23	1.37	17.43	12.89	8345.97	2.50	1458.94
WS2005.d	WAS	0.01	0.90	0.01	0.19	0.41	0.74	8.94	18.14	258.89	2.50	18.20
WS2006.d	DS	0.01	0.42	11.65	0.37	0.17	1.11	5.19	0.01	452.29	2.50	1190.72
WS3007.d	PCS	0.01	0.37	39.58	19.62	1.04	0.52	7.28	16.54	2373.14	2.50	476.41
WS3008.d	WAS	0.01	0.32	0.01	0.24	0.91	0.70	8.58	15.12	279.21	2.50	9.86
WS3009.d	DS	0.20	0.58	4.80	0.26	0.14	1.29	5.00	0.01	446.70	2.50	1265.37
WS4010.d	PCS	0.01	0.33	46.74	24.19	0.80	0.84	14.39	9.02	4830.06	2.50	777.45
WS4011.d	WAS	0.01	0.14	0.01	0.09	0.41	0.39	9.49	15.74	194.51	2.50	9.36
WS4012.d	DS	0.01	2.09	7.65	0.10	0.11	1.19	2.97	0.49	249.12	2.50	841.15
WS5013.d	PCS	0.09	0.35	20.15	7.67	0.76	0.63	12.25	9.02	3824.89	2.50	365.77
WS5014.d	WAS	0.01	0.66	0.16	0.01	0.37	0.58	4.21	4.90	306.84	2.50	17.34
WS5015.d	DS	0.01	0.97	11.64	0.03	0.10	1.21	3.20	0.32	357.94	2.50	841.22
WS6016.d	PCS	0.01	0.25	27.45	2.01	0.79	0.50	12.00	5.93	3448.78	2.50	407.72
WS6017.d	WAS	0.01	0.28	0.09	0.01	0.71	0.86	7.57	8.50	231.20	2.50	5.00
WS6018.d	DS	0.35	2.27	9.17	0.40	0.24	1.33	3.13	0.28	642.13	2.50	716.63

APPENDIX D: QUARTILE ANALYSIS

Liquid Quartile MATLAB Code

The following MATLAB code was used to complete the liquid quartile analysis and plot the data. This analysis was performed to remove outliers from the dataset.

```
clear; clc;
%Reading in data
format long g
fid = fopen('liquid_datafile.m','r');
x = fscanf(fid,'%f',[11 66]);
A = zeros(66,11);
for j = 1:11
    for n = 1:6
        i = 1+(n-1)*11;
        k = n+(j-1)*6;
        A(k,:) = x(j,i:i+10)';
    end
end

%Quartile analysis (Sample Location)
sampleLoc = {'INF 1','INF
 2','PCE','ABE','SCE','UV','W3','GBTU','UP','DWN','BLK'};


figure();
q1 = boxplot(A(1:6,:),'Labels',sampleLoc);
title('Albuterol Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q2 = boxplot(A(7:12,:),'Labels',sampleLoc);
title('Cimetidine Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q3 = boxplot(A(13:18,:),'Labels',sampleLoc);
title('Theophylline Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q4 = boxplot(A(19:24,:),'Labels',sampleLoc);
title('Acetaminophen Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q5 = boxplot(A(25:30,:),'Labels',sampleLoc);
```

```
title('Methylphenidate Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q6 = boxplot(A(31:36,:),'Labels',sampleLoc);
title('Primidone Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q7 = boxplot(A(37:42,:),'Labels',sampleLoc);
title('Citalopram Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q8 = boxplot(A(43:48,:),'Labels',sampleLoc);
title('Sulfamethoxazole Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q9 = boxplot(A(49:54,:),'Labels',sampleLoc);
title('Salicylic Acid Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q10 = boxplot(A(55:60,:),'Labels',sampleLoc);
title('Warfarin Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')


figure();
q11 = boxplot(A(61:66,:),'Labels',sampleLoc);
title('Ibuprofin Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/L)')
```

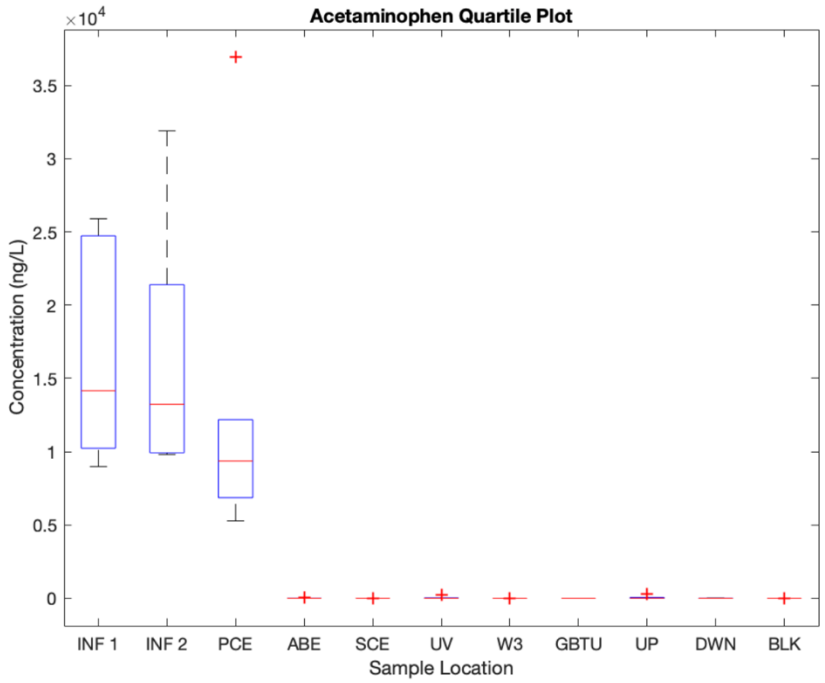

Figure D.1: Acetaminophen Liquid Quartile Plot

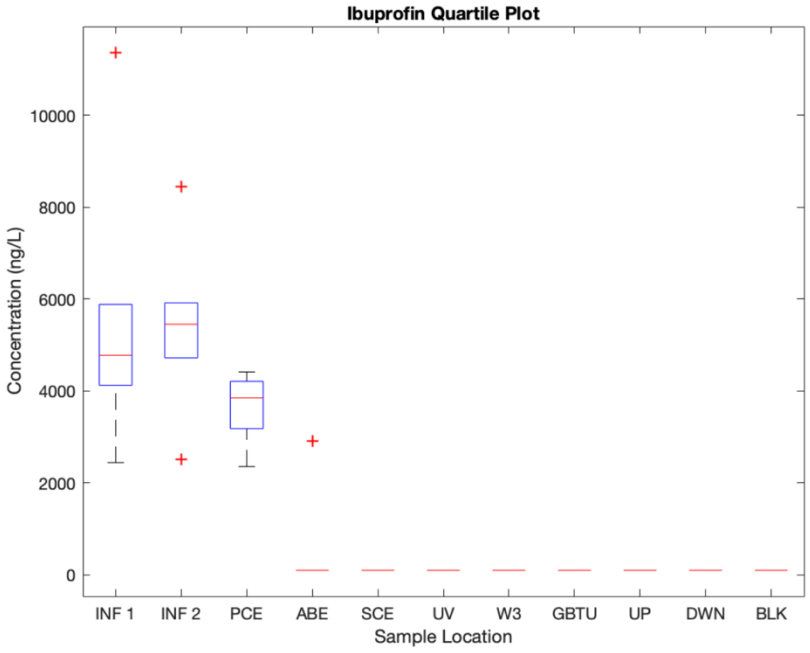

Figure D.2: Ibuprofen Liquid Quartile Plot

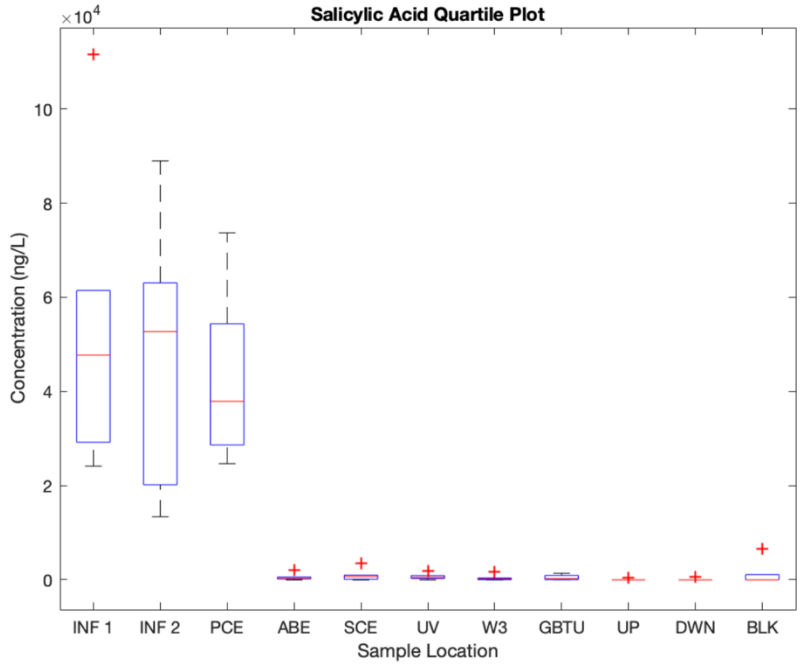

Figure D.3: Salicylic Acid Liquid Quartile Plot

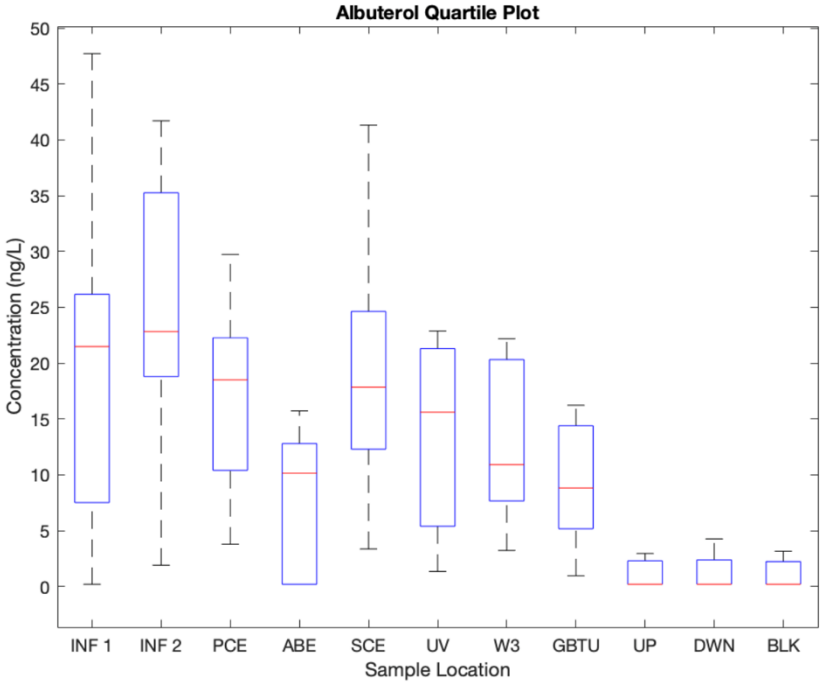

Figure D.4: Albuterol Liquid Quartile Plot

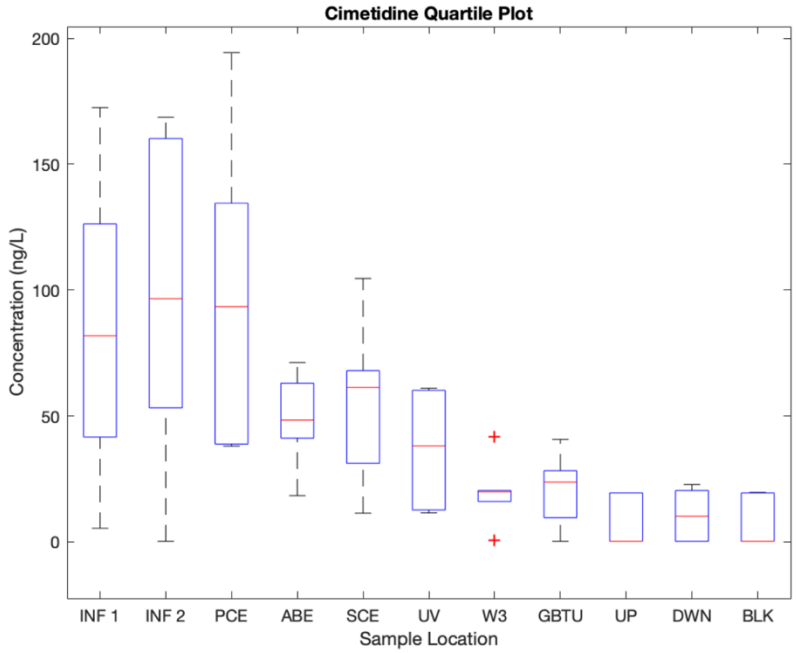

Figure D.5: Cimetidine Liquid Quartile Plot

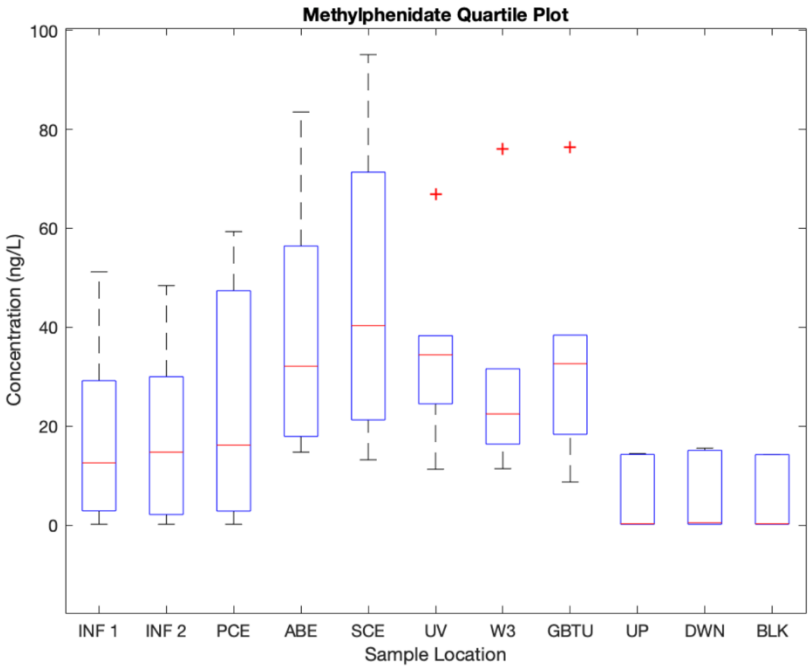

Figure D.6: Methylphenidate Liquid Quartile Plot

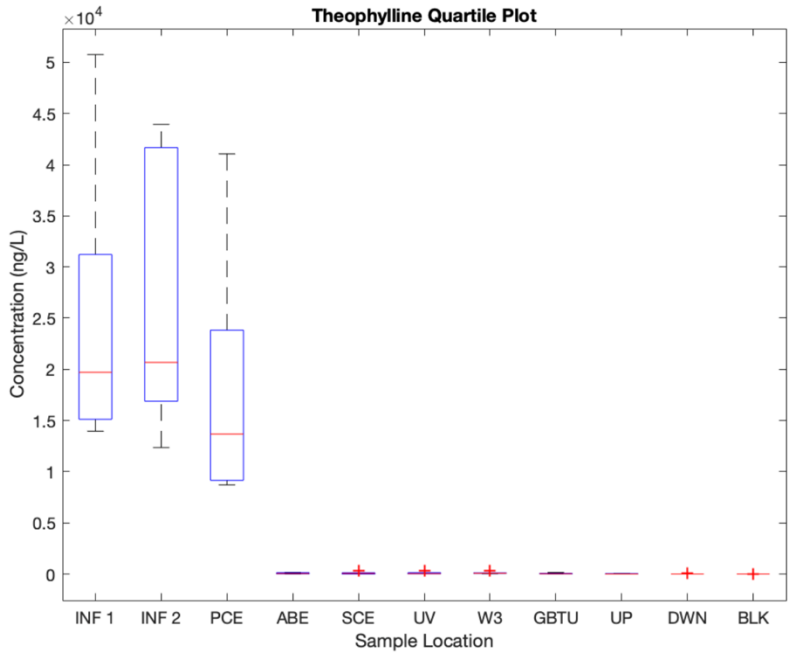

Figure D.7: Theophylline Liquid Quartile Plot

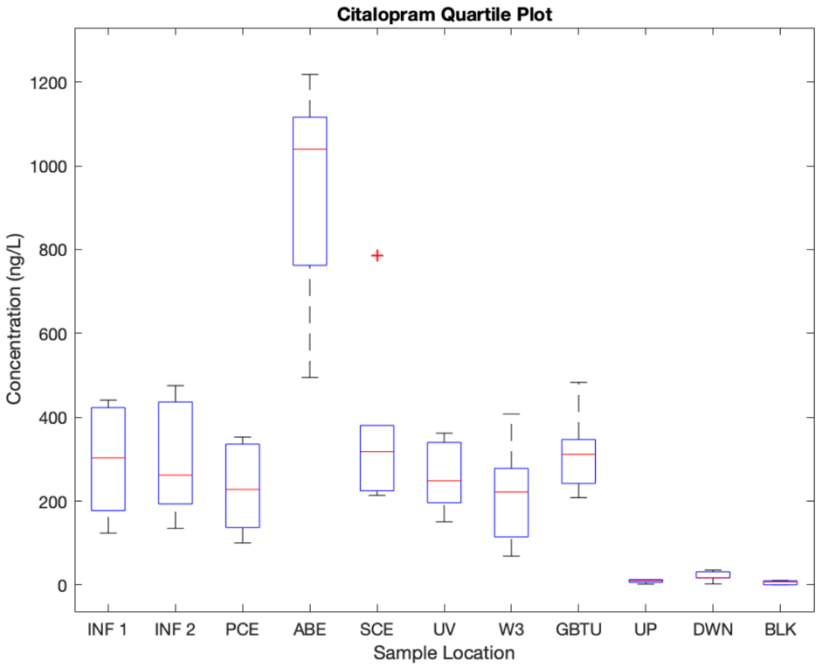

Figure D.8: Citalopram Liquid Quartile Plot

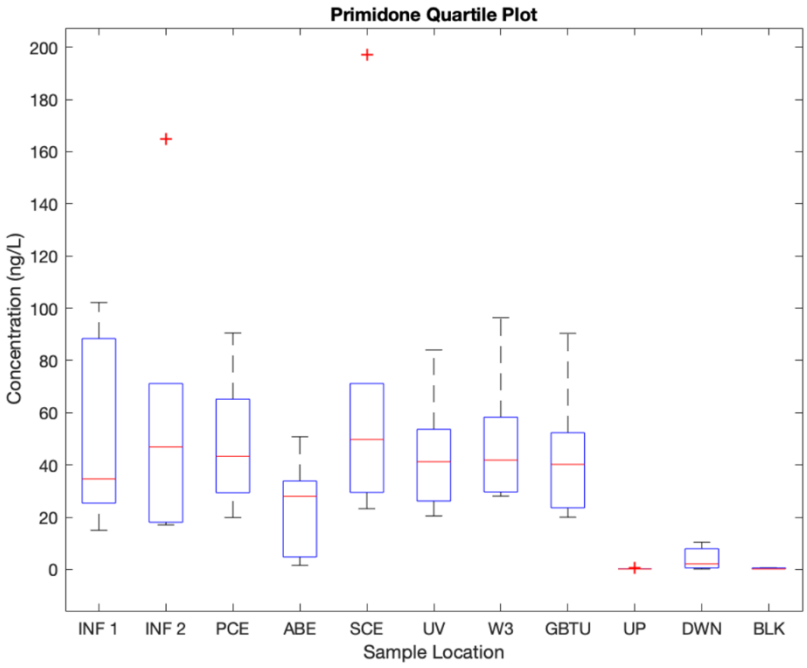

Figure D.9: Primidone Liquid Quartile Plot

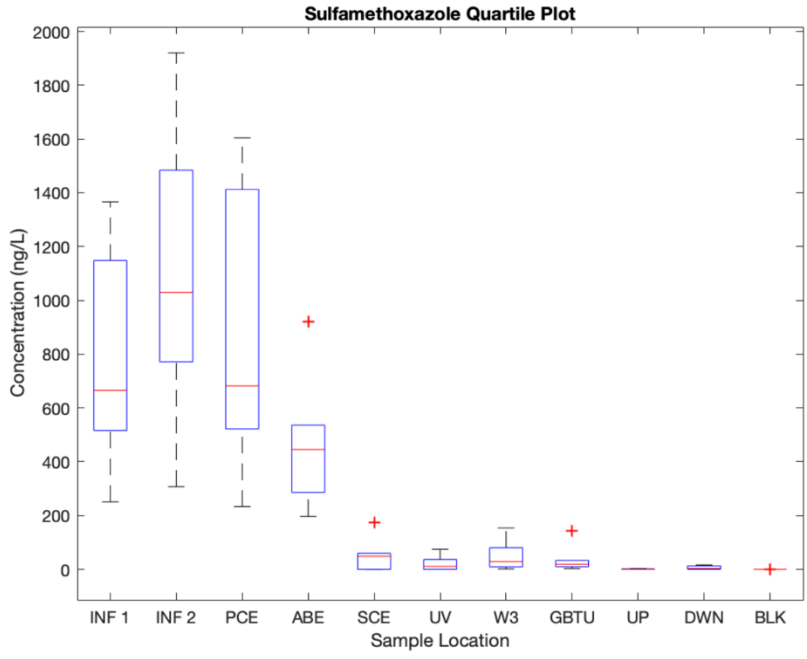

Figure D.10: Sulfamethoxazole Liquid Quartile Plot

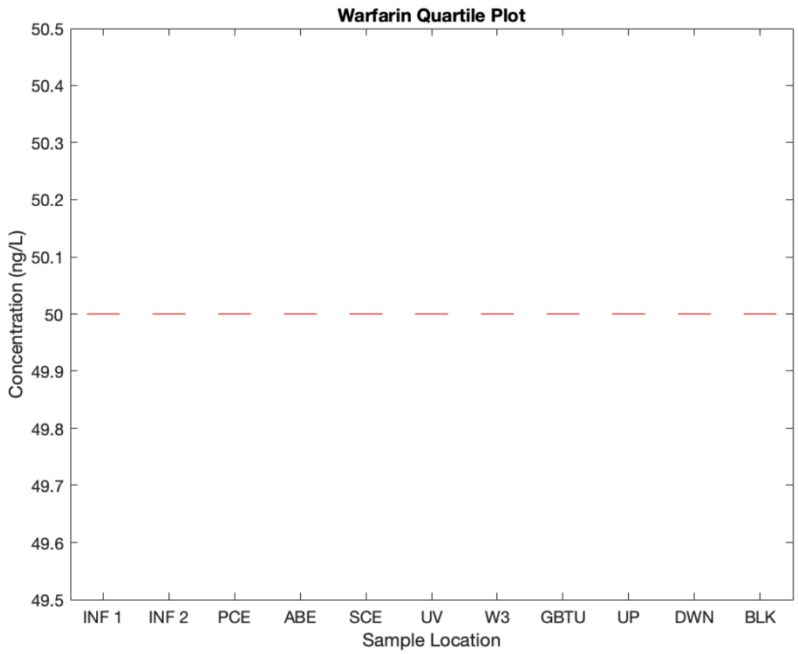

Figure D.11: Warfarin Liquid Quartile Plot

Solids Quartile MATLAB Code

The following MATLAB code was used to complete the solids quartile analysis and plot the data. This analysis was performed to remove outliers from the dataset.

```
clear; clc;
%Reading in data
format long g
fid = fopen('total_solid_datafile.m','r');
x = fscanf(fid,'%f',[11 18]);
A = zeros(66,3);
for j = 1:11
    for n = 1:6
        i = 1+(n-1)*3;
        k = n+(j-1)*6;
        A(k,:) = x(j,i:i+2)';
    end
end

%Quartile analysis (Sample Location)
sampleLoc = {'PS','AS','DS'};

figure();
q1 = boxplot(A(1:6,:),'Labels',sampleLoc);
title('Albuterol (solids) Quartile Plot');
```

```
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q2 = boxplot(A(7:12,:),'Labels',sampleLoc);
title('Cimetidine (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q3 = boxplot(A(13:18,:),'Labels',sampleLoc);
title('Theophylline (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q4 = boxplot(A(19:24,:),'Labels',sampleLoc);
title('Acetaminophen (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q5 = boxplot(A(25:30,:),'Labels',sampleLoc);
title('Methylphenidate (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q6 = boxplot(A(31:36,:),'Labels',sampleLoc);
title('Primidone (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q7 = boxplot(A(37:42,:),'Labels',sampleLoc);
title('Citalopram (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q8 = boxplot(A(43:48,:),'Labels',sampleLoc);
title('Sulfamethoxazole (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q9 = boxplot(A(49:54,:),'Labels',sampleLoc);
title('Salicylic Acid (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')

figure();
q10 = boxplot(A(55:60,:),'Labels',sampleLoc);
title('Warfarin (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')
```

```
figure();
q11 = boxplot(A(61:66,:),'Labels',sampleLoc);
title('Ibuprofin (solids) Quartile Plot');
xlabel('Sample Location')
ylabel('Concentration (ng/g)')
```

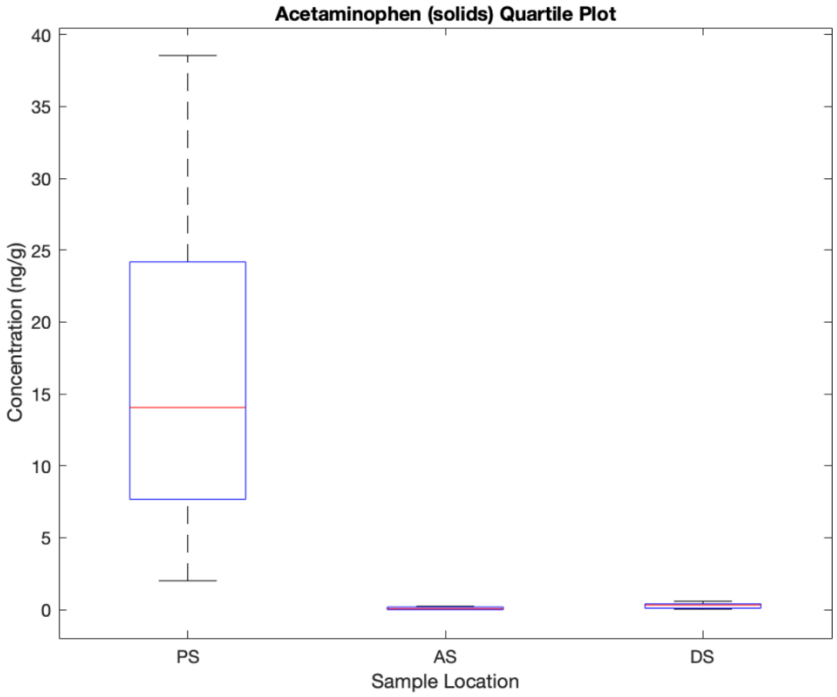

Figure D.12: Acetaminophen Solids Quartile Plot

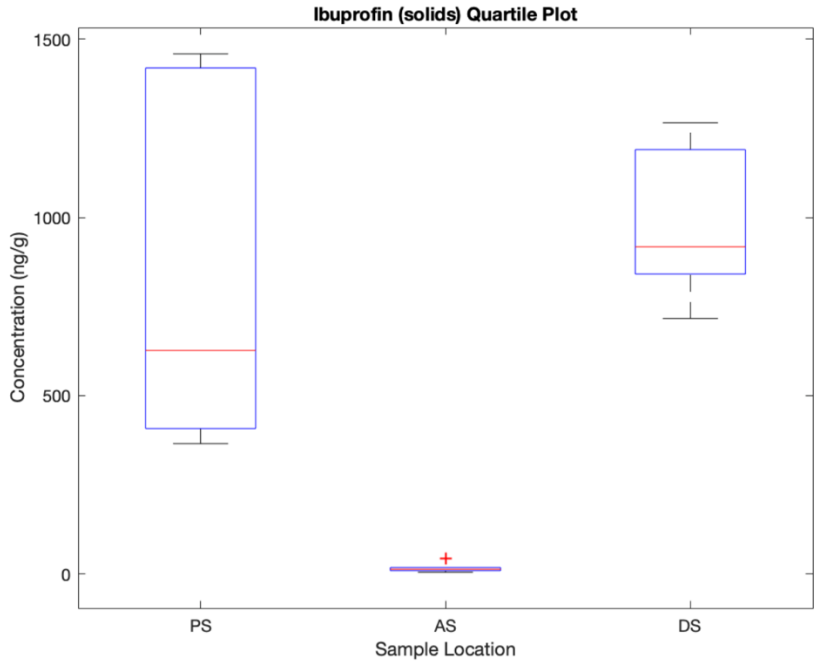

Figure D.13: Ibuprofen Solids Quartile Plot

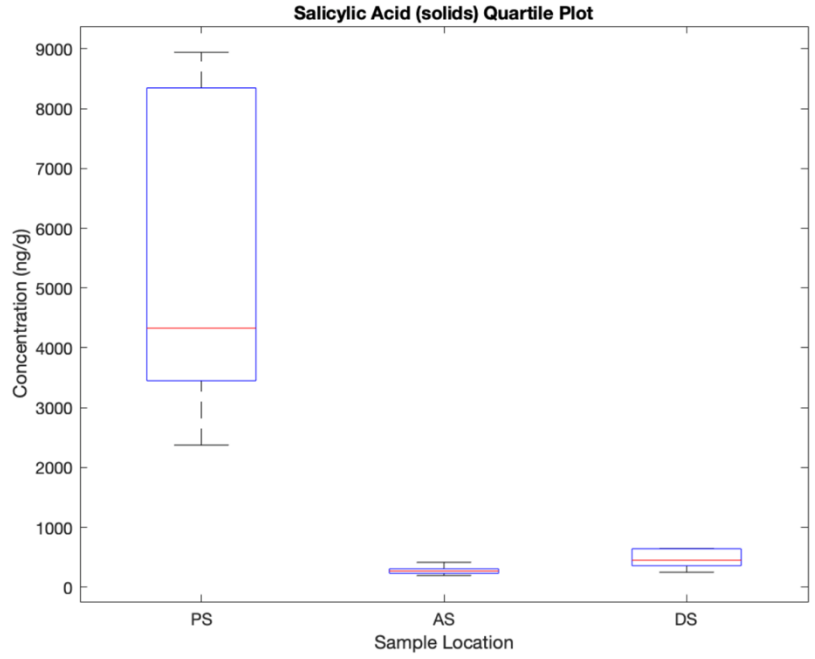

Figure D.14: Salicylic Acid Solids Quartile Plot

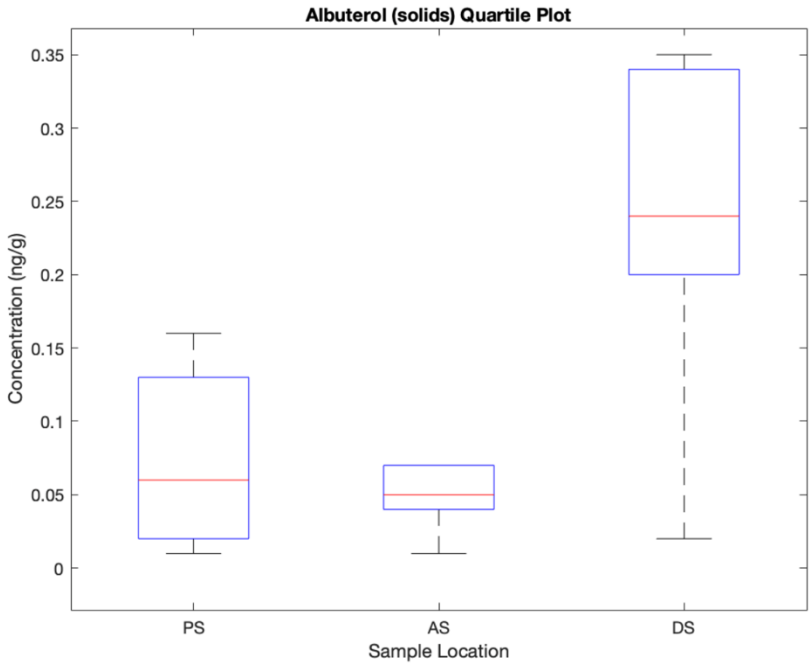

Figure D.15: Albuterol Solids Quartile Plot

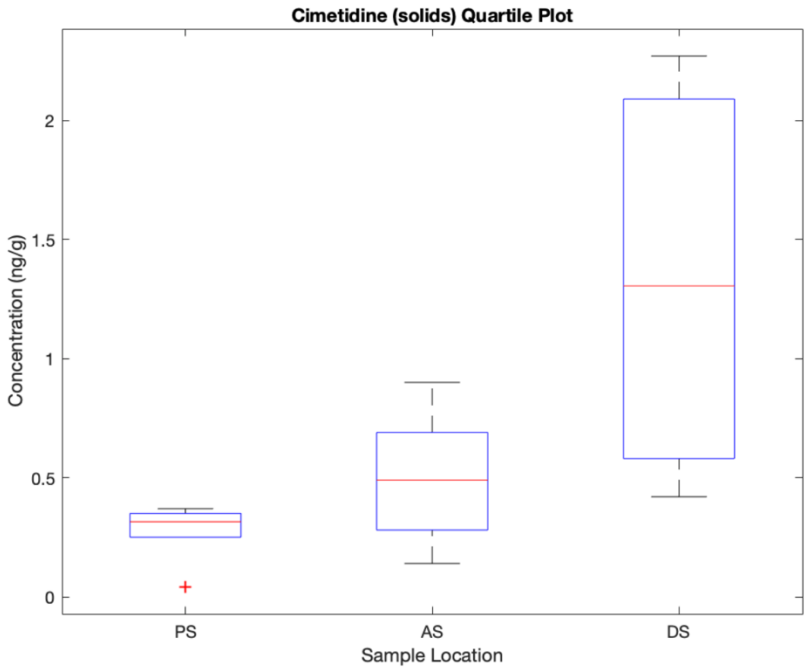

Figure D.16: Cimetidine Solids Quartile Plot

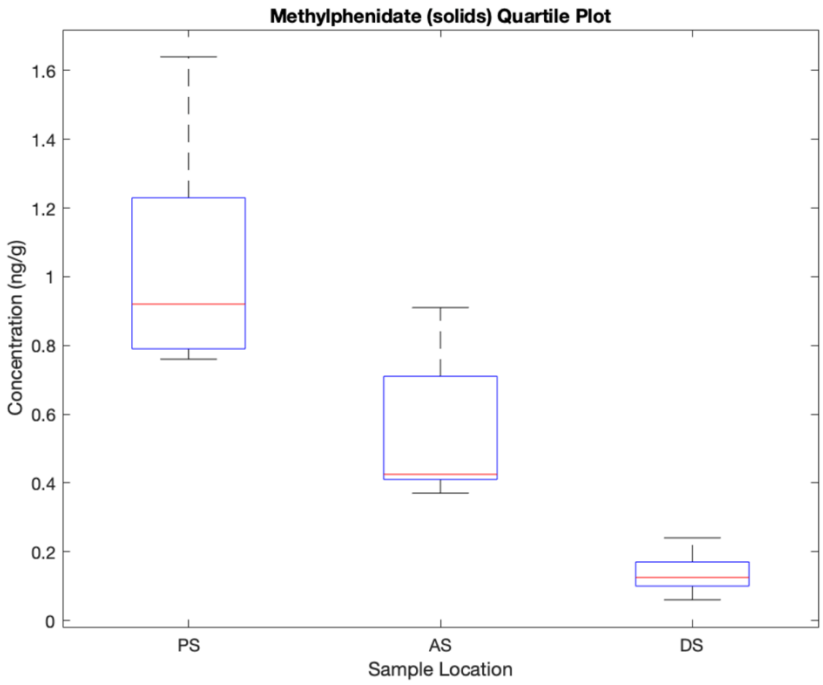

Figure D.17: Methylphenidate Solids Quartile Plot

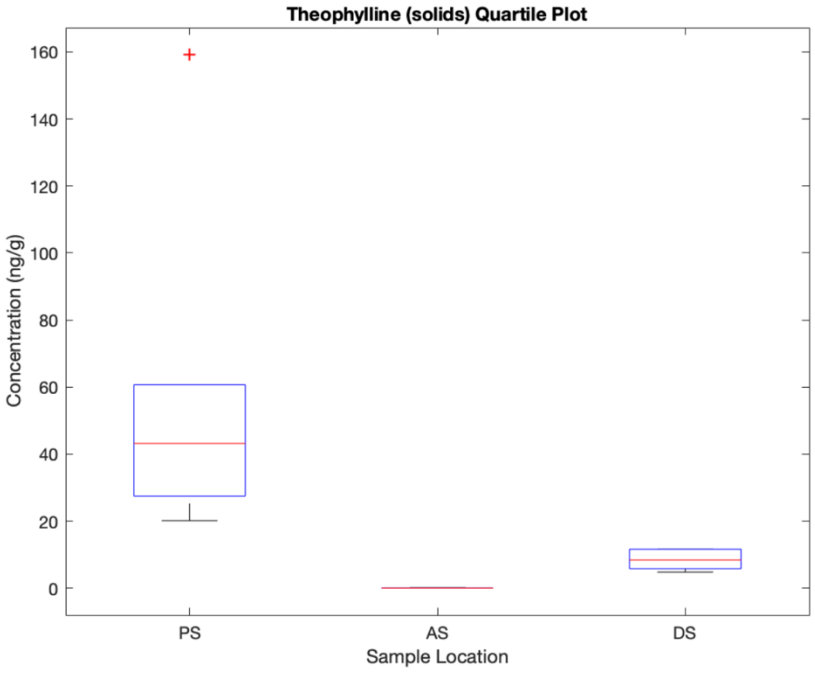

Figure D.18: Theophylline Solids Quartile Plot

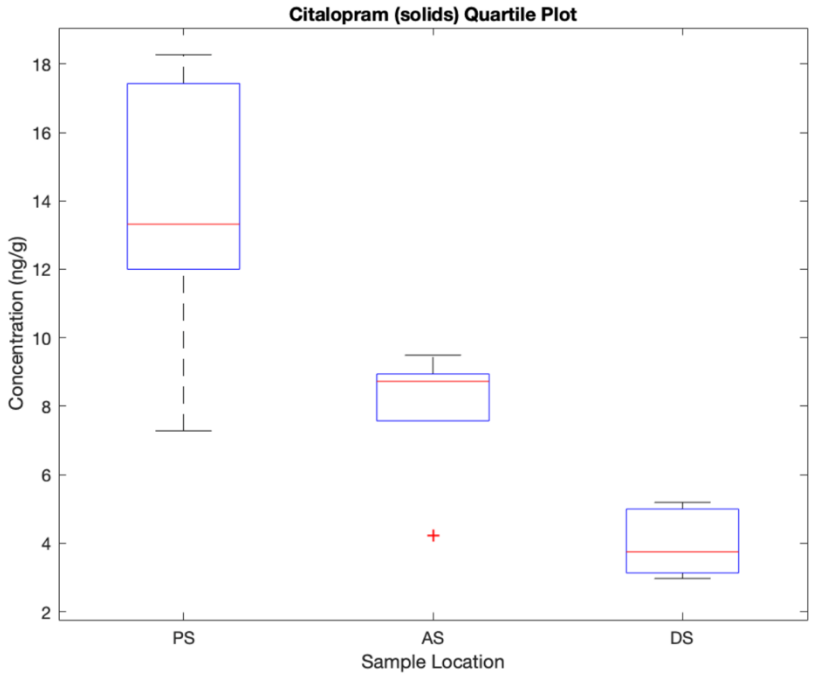

Figure D.19: Citalopram Solids Quartile Plot

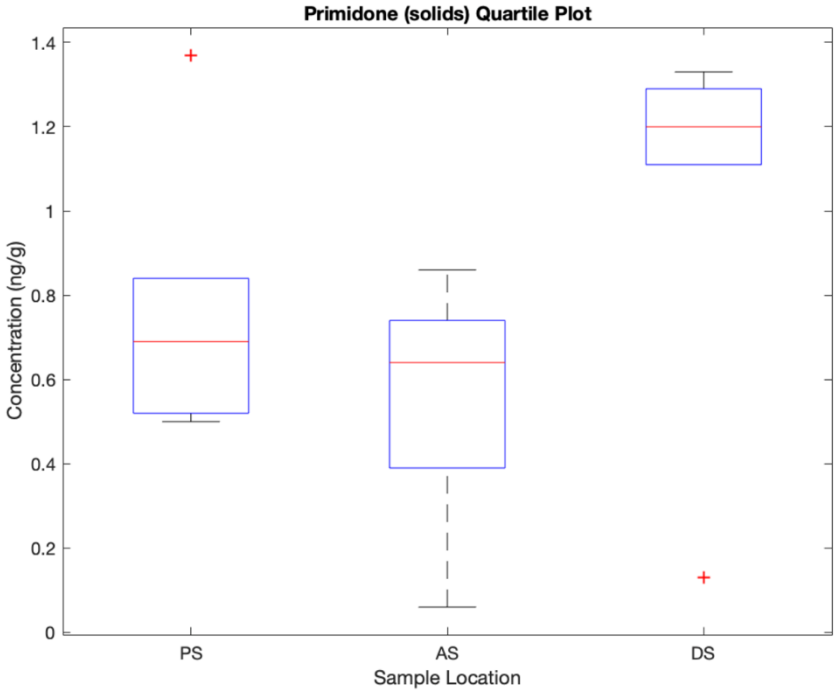

Figure D.20: Primidone Solids Quartile Plot

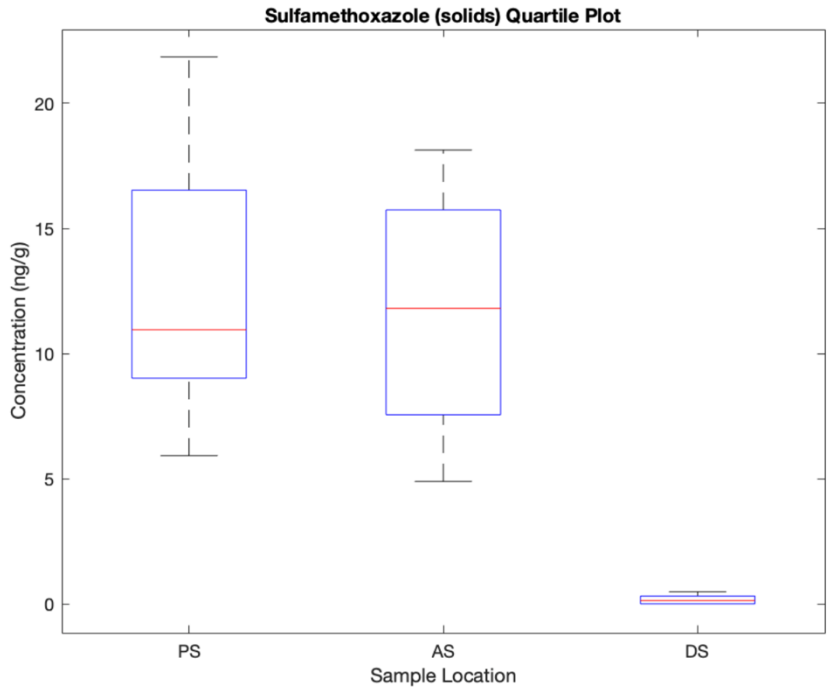

Figure D.21: Sulfamethoxazole Solids Quartile Plot

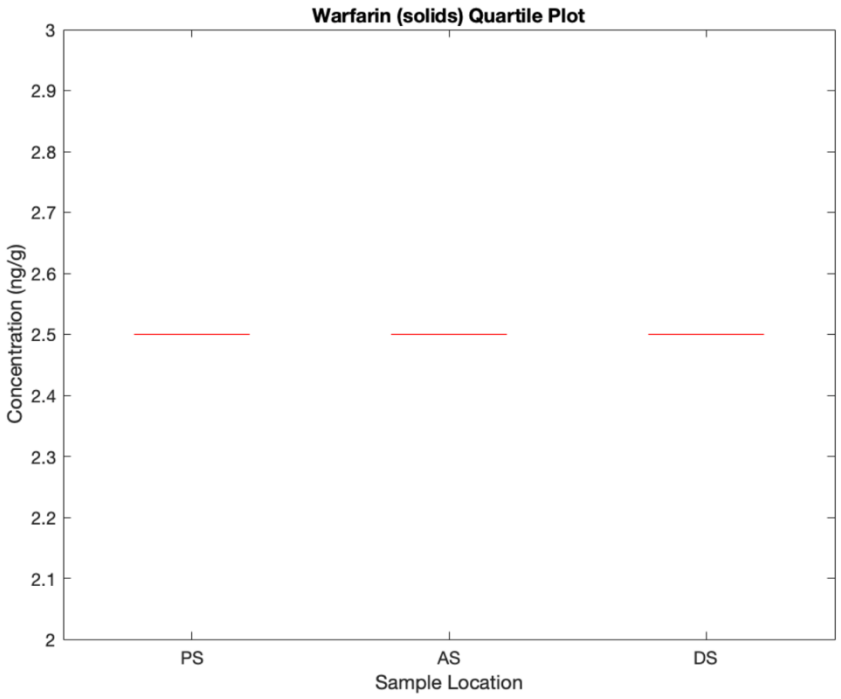

Figure D.22: Warfarin Solids Quartile Plot

APPENDIX E: STUDENT'S T-TEST ANALYSIS

Liquid Student's T-Test MATLAB Code

The following MATLAB code was used to complete the liquid student's t-test analysis. This analysis was used to determine if the liquid concentrations were significantly different.

```
%Student's T-Test Code for the Liquid Samples

clear; clc;

%Reading in data
format long g
fid = fopen('liquid_datafile_no_outliers.m','r');
x = fscanf(fid,'%f',[11 66]);
A = zeros(66,11);
for j = 1:11
    for n = 1:6
        i = 1+(n-1)*11;
        k = n+(j-1)*6;
        A(k,:) = x(j,i:i+10)';
    end
end

%T-test of constituents (Sample Location)
names =
 ["Albuterol" "Cimetidine" "Theophylline" "Acetaminophen"
"Methylphenidate" ...
    "Primidone" "Citalopram" "Sulfamethoxazole" "Salicylic
 Acid" "Warfarin" "Ibuprofin"];

for i = 1:10
    if i >= 7
    [h1(i),p1(i)] = ttest(A(1:6,6),A(1:6,9),'Alpha',.05);
    [h2(i),p2(i)] = ttest(A(7:12,6),A(7:12,9),'Alpha',.05);
    [h3(i),p3(i)] = ttest(A(13:18,6),A(13:18,9),'Alpha',.05);
    [h4(i),p4(i)] = ttest(A(19:24,6),A(19:24,9),'Alpha',.05);
    [h5(i),p5(i)] = ttest(A(25:30,6),A(25:30,9),'Alpha',.05);
    [h6(i),p6(i)] = ttest(A(31:36,6),A(31:36,9),'Alpha',.05);
    [h7(i),p7(i)] = ttest(A(37:42,6),A(37:42,9),'Alpha',.05);
    [h8(i),p8(i)] = ttest(A(43:48,6),A(43:48,9),'Alpha',.05);
    [h9(i),p9(i)] = ttest(A(49:54,6),A(49:54,9),'Alpha',.05);
    [h10(i),p10(i)] = ttest(A(55:60,6),A(55:60,9),'Alpha',.05);
    [h11(i),p11(i)] = ttest(A(61:66,6),A(61:66,9),'Alpha',.05);
    elseif i == 8
    [h1(i),p1(i)] = ttest(A(1:6,6),A(1:6,10),'Alpha',.05);
    [h2(i),p2(i)] = ttest(A(7:12,6),A(7:12,10),'Alpha',.05);
    [h3(i),p3(i)] = ttest(A(13:18,6),A(13:18,10),'Alpha',.05);
    [h4(i),p4(i)] = ttest(A(19:24,6),A(19:24,10),'Alpha',.05);
    [h5(i),p5(i)] = ttest(A(25:30,6),A(25:30,10),'Alpha',.05);
    [h6(i),p6(i)] = ttest(A(31:36,6),A(31:36,10),'Alpha',.05);
    [h7(i),p7(i)] = ttest(A(37:42,6),A(37:42,10),'Alpha',.05);
    [h8(i),p8(i)] = ttest(A(43:48,6),A(43:48,10),'Alpha',.05);
```

```
            [h9(i),p9(i)] = ttest(A(49:54,6),A(49:54,10),'Alpha',.05);
            [h10(i),p10(i)] = ttest(A(55:60,6),A(55:60,10),'Alpha',.05);
            [h11(i),p11(i)] = ttest(A(61:66,6),A(61:66,10),'Alpha',.05);
            else
            [h1(i),p1(i)] = ttest(A(1:6,i),A(1:6,i+1),'Alpha',.05);
            [h2(i),p2(i)] = ttest(A(7:12,i),A(7:12,i+1),'Alpha',.05);
            [h3(i),p3(i)] = ttest(A(13:18,i),A(13:18,i+1),'Alpha',.05);
            [h4(i),p4(i)] = ttest(A(19:24,i),A(19:24,i+1),'Alpha',.05);
            [h5(i),p5(i)] = ttest(A(25:30,i),A(25:30,i+1),'Alpha',.05);
            [h6(i),p6(i)] = ttest(A(31:36,i),A(31:36,i+1),'Alpha',.05);
            [h7(i),p7(i)] = ttest(A(37:42,i),A(37:42,i+1),'Alpha',.05);
            [h8(i),p8(i)] = ttest(A(43:48,i),A(43:48,i+1),'Alpha',.05);
            [h9(i),p9(i)] = ttest(A(49:54,i),A(49:54,i+1),'Alpha',.05);
            [h10(i),p10(i)] = ttest(A(55:60,i),A(55:60,i+1),'Alpha',.05);
            [h11(i),p11(i)] = ttest(A(61:66,i),A(61:66,i+1),'Alpha',.05);
            end

        end

        varNames =

{'Constituent','INF1_INF2','INF2_PCE','PE_ABE','ABE_SCE','SCE_UV','UV_W3',
...'UV_UP','UV_DWN','UP_DWN','DWN_BLK'};

        H = [h1;h2;h3;h4;h5;h6;h7;h8;h9;h10;h11];
        disp(table(names',H(:,1),H(:,2),H(:,3),H(:,4),H(:,5),H(:,6),H(:,7),.
..
            H(:,8),H(:,9),H(:,10),'VariableNames',varNames));

        P = [p1;p2;p3;p4;p5;p6;p7;p8;p9;p10;p11];
        disp(table(names',P(:,1),P(:,2),P(:,3),P(:,4),P(:,5),P(:,6),P(:,7),.
..
            P(:,8),P(:,9),P(:,10),'VariableNames',varNames));

        %AVG between INF1 and INF2
        %T-test of constituents between influent and effluent
        for i = 1:11
            [C(1,i),C2(1,i)] = ttest((A((i+(i-1)*5):(i+(i-
1)*5)+5,1)+A((i+(i-1)*5):(i+(i-1)*5)+5,1))./2,A((i+(i-1)*5):(i+(i-
1)*5)+5,6),'Alpha',.05);
        varNames1 = {'Constituent','INF_UV'};

        disp(table(names',C','VariableNames',varNames1));
        varNames1 = {'Constituent','INF_UV'};

        disp(table(names',C2','VariableNames',varNames1));

        %T-test of constituents temporally
        for i = 1:3
            for j = 1:11
                if i == 2
                elseif i == 3
                [D2(j,i-1),D3(j,i-1)] = ttest((A((j+(j-1)*5)+(i-
1),:)+A((j+(j-1)*5)+i,:))./2,(A((j+(j-1)*5)+(i+1),:)+A((j+(j-1)*5)+i
                else
                [D2(j,i),D3(j,i)] = ttest((A((j+(j-1)*5)+(i-1),:)+A((j
```

```
+(j-1)*5)+i,:))./2,...
            (A((j+(j-1)*5)+(i+1),:)+A((j+(j-1)*5)+i
+2,:))./2,'Alpha',.05);
      end

   end

end

varNames3 = {'Constituent', 'Winter_Spring','Spring_Summer'};
disp(table(names',D2(:,1),D2(:,2),'VariableNames',varNames3));
disp(table(names',D3(:,1),D3(:,2),'VariableNames',varNames3));
```

Table E.1: Liquid Student's T-Test Removal P-Values

Over-The-Counter Drugs and Personal Care Products			
Sample Location	**Acetaminophen**	**Ibuprofen**	**Salicylic Acid**
INF vs PCE	0.015	0.049	0.715
PCE vs ABE	0.002	0.001	0.002
ABE vs SCE	0.316	NA	0.504
SCE vs UV	0.301	NA	0.643
UV vs W3	0.299	NA	0.092
UV vs UP	0.776	NA	0.041
UV vs DWN	0.776	NA	0.041
UP vs DWN	0.776	NA	0.041
INF vs UV	0.003	0.002	0.004

Common Prescription Drugs				
Sample Location	**Albuterol**	**Cimetidine**	**Methylphenidate**	**Theophylline**
INF vs PCE	0.076	0.887	0.331	0.039
PCE vs ABE	0.101	0.088	0.143	0.015
ABE vs SCE	0.157	0.560	0.557	0.520
SCE vs UV	0.465	0.242	0.340	0.883
UV vs W3	0.684	0.089	0.101	0.756
UV vs UP	0.011	0.004	0.005	0.119
UV vs DWN	0.011	0.004	0.005	0.119
UP vs DWN	0.011	0.004	0.005	0.119
INF vs UV	0.155	0.046	0.350	0.007

ABE vs SCE				
Sample Location	**Citalopram**	**Primidone**	**Sulfamethoxazole**	**Warfarin**
INF vs PCE	0.142	0.9711	0.347	NA
PCE vs ABE	0.001	0.077	0.202	NA
ABE vs SCE	0.008	0.096	0.023	NA
SCE vs UV	0.255	0.741	0.494	NA
UV vs W3	0.396	0.055	0.106	NA
UV vs UP	0.001	0.006	0.120	NA
UV vs DWN	0.001	0.006	0.120	NA
UP vs DWN	0.001	0.006	0.120	NA
INF vs UV	0.518	0.598	0.007	NA

Table E.2: Liquid Student's T-Test T Temporal Variation P-Values

Constituent	Temporal Variation Liquid Results	
	Winter vs Spring	Spring vs Summer
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	0.171	0.123
Ibuprofen	0.189	0.351
Salicylic Acid	0.378	0.108
Common Prescription Drugs		
Albuterol	0.011	0.012
Cimetidine	0.004	0.009
Methylphenidate	0.075	0.150
Theophylline	0.105	0.104
Specialized Prescrription Drugs		
Citalopram	0.013	0.348
Primidone	0.008	0.010
Sulfamethoxazole	0.492	0.079
Warfarin	NA	NA

Solids Student's T-Test Analysis MATLAB Code

The following MATLAB code was used to complete the solids student's t-test analysis. This analysis was used to determine if the liquid concentrations were significantly different.

```
%Student's T-Test Code for the Solids Samples

clear; clc;

%Reading in data
format long g
fid = fopen('Total_solid_datafile_no_outliers.m','r');
x = fscanf(fid,'%f',[11 18]);
A = zeros(66,3);
for j = 1:11
    for n = 1:6
        i = 1+(n-1)*3;
        k = n+(j-1)*6;
        A(k,:) = x(j,i:i+2)';
   end
end

%T-test of constituents (Sample Location)
names =
 ["Albuterol" "Cimetidine" "Theophylline" "Acetaminophen"
"Methylphenidate" "Primidone" "Citalopram" "Sulfamethoxazole" "Salicylic
 Acid" "Warfarin" "Ibuprofin"];

for i = 1:3
```

```
    if i == 3
    [h1(i),p1(i)] = ttest(A(1:6,1),A(1:6,i),'Alpha',.05);
    [h2(i),p2(i)] = ttest(A(7:12,1),A(7:12,i),'Alpha',.05);
    [h3(i),p3(i)] = ttest(A(13:18,1),A(13:18,i),'Alpha',.05);
    [h4(i),p4(i)] = ttest(A(19:24,1),A(19:24,i),'Alpha',.05);
    [h5(i),p5(i)] = ttest(A(25:30,1),A(25:30,i),'Alpha',.05);
    [h6(i),p6(i)] = ttest(A(31:36,1),A(31:36,i),'Alpha',.05);
    [h7(i),p7(i)] = ttest(A(37:42,1),A(37:42,i),'Alpha',.05);
    [h8(i),p8(i)] = ttest(A(43:48,1),A(43:48,i),'Alpha',.05);
    [h9(i),p9(i)] = ttest(A(49:54,1),A(49:54,i),'Alpha',.05);
    [h10(i),p10(i)] = ttest(A(55:60,1),A(55:60,i),'Alpha',.05);
    [h11(i),p11(i)] = ttest(A(61:66,1),A(61:66,i),'Alpha',.05);
    else
    [h1(i),p1(i)] = ttest(A(1:6,i),A(1:6,i+1),'Alpha',.05);
    [h2(i),p2(i)] = ttest(A(7:12,i),A(7:12,i+1),'Alpha',.05);
    [h3(i),p3(i)] = ttest(A(13:18,i),A(13:18,i+1),'Alpha',.05);
    [h4(i),p4(i)] = ttest(A(19:24,i),A(19:24,i+1),'Alpha',.05);
    [h5(i),p5(i)] = ttest(A(25:30,i),A(25:30,i+1),'Alpha',.05);
    [h6(i),p6(i)] = ttest(A(31:36,i),A(31:36,i+1),'Alpha',.05);
    [h7(i),p7(i)] = ttest(A(37:42,i),A(37:42,i+1),'Alpha',.05);
    [h8(i),p8(i)] = ttest(A(43:48,i),A(43:48,i+1),'Alpha',.05);
    [h9(i),p9(i)] = ttest(A(49:54,i),A(49:54,i+1),'Alpha',.05);
    [h10(i),p10(i)] = ttest(A(55:60,i),A(55:60,i+1),'Alpha',.05);
    [h11(i),p11(i)] = ttest(A(61:66,i),A(61:66,i+1),'Alpha',.05);
    end

end

varNames = {'Constituent','PCS_WAS','WAS_DS','PCS_DS'};
H = [h1;h2;h3;h4;h5;h6;h7;h8;h9;h10;h11];
disp(table(names', H(:,1),H(:,2),H(:,3),'VariableNames',varNames));

P = [p1;p2;p3;p4;p5;p6;p7;p8;p9;p10;p11];
disp(table(names', P(:,1),P(:,2),P(:,3),'VariableNames',varNames));

%Every two months
for i = 1:3
    for j = 1:11
        if i == 2
        elseif i == 3
        [D2(j,i-1),D3(j,i-1)] = ttest((A((j+(j-1)*5)+(i-1),:)+A((j
+(j-1)*5)+i,:))./2,...
            (A((j+(j-1)*5)+(i+1),:)+A((j+(j-1)*5)+i
+2,:))./2,'Alpha',.05);
        else
        [D2(j,i),D3(j,i)] = ttest((A((j+(j-1)*5)+(i-1),:)+A((j
+(j-1)*5)+i,:))./2,...
            (A((j+(j-1)*5)+(i+1),:)+A((j+(j-1)*5)+i
+2,:))./2,'Alpha',.05);
        End
    end
end

varNames3 = {'Constituent', 'Winter_Spring','Spring_Summer'};
disp(table(names',D2(:,1),D2(:,2),'VariableNames',varNames3));
disp(table(names',D3(:,1),D3(:,2),'VariableNames',varNames3));
```

Table E.3: Solids Student's T-Test Removal P-Values

Constituent	Temporal Variation Liquid Results	
	Winter vs Spring	Spring vs Summer
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	0.171	0.123
Ibuprofen	0.189	0.351
Salicylic Acid	0.378	0.108
Common Prescription Drugs		
Albuterol	0.011	0.012
Cimetidine	0.004	0.009
Methylphenidate	0.075	0.150
Theophylline	0.105	0.104
Specialized Prescrription Drugs		
Citalopram	0.013	0.348
Primidone	0.008	0.010
Sulfamethoxazole	0.492	0.079
Warfarin	NA	NA

Table E.4: Solids Student's T-Test Temporal Variation P-Values

Constituent	Temporal Variation Solids Results	
	Winter vs Spring	Spring vs Summer
Over-The-Counter Drugs and Personal Care Products		
Acetaminophen	0.345	0.007
Ibuprofen	0.469	0.188
Salicylic Acid	0.389	0.205
Common Prescription Drugs		
Albuterol	0.986	0.419
Cimetidine	0.815	0.271
Methylphenidate	0.725	0.344
Theophylline	0.500	0.561
Specialized Prescription Drugs		
Citalopram	0.375	0.86
Primidone	0.735	0.343
Sulfamethoxazole	0.806	0.210
Warfarin	NA	NA