Quality of Wastewater in the El Paso, Texas Region--Analysis of Pharmaceuticals and Personal Care Products using High Performance Liquid Chromatography Tandem Mass Spectrometry

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QUALITY OF WASTEWATER IN THE EL PASO, TEXAS REGION-ANALYSIS OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Dedication

To my loving and wonderful mother Elva Guerrero
And father Rudy Guerrero Sr., my brothers Josh B. and Ruben Guerrero,
my two sisters Celia and Samantha Guerrero
To my entire Family (Guerrero, Calderon Bachman and Dick Mayers Family)
To my loving, beautiful and understanding
girlfriend Lacey Mills
In Loving memory of: my grandfathers
Victor Calderon and Paulino Guerrero
My uncles Manuel Guerrero and Tim Roach
My nino Jose Guerrero
My abuelita Manuela Fuentes
My godson Ryan Anthony Bluth
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By

Rodolfo Guerrero Jr., B.S.

THESIS
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Abstract

Pharmaceuticals and Personal Care Products (PPCPs), such as household cleaning materials, prescription medication, and personal hygiene products, are pollutants of emerging concern that are found in 80% of our water system. Because of their applications, PPCPs are subsequently being released into wastewater; however, current wastewater treatment facilities are not regulated or designed to remove PPCPs. Therefore, these compounds are discharged into the environment on a continual basis. The effects of PPCPs on human health are not yet well known; however, recent research has found that exposure to these chemicals has some adverse developmental effects on aquatic species at low concentrations in lab exposure studies.

Present research on PPCPs focuses on their occurrences, concentrations, fate, and transport in the environment. The major goal of this research was to analyze the presence of PPCPs in wastewater and river samples collected in the El Paso, Texas area. The objective of this study is to (1) optimize an existing analytical method to obtain higher sensitivity, (2) establish a baseline of concentrations of PPCPs in this border region, and (3) assess the impacts of PPCP in wastewater bio-solids from wastewater treatment plants in the El Paso, TX.

This research was modeled after the Environmental Protection Agency (EPA) Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by High-Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS). The principle target compounds for this study are acetaminophen, fluoxetine, ciprofloxacin, caffeine, trimethoprim, sulfamethazine, codeine, cotinine, and erythromycin. Samples were prepared by solid-phase extraction (SPE) and analyzed on a Linear Ion Trap (LTQ) for the presence of the target analytes. A preliminary study on the influent, effluent and sludge wastewater samples
collected from two wastewater facilities in the El Paso, TX region showed the presence of PPCPs in the ppt (ng/L) to ppq (pg/L) range. Fifty-four wastewater samples and six sludge samples have been processed for analysis and are being stored for further study once instrumentation time has been allotted. These samples will help to form a baseline and better understanding of the presence of PPCPs. The data will provide valuable information for future research addressing the concern of PPCPs on human health and water quality.
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1 Introduction

Water plays a vital function for humans in the digestion of food, supplying of nutrients in the body to vital organs and maintaining metabolism cycles within the body.\(^1\,2\,3\) Furthermore, it plays a major role in dissolving gases such as, oxygen and carbon dioxide, in aquatic ecosystems, which are utilized by photosynthetic and non-photosynthetic organisms.\(^3\,4\,5\) Water is mainly used in the irrigation of fields, which consumes 80 percent of freshwater resources in the United States; however, it is also being consumed for domestic and commercial uses.\(^6\,7\,8\) As the world population continues to rise so too does the demand for water. The world's supply of clean, fresh water is decreasing. Developing new processes in order to reuse depleting resources is a recurring theme in many aspects of academia and environmental agencies, such as the Environmental Protection Agency (EPA) and World Health Organization (WHO).

The city of El Paso with its twin city, Ciudad Juarez, Mexico, is one of the largest semi-arid international border communities in the world. In this desert area, it is important to find ways to recycle and reuse water. The Rio Grande forms the international boundary between the U.S. and Mexico, separating El Paso and Juarez, and serves as a fresh water source. Currently El Paso has three water sources: the Hueco Bolson (groundwater), the Mesilla Bolson (groundwater) and the Rio Grande (surface water).\(^7\,8\,9\) The Hueco Bolson and the Rio Grande supply water to the El Paso community and the majority of the Ciudad Juarez community. It is estimated that the average resident in El Paso and Ciudad Juarez consumes approximately 175 gallons per day and 88 gallons per day respectively.\(^7\,9\,10\)
In the past decade, the United States saw a rise in population from 281.4 million in 2000 to 308.7 million in 2010 (Figure 1). The highest of this population increase occurred in the state of Texas, which observed an increase from 20.8 million in 2000 to 25.1 million in 2010 (Figure 2). (11) This increase in population along with the current use of water has hydrologists estimating that the Hueco Bolson will be nearly depleted by the year 2025. (7,9) Furthermore, with an increase of population comes a rise in the use of water resources, which may force many states to find other expensive sources of water supplies and increase in the cost of transportation of distant water supplies.

![United States Population Growth](image)

**Figure 1.** U.S. population growth from 1910 to 2010. Source: U.S. Census Bureau, Data Base 12-21-10 (11)
This observed population growth also provides a driving force for the need of continued assurance in both the quality and integrity of groundwater, wastewater and surface water. The quality of the water resources is affected by the growth of bacterium, viruses, protozoan and helminthes are naturally occurring in the environment, the release of pesticides, as well as the veterinary use of hormones, used on livestock in confined animal feeding operations (CAFOs) for enhanced growth, and antibiotics, for disease control. These issues, along with the discharge of toxic chemicals from industrial sources into the environment and aquatic ecosystems, pose a health risk on all living organisms. Most wastewater treatment plants (WWTPs) are equipped for water treatment using a secondary or tertiary process. The secondary
process (Figure 3) consists of bar screens for the removal of large trash from the inflow of water, followed by basins are used for the separation of water from sand and grit, and then a pre-aeration tank in which air is used for the removal of gases. Subsequently, water enters a primary clarifier which allows solids in wastewater to settle out to form sludge. The water is then sent to an aeration tank in which oxygen is used for bacteria to thrive and consume organic matter that is present. The influent is then sent into a secondary clarifier where bacteria may settle out. Finally, the treated water is disinfected with chlorine and distributed. \(^{(13)}\) The secondary process (Figure 4) and entails much of the same process as the previous treatment; however, three important steps are used after the secondary clarifier stage. First, a filtration system is used to remove light particles, which do not settle, then an ultraviolet light is used for disinfection and finally water is re-aerated to ensure oxygen levels meets standards before distribution. \(^{(14)}\)
Figure 3. Secondary Treatment Process. Source: Roberto R. Bustamante Treatment Plant (13)
Figure 4. Secondary Treatment Process. Source: Northwest Wastewater Treatment Plant (14)
Modern sewage treatment processes must meet regulations set forth by the EPA before water can be discharged into the environment or for human consumption. Many of the technologies used for disinfection and sanitization prevent, the growth of most bacteria and the possibility for contamination of water supplies. Wastewater treatment facilities have improved dramatically since they first appeared. They have become the frontrunners in providing protection against toxic chemicals and parasites, which can be harmful to all living organisms that can come into contact with treated water. However, even with the vast improvements seen throughout the years in water treatment, some chemicals can still go undetected or untreated and released into the environment. Among those, a new class of contaminants of emerging concern known as Pharmaceuticals and Personal Care Products (PPCPs) are entering our water systems due to their persistence to modern water treatment processes causing concerns around the globe.

1.1 PPCPs

PPCPs are contaminants of emerging concern, which are defined as chemicals coming from domestic, municipal, industrial, and agricultural sources with the potential to have adverse environmental and health effects that are not commonly monitored. They are commonly washed down sinks or flushed down toilets in the form of complete and incomplete metabolized drugs in human and animal waste. The metabolized intermediates can be even more stable than the original parent compounds and have the potential for bioaccumulation. PPCPs are a mixture of an enormous class of compounds that vary by molecular weight, structure and functionality. They are commonly categorized into two categories: pharmaceuticals which include over-the-counter, prescription, hormone supplements, chemo-, and veterinary drugs, and personal care products such as cosmetics, fragrances, cleaning products, and personal hygiene
products. Of the many compounds under investigation erythromycin, an antibiotic drug is the only compound listed under the EPAs contaminates candidate list \(^{(18)}\) leading toward possible regulation under the Safe Drinking Water Act. According to the National Center for Health Statistics, there was a noticeable increase in the United States prescription medications.\(^{(19)}\) Americans using at least one prescription medication increased by 10 percent, 20 percent for those using two or more and 70 percent using five or more.\(^{(19)}\) A rise was also seen in veterinary medicine in which the average household had an expenditure of 366 dollars for all pets for veterinary treatment.\(^{(20)}\) The United States is one of the largest markets for cosmetics and toiletries with more than 45 million dollars in annual sales.\(^{(21)}\)

PPCPs are comprised of thousands of chemicals and their effects are continually being researched. The analytes tested for this research were selected based on their availability as standard materials; selected analytes are shown in Table 1.
Table 1. Selected Analytes (Group 1 Acidic Compounds Electrospray Ionization (ESI)(+))

<table>
<thead>
<tr>
<th>Group 1 (ESI+)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Acetaminophen (Analgesic/Antipyretic)</td>
<td>Fluoxetine (Antidepressant)</td>
<td>Sulfamethazine (Antibiotic)</td>
</tr>
<tr>
<td><img src="image" alt="Acetaminophen" /></td>
<td><img src="image" alt="Fluoxetine" /></td>
<td><img src="image" alt="Sulfamethazine" /></td>
</tr>
<tr>
<td>Ciprofloxacin (Chemotherapeutic Antibiotic)</td>
<td>Erythromycin (Antibiotic)</td>
<td>Codeine (Pain Reliever)</td>
</tr>
<tr>
<td><img src="image" alt="Ciprofloxacin" /></td>
<td><img src="image" alt="Erythromycin" /></td>
<td><img src="image" alt="Codeine" /></td>
</tr>
<tr>
<td>Cotinine (Metabolite of Nicotine)</td>
<td>Caffeine (Stimulant)</td>
<td>Trimethoprim (Antibiotic)</td>
</tr>
<tr>
<td><img src="image" alt="Cotinine" /></td>
<td><img src="image" alt="Caffeine" /></td>
<td><img src="image" alt="Trimethoprim" /></td>
</tr>
</tbody>
</table>

1.2 PPCPs and Water Regulations

PPCPs are found in the environment at trace level amounts (ng/L). Currently, unlike many pesticides, there is no regulation for these compounds and once they are introduced into the sewage system they are not treated for by wastewater facilities due to the fact that they are at trace level concentrations. These compounds are then allowed to be discharged back into both the environment and water supplies on a continual basis, which causes a concern of long-term and generational exposure and the effects. Although many PPCPs can be degraded in the environment by a change in pH, stereo-chemical structure, chemical nature and soil organic matter (SOM). With the continual replenishment from effluent of wastewater treatment
processes there are still compounds entering groundwater, surface water and aquifers. Before 1999 most of the research on PPCPs took place throughout Europe in groundwater, wastewater and surface water. (16) The discoveries of PPCPs in Europe’s water systems lead to the testing for PPCPs in many other industrialized countries water systems throughout the world. From 1999 to 2000 the United States Geological Survey (USGS) collected and analyzed 139 susceptible streams in 30 states throughout the United States. (22) It was found that in 80 percent of the samples analyzed that one or more PPCPs were present. (22) Mixtures of contaminants were also present: 75 % of the samples contained more than one PPCP, 50 % had seven or more and 34 % contained ten or more contaminants. (22) Since the discovery of PPCPs the majority of research is now focused on the effects and development of new ways to remove these compounds. In Japan a new approach has been taken to better understand the exact sources of these compounds by using 13 PPCPs as biomarkers for the detection of sewage contamination. (25) Of the 13 PPCPs under investigation carbamazepine and crotamiton showed the most promise throughout seasons showing consistency and not largely affected by soil absorption or biodegradation. Both showed consistency in freshwater and coastal water environments, combining carbamazepine and cromaiton could help detect in the leakage of septic sewage systems and other sources of contamination. (25) PPCPs are continually discharged into the environment, the concerns of their effects on the environment and human health is now the focus of research performed by the EPA and WHO for the development of methods for detection and removal.
1.3 Environmental Fate and Transport of PPCPs

The continual release and detection of these compounds in our water bodies has led to research looking at the persistence and mobility of these compounds once introduced into the environment. PPCPs contain such a large and broad category of compounds which makes it difficult to model a specific pathway once introduced into the environment. With varying structures and chemical properties the fate and transport of PPCPs vary between each compound. PPCPs once in the environment may undergo alterations such as degradation from ultraviolet light, mineralization, and the potential to be accumulated in plants and aquatic organisms. For PPCPs traditional methodology to illustrate the movement of these compounds through soil and water matrices once introduced has yet to be established, unlike the class of pesticides. For instance the modeling of the movement of certain pesticides look at the point of origin, the persistence, introduction and range of dispersion and the affinity for that compound to bind to substrates. PPCPs, which comprise a large class, do not all fit into one model. Some may degrade once introduced into the environment; however, many do not and are able to form complexes with organic and inorganic soil matter. Many of these interactions not only take place due to hydrophobic interactions but are driven by other means such as cation exchange, bridging and hydrogen bonding. For example ciprofloxacin (antibiotic) was studied in 30 different soil types from the eastern United States to see the major contributing factors (cation exchange, cation bridging and surface complexation) in its absorption to the soils. Cation exchange was found to be the main driving force behind the sorption of ciprofloxacin in the soils. In addition, it was found that ciprofloxacin in certain soils with a variable pH range would limit or slow its activity. This was seen especially for soils
such as vertisols, which are clay rich and have a high amount of cation exchange sites\textsuperscript{(26)}. Ciprofloxacin would then be competing with other mineral and metals for complex formation in these soils, in which the trace amounts of ciprofloxacin in the environment would be out competed. However the opposite was seen in alkali soils, particularly those impacted by liming which had small effects on ciprofloxacin’s activity. Modeling the pathways of PPCPs once introduced into the environment will help to better understand the fate and mobility of these compounds which is vital in the development of methods for removal and detection.

When conditions are optimum PPCPs will bind to minerals and metals present both in water and soil. These complexes play a large role in the misconception that PPCPs at these trace levels are readily biodegradable. However recent studies have shown that the non-persistent behavior seen is due to the formation of these complexes.\textsuperscript{(12, 22, 26)} The active ingredient and its metabolite may bind and form these complexes, which can limit its mobility and effects on the environment. These complexes can increase the persistence of PPCPs in the soil for several years; possibly extend their half-lives.\textsuperscript{(12, 24)} The bound residues however are not completely inactive; changes in the environmental conditions such as a change in temperature and pH can release them posing an environmental and human health risk. The fate and transport of PPCPs are mostly affected by pH, stereo-chemical structure, chemical nature and soil organic and inorganic matter.\textsuperscript{(12)} Unfortunately not all PPCPs will behave similarly, so more work of modeling and fate would be required for this large class of compounds.
1.4 Environmental and Human Health Effects of PPCPs

The effects of PPCPs on human health are not well known, however, research on the potential effects on human tissue has begun to draw much attention. Pomati et al. looked at the effects of PPCPs in embryonic kidney cells (HEK293), ovarian tumor cells (OVCAR3) and E. coli (27) to a mixture of 13 pharmaceuticals containing atenolol, bezafibrate, carbamazepine, cyclophosphamide, ciprofloxacin, furosemide, hydrochlorothiazide, ibuprofen, lincomycin, ofloxacin, ranitidine, salbutamol, and sulfamethoxazole at concentrations normally found in the environment in the parts per billion (ppb) and parts per trillion (ppt) ranges. (27) All cells were tested with both a mixture of PPCPs and each PPCP individually. Four (atenolol, bezafibrate, ciprofloxacin, and lincomycin) of the thirteen PPCPs had statistically significant effects on cell proliferation, which could be due to the specific target sites available from the cells lines used. (27) Antagonistic effects seemed to be the contributing factor for significant responses to the mixture of PPCPs. (27) Scientists at the Helmholtz research center found that low-levels of pain relievers caused an anti-inflammatory like response in human blood cells (28).

A study conducted between 2000 and 2007 looked at the effect of diclofenac (anti-inflammatory) on vultures who fed on the dead carcasses of treated cows in India. It was found that the gypsy vultures who fed on the treated livestock accumulated 100 micrograms per kilogram which caused renal failure and a 40 percent decline per year in the population. (29) This study shows the potential for bioaccumulation of PPCPs in a food web which could drastically affect all populations.
There is a wide variety of ecotoxicity methods used to address the effects of PPCPs on aquatic organisms (fish, algae and invertebrates). The specificity of the drugs target binding site as well as the metabolites which are formed after they have reacted must be taken into account when developing the experiment and species to be exposed. Much of the concern comes from the bioaccumulation of PPCPs, which are fat-soluble, and build up in aquatic organisms through continual exposure. Many of these compounds are found present in the environment as the original compound or in a mixture usually at the ppt (ng/L) range. Many PPCPs have been found to be endocrine disruptors which can mimic and block hormones and can have a negative effect on the processes needed by the body. In 1999 the USGS tested 139 rivers and streams located in the United States. In all streams tested at least one strain of ampicillin-resistant bacteria was present in the samples obtained. A research group at the University of Georgia in 2003 exposed tadpoles to Prozac (fluoxetine/antidepressant) at 30ppb (concentrations above those found in the environment) and on the 57th day of exposure, deformities and slowed development were observed. In 2003 the USGS conducted a survey on small-mouth male bass in the Potomac River and discovered the feminization of male fish. Fish sampled in areas with high agricultural industry and urban population showed 75% intersex male fish, 14 to 35% in less populated and farmed sites and 47-77% in the South Branch of the Potomac River. Despite the results of the research conducted concerning PPCPs, many in the scientific community have questioned the validity of whether these compounds are the contributing factor for the observed developmental effects since many of the experiments are conducted at much higher levels than those found in the environment. This lead to a real-life settings and scenarios used to show the impact of selected analytes on aquatic organisms. In
2007 Dr. Karen A Kidd and colleagues of the Canadian Rivers Institute applied a synthetic estrogen found in birth control in ppt (5-6 ng/L) concentrations to the experimental lakes found in Canada over three summers.\(^{(32)}\) After the experimental period, it was found that male minnows exposed over the experimental period to even these very minuscule concentrations began to develop female characteristics and the population slowly began to decline.\(^{(32)}\) These studies have now led to a more extensive amount of research being placed into the study of PPCPs and their effects. The EPA has taken a major role in assessing the risks and method development for the detection of PPCPs through scientific collaborations and the funding of research.\(^{(33)}\) Two pilot studies were conducted by the Office of Science and Technology (OST) with the involvement of Baylor University, EPA Great Lakes National Program Office, Metropolitan Water Reclamation District of Greater Chicago and New Mexico Environmental Department; one was performed in 2006 for 24 commonly used PPCPs in wastewater, fish fillets and tissues at five sites. The results showed 17 of the 24 selected pharmaceuticals and 10 of the 12 personal care products tested for were not detected in fish fillets and tissues while none of the PPCPs were detected in the composite water samples collected.\(^{(33)}\)
Figure 5 shows the sites of a second pilot study being conducted since 2008 on 150 urban river sites for 20 pharmaceuticals and 15 personal care products in fish fillets and tissue to determine the presence of PPCPs. The results of the pilot study are to be released in 2011. These pilot studies will be used to gauge the presence and importance for further testing and method development. A great deal of the research being conducted shows that much more attention must be paid to PPCPs and their effects that can be detrimental to human health and the health of the ecosystems which may be detrimental.

1.5 Current PPCP detection methods

The concerns of the emerging contaminants, PPCPs, have pushed for the development of analytical equipment and methods for the detection of these trace micro-pollutants by the EPA and many government agencies. There have been numerous advances in the analytical equipment being utilized for the analysis of PPCPs in wastewater, surface waters, biosolids and sediment and sample preparation must be carried out before they can be analyzed in the
instrument of choice.\textsuperscript{(12,36,38)} Currently, there are no set standardized methods for multi-class analysis without the loss of sensitivity or selectivity, mainly due to the fact that many of these compounds have different chemical properties and behave differently in the complex matrices.\textsuperscript{(12,67)} However, there are many methods developed for the specific analysis of specific classes of compounds (such as, acidic pharmaceuticals, estrogens, personal care products, etc.).\textsuperscript{(24, 35)} Sample preparation processes are to ensure the removal of interferences (such as natural organic matter (NOM)), convert the analyte to suitable form as well as preserve and increase analyte concentration. Commonly used techniques include solid-phase extraction (SPE) or solid-phase micro extraction (SPME). Many parameters must be considered to obtain the best possible results such as the sorbent, cartridge volume, sample volume, and elution rate. SPE is the more commonly and widely used method for extraction due to the fact that SPME has limited sorbent coatings and small sorbent capacity.\textsuperscript{(34, 36, 38)} This becomes important due to the fact that many environmental contaminants are found at trace amounts in the ppt or even ppq range. In order to achieve enough sensitivity samples must be properly filtered to remove particulates, and a SPE cartridge is used to further remove compounds in the matrix which may interfere when analysis is performed on the mass spectrometer. SPE cartridges contain a solid stationary phase which helps to retain analytes of interest and allow interferences to run through. When the sample is run through the SPE cartridge entirely, appropriate organic or organic solvents mixture is used to elute the analytes of interest from the cartridge to be collected and analyzed.\textsuperscript{(36,38)} The clean up step and use of SPE is a vital step for the analysis of environmental samples. If this is not done correctly the analytes of interest may be lost due to absorption to experimental equipment or compounds that may interfere with the analysis are present.\textsuperscript{(36, 38)}
The two most common instruments for the analysis of PPCPs are HPLC-MS/MS (used primarily when looking at non-volatile polar compounds) and gas chromatography coupled to a mass spectrometer (GC-MS) (used primarily for volatile non-polar compounds). The determination of which technique to use depends on both the analytes of interest and their physical properties. The presented research utilized the Linear Ion Trap (LTQ) Mass Spectrometer due to its low solvent usage, no derivitization step, analytes low vapor pressure and Select Reaction Mode (SRM) for high sensitivity and resolution. The LTQ utilizes the electrospray ionization (ESI) technique. The use of the tandem mass spectrometry allows for the identification of both parent compounds and daughter compounds, which can then be used as a fingerprint for each specific compound and allow for analysis when the co-elution of similar mass to charge ratio compounds occur. The HPLC allows for the separation of the analytes of interest after elution through the column by the aqueous mobile phases. Retention times as well as the mass spectra of PPCPs were used for the identification of the PPCPs of interest. The capillary column used for the HPLC-MS/MS was fritted and packed using an in lab column packing procedure with C18 resin used in the original EPA methods.

1.6 Significance of PPCP research

In 2008 the Associated Press conducted a literature review on all research and data collected on water systems for PPCPs. Figure 6 shows the results and detection of PPCPs for those areas.
The assessment of PPCP concentrations in this area is needed to understand the quality of water sources, as well as to help prevent potential effects of PPCPs on public health and the environment in this region. This research will be used to establish a baseline study on PPCPs in wastewater and freshwater in this geographic area.

1.7 Objectives

The major goal of this study was to establish a baseline concentrations of PPCPs present in both wastewater and river water. This study would reveal the most commonly detected PPCPs and uncover possible sources of PPCPs as well as any effects present in the area. El Paso Water Utilities (EPWU) facilities meet all current EPA regulations and standards and must report on the analysis of drinking water annually. However, there are no current regulations for the trace...
amount of PPCPs so current systems are not equipped for their removal. Results can lead to
determination of how efficient the wastewater treatment facilities in the El Paso, Texas region at
removing PPCPs.

This research optimized the current EPA Method 1694 with the use of nano spray
technology. The method will be used as a guideline to optimize the Linear Ion Trap (LTQ) mass
spectrometer coupled with nano Liquid Chromatography (nano LC) for the detection of PPCPs
in water and sludge samples collected. This research involved several techniques to improve
sensitivity, reduce the use of organic solvent, and therefore, are more cost effective.

1.8 Hypothesis

Previous studies have shown that wastewater treatment facilities are unable to remove
these compounds—released at very low concentrations. We expect PPCPs would be detected in
wastewater effluent. In addition, higher levels of PPCPs would exist in the river samples near
the output of the wastewater facilities while sites farther away from these facilities will have
lower trace levels of PPCPs. Current literature also suggests the absorption of PPCPs to sludge,
which limits its activity and degradation. A higher concentration and number of PPCPs will be
present in sludge and soil samples.
2 Experimental

2.1 Materials

PPCP standards (acetaminophen, caffeine, ciprofloxacin, codeine, cotinine, erythromycin, fluoxetine, sulfamethazine and trimethoprim) and labeled standards (fluoxetine-D₅, cotinine-D₃, ¹³C₃-caffeine and ¹³C₃-atrazine) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) and Cerilliant Corporation (Round Rock, TX). HPLC grade solvents, liquid reagents and solid reagents were from Sigma Aldrich (St.Louis, MO, USA) and Fisher Scientific. The sources of other materials used in this research are described as follows: Oasis HLB SPE cartridges (Part No. 186000117) by Waters (Milford, MA, USA); 2 mL Screw Cap vials with septa (Part No. 5182-0869 500 piece kit) by Agilent (Santa Clara, CA, USA); Picotip emitters (FS360-100-30-D-20) by New Objective; Silica tubing (TSP075375) by Polymicro Technologies (Phoenix, AZ, USA). GF/F Whatman Glass Microfibre filter (70 mm) from Voigt Global Distribution Incorporated (Lawrence, Kansas, USA) (Part No. 1825070); and 15 mL Polystyrene Conical tubes by Becton Dickinson (Franklin Lakes, NJ, USA).

2.2 Instrumentation

2.2.1 High Performance Liquid Chromatography

Tandem Mass Spectrometry (HPLC MS/MS)

The HPLC (Eksigent, nano LC —Figure 8) coupled with a 11 cm C-18 column packed with a 3 micron bead resin and two mobile phases (A: 100% Acetonitrile/ B: Reagent water containing 0.3%Formic Acid /0.1% Ammonium Formate) with a developed gradient method allowed for the separation of target analytes. Utilization of the LTQ provided additional measures for the identification of target compounds found in the samples. The LTQ (ETD XL,
Thermo Scientific----Figure 7) uses a nanospray setup which allows for greater sensitivity at smaller concentrations. The nanospray setup also allows for smaller quantities of solvent to be used. The tandem mass spectrometer allows the fragmentation of molecules to obtain daughter peaks of the parent molecule (in case of a mixture of compounds with similar Parent Ions) and provide the sensitivity needed for the detection of PPCP at low concentrations.

Figure 7. LTQ XL/ETD

Figure 8. Eksigent nano LC.
2.3 **Solid Phase Extraction (SPE)**
SPE was used to clean samples both before and after sample preparation and uses packing silica made of small beads to help retain samples of interest. These target compounds (PPCPs) are retained in the cartridge until eluted by a designated solution such as methanol (Figure 9).

![Figure 9. 12 port SPE apparatus](image)

The extracts were then filtered both before and after preparation to ensure all visible particulates were removed. This is to ensure particulates will not enter the column attached to the HPLC and damage any components.

2.4 **Column packing pressure chamber**
The column packing pressure chamber will be used to pack 75 µm (inner diameter) silica tubing (Polymicro Technologies) with 3 micron sized beads (Restek, C18, 3µm resin). This
device allows an in-laboratory production of a numerous amount of columns and cut costs from purchasing a nano-capillary column, which may cost anywhere from $300 to $400 dollars.

![Figure 10. Nanobaume column packing chamber.](image)

Approximately 25 to 30 cm of empty silica capillary tubing (75 µm Inner Diameter) in length was cut. The column must be fritted to ensure that packing resin is kept inside tubing. A 3:1 mixture of Kasil (PQ Corporation, Valley Forge, PA, USA) to Formamide (VWR, Arlington Heights, IL, USA) was prepared to create the column frit at one end. Once the two agents have been combined, vortex (VWR: mini vortexer) the mixture for 10 seconds and then centrifuge (VWR: mini centrifuge C-1200) for 15 s. The columns should then be dipped into the top layer, in which capillary action will draw liquid into capillary (this should be done quickly because mixture will solidify) followed by being heated on a hot plate at 110° C for 4 hrs. After the frit has been made, a vial (Agilent vials and caps) is filled with acetonitrile and a stir bar is placed inside and into the pressure bomb (nanobaume, Figure 10). The capillary with the frit end up is placed through the fitting (approximately 3-5 mm from bottom of vial) and 500-600 psi of nitrogen gas is applied to the pressure bomb, which forces the acetonitrile up and through the column. If fritted properly, a bead of acetonitrile forms at the top of column and allowed to flow
for 5 minutes to clear any contaminants inside the column. After conditioning has been completed, 10 milligrams of resin (Restek, C18, 3µm) is placed into the vial with 750 µL of acetonitrile and a stir bar. The whole apparatus is then placed on a stir plate to allow the stir bar to spin and keep the resin suspended. Again, 500-600 psi of nitrogen gas is applied and resin is allowed to fill the column to the desired length. Once the column has been packed successfully, pressure is slowly released to ensure resin does not descend to create gaps. The column is then connected to the HPLC and run overnight with acetonitrile. Appropriate mobile phases are then placed into HPLC reservoir bottles and a blank run method using the experimental gradient is allowed to run overnight to condition the column. After the conditioning process, column is ready to be applied for the running of both standards and samples.

### 2.5 Cleaning of Sample Jars

All amber jars (Fisher) used for the collection of aqueous and solid samples were cleaned based off of the EPA 600 Method. Amber jars were first rinsed with tap water to loosen any particulates that may be present along the walls of the jar. Then glassware detergent (Alconox Cleaning Concentrate) was used to scrub and ensure all deposits were removed from the walls (inside and out). Jars were then completely rinsed until all soap was flushed from the jars. They were then rinsed again with de-ionized water (~3 to 4 times). The jars were then rinsed with cleaning acetone to ensure all trace residues were flushed out of the jars. Jars were then placed into the oven to bake (Furnace: Barnstead Thermodyne 62700), set at a max temperature of 400°C, for a total time of 3 hrs. Once the bake cycle had completed jars were allowed to sit until room temperature was reached, and covered with aluminum foil and stored until needed. Before, sample collection stored jars were rinsed with methanol (solvents used for analysis).
2.6 Sampling Areas

The two wastewater treatment plants in which samples were obtained from are the Northwest Water Plant, and the Roberto R Bustamante Plant. Water samples were also collected at the following sites: Rio Bosque Wetlands, Percha Dam, Ascarate Lake and areas along the Rio Grande (Figure 11). Both wastewater treatment processes are secondary treatments, however, the Northwest plant uses further disinfection using Ultraviolet light. Northwest Wastewater Treatment Plant has a capacity to treat 17.5 Million Gallons per Day (MGD) and discharges into the Rio Grande or reused to irrigate schools, golf courses, and parks. The Bustamante Wastewater Treatment Plant has a capacity to treat 39 Million Gallons per Day (MGD) and is discharged into the Riverside canal used for irrigation and 45% of effluent is redirected to the Rio Bosque wetlands.
Figure 11. Sampling Areas.
2.7 Aqueous Sample Preparation

A flow chart of the sample preparation is illustrated in Figure 12. Briefly, all samples collected on site were placed into glass amber bottles (Sigma-Aldrich) and put on ice to slow the degradation process. Once samples reached the lab they were placed into a freezer at -80 °C (VWR freezer model: 5703) until ready for preparation for analysis. Samples were then filtered through 70 mm filter paper (Whatman) to remove any particulates that may be present. Once filtered and particulates removed the samples were then pH adjusted to 2 (Thermo Orion 2 STAR) using 6 N hydrochloric acid (Titristar/EMD). The filtered sample was homogenized and separated into acid and base aliquots. Then 500 mg of tetrasodium EDTA (Fluka Analytical) was added to the acidic aliquots acting as a chelating agent to release analytes that maybe attached to minerals or other interferences present in the sample. The samples were shaken periodically and allowed to equilibrate for approximately 2 hrs. Samples were then spiked with surrogate standards ($^{13}$C$_3$-Caffeine: 10µL, Cotinine-D$_3$: 15µL, Fluoxetine-D$_5$: 50 µL) and internal standard ($^{13}$C$_3$-Atrazine: 15µL) followed by Solid Phase Extraction using hydrophilic-lipophilic balance sorbent cartridges (Waters HLB Oasis).
Figure 12. Preparation of aqueous samples. Source: EPA Methods 1694
2.8 **Solid Sample Preparation**

All sediment and biosolid samples collected were placed into a freezer to slow the degradation of target analytes until samples were ready for preparation for analysis (Figure 13). From the bulk material, 5 to 10 g was weighed out into a clean beaker or weighing plate and dried for 12 hours at 110°C (VWR: Model-1326 Gravity Convection Oven) and cooled in a desiccator. The dried aliquot was again weighed, and the percent solid is calculated using equation 1 (See Appendix B).

$$\text{%Solids} = \frac{\text{Weight of sample aliquot after drying (g)}}{\text{Weight of sample aliquot before drying (g)}} \times 100$$

**Equation 1.** Determination of percent solids.

The sample should be homogenized using a mortar and pestle or a high speed heavy duty blender to obtain particles of solids of 1 mm or less in size. All size-reducing sample preparation was done under the fume hood to prevent contamination. Into a 50 mL centrifuge tube (Becton Dickinson), 1 g of the dried homogenized sample and 15 mL of pH 2 adjusted phosphate buffer were added, and vortexed (VWR: mini vortexer) for 5 min. Additional buffer might be added to maintain pH of the sample mixture at 2. This solid slurry was extracted three times with acetonitrile and sonication. For the first and second extraction, 20 mL of acetonitrile were added to the sample(s), sonicated (Branson Sonicator 2510) for 30 min, and centrifuged (Thermo Scientific Sorall T1 centrifuge) for 5 min at 3000 rpm. The supernatants were decanted and placed into clean 250 mL round-bottom flasks (Kimax: flat bottom flask 500mL). Again 15 mL of the phosphate buffer was added, vortexed to re-suspend the samples and adjusted to a pH
of 2 with 6N HCl. For the third extraction, only 15 mL of acetonitrile was used. If particulates were visible in the extract, sample(s) can be filtered through a 110-mm filter and the filter was rinsed three times by reagent water. The extracts were concentrated to a final volume of 20-30 mL by rotary evaporation (Buchi Rotavapor R-205) at 50°C. The samples were then re-suspended in 200 mL of reagent water and 500 mg of tetra-sodium EDTA and swirled to mix. Solid Phase extraction was used to further filter samples and remove interferences using hydrophilic-lipophilic balance sorbent cartridges.
Figure 13. Preparation of Solid Samples. Source: EPA Methods 1694
2.9 Solid Phase Extraction Preparation

After samples were filtered and spiked with known concentrations of labeled target PPCPs as surrogates, each aliquot was run through Oasis HLB SPE cartridges (20cc/1g 60µm) the extraction of PPCPs. Each cartridge will be conditioned with 20 mL of methanol (Sigma-Aldrich) and 6 mL of reagent water (Sigma-Aldrich). Conditioning for the acid aliquots requires an extra step of 6 mL of reagent water with the pH adjusted to 2. Once the cartridges have been conditioned, the samples may be loaded onto the conditioned cartridge. The cartridges were then placed onto a SPE apparatus (Supelco Visiprep Large Volume Sampler: 57275) connected to a vacuum and filtering flask (EYELA Aspirator) and filtering flask (2000 mL Kimax Filtering Flask No: 27065), which allowed the sample to pass through the cartridge automatically at a rate of 5-10 mL/min. Once the sample had passed through the cartridge, it was rinsed with 6 mL of reagent water to remove the EDTA. The cartridges were allowed to dry for approximately 5 min. The target analytes were eluted from the absorbent using 12 mL of methanol. Next, the collected extracts were concentrated by completely drying samples with nitrogen using a nitrogen concentrator. The dried samples were then reconstituted to a final volume of 100 µL, including surrogate standards (^{13}C₃-Caffeine: 10µL, Cotinine-D₃: 15µL, Fluoxetine-D₅: 50 µL) and internal standard (^{13}C₃-Atrazine: 15µL). The samples were then run through the HPLC MS/MS.
### 2.10 SPE Recovery

A recovery test acts as a lab blank and will confirm the ability of the clean up step to minimize the matrix effect and possible signal suppression. Lab blanks were put through the same process as the actual samples collected. Two-one liter amber jars, used for actual sample collection, where filled with Deionized water (DI) placed into the fridge overnight at 4°C and processed the next day. They were filtered and pH adjusted as if real samples. The blank samples were spiked with known concentrations of isotope labeled compounds and the target analytes (Table 2) then run through the SPE cartridge as described previously. Analytes were then eluted using methanol, the extract was collected and the solvent evaporated to dryness. The samples were then reconstituted with 95% LC-MS grade water and 5% LC-MS grade Acetonitrile to make a total volume of 100 µL.

**Table 2.** Concentrations of stock standards for native and labeled compounds and amount spiked to cartridge for SPE Recovery test to achieve ~1pmol/µL concentration at a final volume of 100 µL.

<table>
<thead>
<tr>
<th>Native Compounds</th>
<th>Stock Standard (µg/mL)</th>
<th>Spiked Volume (µL)</th>
<th>Final Concentration (pmol/µL) at 100 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>100</td>
<td>1</td>
<td>1.985</td>
</tr>
<tr>
<td>Caffeine</td>
<td>25</td>
<td>1</td>
<td>1.268</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>8.75</td>
<td>5</td>
<td>1.320</td>
</tr>
<tr>
<td>Codeine</td>
<td>5</td>
<td>10</td>
<td>1.670</td>
</tr>
<tr>
<td>Cotinine</td>
<td>2.5</td>
<td>10</td>
<td>1.419</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>5</td>
<td>20</td>
<td>1.363</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>2.5</td>
<td>20</td>
<td>1.617</td>
</tr>
<tr>
<td>Sulamethazine</td>
<td>1</td>
<td>30</td>
<td>1.078</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.5</td>
<td>30</td>
<td>0.517</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Labeled Compounds</th>
<th>Stock Standard (µg/mL)</th>
<th>Spiked Volume (µL)</th>
<th>Final Concentration (pmol/µL) at (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{13}$C$_3$-Caffeine</td>
<td>3</td>
<td>10</td>
<td>1.522</td>
</tr>
<tr>
<td>Cotinine-D$_3$</td>
<td>2</td>
<td>15</td>
<td>1.674</td>
</tr>
<tr>
<td>Fluoxetine-D$_3$</td>
<td>1</td>
<td>50</td>
<td>1.233</td>
</tr>
</tbody>
</table>
2.11 Mass Spectrometer and HPLC Parameters

Nine target compounds were selected based on their commercial availability and applications as antibacterial products, anti-inflammatory products, anticoagulants, antidepressants, stimulants, analgesics and metabolites. The nine standard solutions were prepared at 10 pmol/µL (Table 3) followed by a series of dilutions (Figure 14). These standards were used to determine the range of the mass spectrometer.

Table 3. Volumes used to prepare a stock standard mixture (10 pmol/µL) of native and isotope standards and mixture used for dilutions to create mixtures of 5 pmol/µL to 1 fmol/µL

<table>
<thead>
<tr>
<th>Native Compound</th>
<th>10 pmol/µL</th>
<th>5 pmol/µL</th>
<th>1 pmol/µL</th>
<th>100 fmol/µL</th>
<th>10 fmol/µL</th>
<th>1 fmol/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>30.23 µL</td>
<td>1600 µL</td>
<td>1800 µL</td>
<td>1800 µL</td>
<td>1800 µL</td>
<td>1800 µL</td>
</tr>
<tr>
<td>Caffeine</td>
<td>38.84 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>73.58 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>5.98 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>3.52 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>146.84 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>6.91 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>55.66 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>116.12 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isotope Standards</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13C3-Caffeine</td>
<td>39.43 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine-D3</td>
<td>35.84 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine-D5</td>
<td>80.97 µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>Analysis Volume</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>
Figure 14. Preparation of Standard Solutions for calibration.
After the determination of the lowest and highest concentration attainable by the LTQ a new range of concentration mixtures were created. This range allowed for the enhancement of sensitivity to cover the range more specific to actual environment levels of 1fmol/µL-1pmol/uL (Table 4).

Table 4. Volumes used to prepare a stock standard mixture (800 fmol/µL and 600 fmol/µL) of native and isotope standards and mixtures used for dilutions to create 400 fmol/µL to 1fmol/µL.

<table>
<thead>
<tr>
<th>Native Compound</th>
<th>800 fmol/µL</th>
<th>600 fmol/µL</th>
<th>400 fmol/µL</th>
<th>200 fmol/µL</th>
<th>100 fmol/µL</th>
<th>10 fmol/µL</th>
<th>1 fmol/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Caffeine</td>
<td>155 µL</td>
<td>130 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>120 µL</td>
<td>80 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Codeine</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Cotinine</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>157 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>161 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Isotope Standards</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{13}$C$_3$-Caffeine</td>
<td>158 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Cotinine-D$_3$</td>
<td>160 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Fluoxetine-D$_5$</td>
<td>153 µL</td>
<td>120 µL</td>
<td>1333 µL</td>
<td>500 µL</td>
<td>250 µL</td>
<td>100 µL</td>
<td>200 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
<td>2 mL</td>
</tr>
<tr>
<td>Analysis Volume</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

Samples were then placed onto nano LC autosampler and run from the lowest concentration to the highest concentration and injected through the column and into the mass spectrometer (MS). The MS and HPLC parameters are modeled around those set by the EPA method with adjustments to the flow rate, injection volume, composition of mobile phases and use of the nanospray set-up (Table 5).

To ensure proper detection of analytes and deter false positives the use of fragmentation patterns of the parent peak as a fingerprint for each compound was done through direct injection.
and increasing the collision energy. Samples were injected directly into the system using the syringe pump at 0.500 µL/min until the parent peaks were identified. Once parent peaks were identified the collision energy was increased until a noticeable change of intensity approximately 1.0E+3 or higher was achieved along with the presence of the daughter ion peak. Figure 15 shows both views of the LTQ Mass Spectrometer the collision cell is where the parent ions selected are hit with nitrogen gas for further fragmentation to form the daughter peaks the ion trap is used to only collect those ions selected and eject all others allowing for greater sensitivity to be achieved.

![Diagram of Linear Ion trap (LTQ) with collision cell](image)

**Figure 15.** Diagram of Linear Ion trap (LTQ) with collision cell (39)
Table 5. HPLC Parameters (Group 1 Acidic Extraction, Positive electrospray ionization ESI (+)).

<table>
<thead>
<tr>
<th>LC Gradient</th>
<th>LC Flow Rate (nL/min)</th>
<th>LC Conditions (Eksigent NanoLC)</th>
<th>MS Conditions (LTQ XL/ETD)</th>
<th>Mobile Phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (mins)</td>
<td>Flow Mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>95% A 5% B</td>
<td>300</td>
<td>Flow Rate: 300 nL/min</td>
<td>Solvent A: 0.3% Formic Acid 0.1% Ammonium Formate in LC-MS Grade Water</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pressure</td>
<td>Solvent B: 100% ACN LC-MS Grade/0.1% Formic Acid</td>
</tr>
<tr>
<td>4.0</td>
<td>95% A 5% B</td>
<td>300</td>
<td>Autosampler Tray Temp.: 4°C</td>
<td></td>
</tr>
<tr>
<td>22.5</td>
<td>12% A 88% B</td>
<td>300</td>
<td>Column Length: 13 cm</td>
<td></td>
</tr>
<tr>
<td>23.0</td>
<td>100% B</td>
<td>300</td>
<td>Injection Volume: 1µL</td>
<td></td>
</tr>
<tr>
<td>26.0</td>
<td>100% B</td>
<td>300</td>
<td>Source Temp.</td>
<td></td>
</tr>
<tr>
<td>26.5</td>
<td>95% A 5% B</td>
<td>300</td>
<td>Desolvation Temp.</td>
<td></td>
</tr>
<tr>
<td>33.0</td>
<td>95% A 5% B</td>
<td>300</td>
<td>Ionization: ESI (+)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acquisition: 40 mins.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3 Results and Discussion

3.1 Limit of Detection and Calibration Curve

As a preliminary study for testing the instrument detection limit for the analytes, a series of standard solutions of each target PPCP from 10 pmol/µL to 1 fmol/uL (Table 3 and Table 4) were prepared and directly injected into the mass spectrometer through Electrospray Ionization ESI. The parent ion peaks were first identified for each standard through direct injection of the standards using the syringe pump at a flow rate of 0.500 µL/min. The collision energy which induces fragmentation was increased until the daughter peak achieved an intensity of approximately 1.0E+3 or higher. This would allow for positive identification of each peak as well as the energy needed to fragment the parent peak (Table 6).

Table 6. Parent ions, Daughter ions and Collision Energies for Target analytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent Ion (m/z)</th>
<th>Daughter Ion (m/z)</th>
<th>Collision Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>152.2</td>
<td>110.14</td>
<td>25</td>
</tr>
<tr>
<td>Caffeine</td>
<td>195.0</td>
<td>138.14</td>
<td>45</td>
</tr>
<tr>
<td>Codeine</td>
<td>300.0</td>
<td>152.88</td>
<td>23</td>
</tr>
<tr>
<td>Cotinine</td>
<td>177.0</td>
<td>98.13</td>
<td>22</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>734.4</td>
<td>158.27</td>
<td>21</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>310.3</td>
<td>148.16</td>
<td>55</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>279.0</td>
<td>156.22</td>
<td>35</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>291.0</td>
<td>230.22</td>
<td>50</td>
</tr>
</tbody>
</table>

Standard linear curves were then constructed based on the intensity of the peaks and the concentrations to have a correlation coefficient ($r^2$) value of at least 0.95 (Table 7). These standards were used to determine the linear range of detection of the LTQ mass spectrometer. Figure 16 shows the calibration curve of cotinine as an example, and the curves for the rest of the analytes are included in Appendix A.
Figure 16. Linear curve of Cotinine Peak Intensity vs. Concentration 1fmol/µL-10pmol/µL

Table 7. Correlation coefficient for Calibration Standards using peak intensity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Correlation coefficient ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.95</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.97</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.96</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.99</td>
</tr>
<tr>
<td>Cotinine</td>
<td>0.96</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.99</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.95</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0.98</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Target analytes were first identified based on the parent mass to charge ratio (m/z) peaks to ensure sensitivity levels were attainable. It was determined that concentrations above 1pmol/µL oversaturated the column causing tailing and co-elution of compounds. The linear ranges for the
analytes were determined to be 1fmol/µL-1pmol/µL which covers the range more specific to actual environmental levels.

Once the detection of the 9 PPCPs on the LTQ mass spectrometer were optimized, a LC method was tested with a controlled gradient of 95% A and 5% B (A: 100% Acetonitrile/ B: Reagent water 0.3% Formic Acid 0.1% Ammonium Formate) and slowly increased to 100% B where it held for 3 mins and back to 95% A and 5% B for the remainder of the run time (40 mins total). A constant flow rate of 300 nL/min was used for separating target PPCPs in the column. The chromatogram of the mixtures of target PPCPs, showing the selected parent ions m/z and daughter ions m/z peaks, were used to specify retention times and identify target analytes (Figure 17). The peak areas based on the m/z value of daughter ions m/z and retention times were obtained as shown in Table 8 and then later used for the identification of target analytes in real samples.
Figure 17. Chromatogram of Target analytes for Identification of peak and retention times
Table 8. Compounds with Retention Times for Target PPCPs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>7.56</td>
</tr>
<tr>
<td>Caffeine</td>
<td>17.42</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30.77</td>
</tr>
<tr>
<td>Codeine</td>
<td>11.23</td>
</tr>
<tr>
<td>Cotinine</td>
<td>6.06</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>27.41</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>29.74</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>16.44</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>19.14</td>
</tr>
</tbody>
</table>

Once all mixtures of the compounds had run through the system, the Xcalibur analysis program\(^{(75)}\) was used to further analyze the chromatograms produced for the mixtures of the analytes of interest. Xcalibur Analysis is a program used for the quantification process and simultaneous processing of multiple scans. After run completion the program allows for further analysis from peak selection, integration, retention time identification and signal to noise ratio. The chromatogram for the selected daughter ion fragmented from the parent ion was used to obtain the peak areas (Figure 19; Appendix C) and produce calibration curves with a correlation coefficient \(r^2\) value of 0.95 or greater as shown in Table 9 and a linear regression equation was also obtained to be used for the quantification of analytes detected in the real samples. Figure 18 shows the calibration curve of erythromycin as an example, and the curves for the rest of the analytes are included in Appendix A. All curves were fitted with 95% confidence intervals to show the relation of the points along the calibration curve and lie within the range of the standards created.
Figure 18. Peak Area vs. Concentration (1 fmol/µL to 1 pmol/µL) with 95% confidence intervals

Table 9. Correlation Coefficient for calibration standards using peak area

<table>
<thead>
<tr>
<th>Compound</th>
<th>Correlation coefficient ($r^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.995</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.995</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.994</td>
</tr>
<tr>
<td>Cotinine</td>
<td>0.992</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.999</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.999</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>0.995</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.992</td>
</tr>
</tbody>
</table>
Figure 19. Chromatogram showing the area selection for target analyte peaks.
3.2 Comparison between Methods

The EPA leads the way when managing emerging contaminants and developing the foundation of methods used for their analysis and detection. This research used the EPA method 1694: Pharmaceutical and Personal Care Products in Water, Soil, Sediment and Biosolids by HPLC-MS/MS as a template. This is research aimed at implementing green chemistry concepts to minimize the use of large volume amounts of organic solvents and the production of toxic waste without affecting instrument sensitivity and reproducibility. This was achieved through the modification of concentration and volume re-suspension of samples and the instrumentation used for analysis.

The EPA methods calls for the use of a tandem mass spectrometer (MS/MS) equipped with ESI interface, negative and positive mode capability, collision cell and the ability to produce parent-daughter transitions of the compounds. The instrument recommended was a Quattro Ultima triple quadrupole MS or equivalent. The MS/MS must be coupled to a HPLC system with multi-segment gradient capability for proper separations of the target compounds using the specified parameters listed in the method. These systems are effective in meeting the criteria previously mentioned such as being equipped with ESI interface, fragmentation capable and multi-segment gradient HPLC as outlined by the EPA.

In this study the use of the thermo LTQ-ETD/XL coupled to an eksigent nano-LC was used to perform the analysis of the standards and samples collected. The Thermo LTQ-ETD/XL allows for the specific fragmentation of target ions. The selected peak of the target analyte can be confirmed by the specific fragments the parent ions and the expected daughter ions. Figure
is an example chromatogram of fluoxetine which has an m/z value of 310.3 for the parent compound and 148.16 for the daughter compound. The compound can be identified in the chromatogram as the single most abundant peak at a retention time of 29.74 for the surrogate standard used to determine retention time and sensitivity.

There are three different interfaces for sample introduction to the mass spectrometer: the triversa (chip-based electrospray ionization), atmospheric-pressure chemical ionization (APCI) and electrospray ionization (ESI). ESI was used for the analysis of wastewater and sludge samples collected. This is a soft ionization technique in which the ions are formed without breaking apart bonds. The LTQ ESI interface uses pico-tip emitters with an inner bore size of 10 ±1µm which allows for smaller volumes to be used to minimize signal suppression and sensitivity. Coupled to the nano-LC which uses a flow in nL/min ensures smaller volume amounts of organic solvents used when running the gradient. Table 10 compares the volumes between the EPA and the parameters used in this study. As can be seen above the volumes and flow rates were molded to the system LTQ-nano LC system to obtain the best results. The standards used were also altered to prevent overloading of the column to ensure proper separation of the target compounds and identification of the spiked isotopes and surrogate compounds. The method used for this study is more sensitive compared to the EPA and therefore allows for the range of calibration standards to focus on the lower concentration levels of ppt (ng/L) to ppq (pg/L) (Table 11 and Table 12).
A recovery test was also performed to confirm the ability of the clean up step to minimize the matrix effect and possible signal suppression as well as enhance the ability to detect these trace contaminants.

Even though SPE has a larger range of commercially available sorbents allowing for a wider range of analytes being collected it does have its disadvantages. One being the selection of the eluting solvent, which if not chosen properly will either not elute or elute too quickly the analytes of interest during collection. Another disadvantage is the lowering of pH during this step which is used to activate the cartridge which may also allow for the co-elution of NOMs. Once extracted samples are then concentrated, the samples are dried completely under a nitrogen flow and re-suspended in proper solvent. After the extract is obtained it can then be run through the selected instrument for analysis. Knowing the amount of standards spiked to the cartridge would then allow for the recovery of these compounds to be determined after the extraction process. This process would also help to identify weaknesses such as absorption to glassware, loss during evaporation stage or during transfer of the liquid, determination of how clean the samples are after filtering and if any changes should be modified to improve the recovery of the target analytes.

Table 10. Comparison to EPA volumes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EPA</th>
<th>This Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection Volume</td>
<td>15 µL</td>
<td>1µL</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>mL/min</td>
<td>nL/min</td>
</tr>
<tr>
<td>Total Volume Sample</td>
<td>1 mL</td>
<td>100 µL</td>
</tr>
</tbody>
</table>
Figure 20. Identification and selection of Daughter ion (Spectra and Chromatogram)
Table 11. EPA calibration standards (CS)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration</th>
<th>CS-1</th>
<th>CS-2</th>
<th>CS-3</th>
<th>CS-4</th>
<th>CS-5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetaminophen</strong></td>
<td>ng/mL</td>
<td>50</td>
<td>150</td>
<td>750</td>
<td>2500</td>
<td>10000</td>
</tr>
<tr>
<td>MW:151.16256</td>
<td>pmol/µL</td>
<td>0.331</td>
<td>0.992</td>
<td>4.962</td>
<td>16.538</td>
<td>66.154</td>
</tr>
<tr>
<td><strong>Caffeine</strong></td>
<td>ng/mL</td>
<td>12.5</td>
<td>37.5</td>
<td>18.7</td>
<td>62.5</td>
<td>250</td>
</tr>
<tr>
<td>MW:194.19</td>
<td>pmol/µL</td>
<td>0.064</td>
<td>0.193</td>
<td>0.096</td>
<td>0.322</td>
<td>1.287</td>
</tr>
<tr>
<td><strong>Codeine</strong></td>
<td>ng/mL</td>
<td>2.5</td>
<td>7.5</td>
<td>37.5</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>MW:299.364</td>
<td>pmol/µL</td>
<td>0.008</td>
<td>0.025</td>
<td>0.125</td>
<td>0.418</td>
<td>1.670</td>
</tr>
<tr>
<td><strong>Cotinine</strong></td>
<td>ng/mL</td>
<td>1.25</td>
<td>3.75</td>
<td>18.7</td>
<td>62.5</td>
<td>250</td>
</tr>
<tr>
<td>MW:176.22</td>
<td>pmol/µL</td>
<td>0.007</td>
<td>0.021</td>
<td>0.106</td>
<td>0.355</td>
<td>1.419</td>
</tr>
<tr>
<td><strong>Erythromycin</strong></td>
<td>ng/mL</td>
<td>0.25</td>
<td>0.75</td>
<td>3.75</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>MW:219.5</td>
<td>pmol/µL</td>
<td>0.0003</td>
<td>0.001</td>
<td>0.005</td>
<td>0.017</td>
<td>0.068</td>
</tr>
<tr>
<td><strong>Fluoxetine</strong></td>
<td>ng/mL</td>
<td>1.25</td>
<td>3.75</td>
<td>18.7</td>
<td>62.5</td>
<td>250</td>
</tr>
<tr>
<td>MW:309.33</td>
<td>pmol/µL</td>
<td>0.004</td>
<td>0.012</td>
<td>0.060</td>
<td>0.202</td>
<td>0.808</td>
</tr>
<tr>
<td><strong>Sulfamethazine</strong></td>
<td>ng/mL</td>
<td>0.5</td>
<td>1.5</td>
<td>7.5</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>MW:278.36</td>
<td>pmol/µL</td>
<td>0.002</td>
<td>0.005</td>
<td>0.027</td>
<td>0.090</td>
<td>0.359</td>
</tr>
<tr>
<td><strong>Trimethoprim</strong></td>
<td>ng/mL</td>
<td>1.25</td>
<td>3.75</td>
<td>18.7</td>
<td>62.5</td>
<td>250</td>
</tr>
<tr>
<td>MW:290.3</td>
<td>pmol/µL</td>
<td>0.004</td>
<td>0.013</td>
<td>0.064</td>
<td>0.215</td>
<td>0.861</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td>ng/mL</td>
<td>4.4</td>
<td>13.1</td>
<td>65.6</td>
<td>218</td>
<td>875</td>
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<tr>
<td>MW:385.8</td>
<td>pmol/µL</td>
<td>0.011</td>
<td>0.034</td>
<td>0.170</td>
<td>0.565</td>
<td>2.268</td>
</tr>
<tr>
<td><strong>Cotinine-D&lt;sub&gt;3&lt;/sub&gt;</strong></td>
<td>ng/mL</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>MW:176.22</td>
<td>pmol/µL</td>
<td>0.284</td>
<td>0.284</td>
<td>0.284</td>
<td>0.284</td>
<td>0.284</td>
</tr>
<tr>
<td><strong>Fluoxetine-D&lt;sub&gt;5&lt;/sub&gt;</strong></td>
<td>ng/mL</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>MW:309.33</td>
<td>pmol/µL</td>
<td>0.081</td>
<td>0.081</td>
<td>0.081</td>
<td>0.081</td>
<td>0.081</td>
</tr>
<tr>
<td><strong>13C&lt;sub&gt;3&lt;/sub&gt;-Caffeine</strong></td>
<td>ng/mL</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>MW:194.19</td>
<td>pmol/µL</td>
<td>0.386</td>
<td>0.386</td>
<td>0.386</td>
<td>0.386</td>
<td>0.386</td>
</tr>
<tr>
<td><strong>13C&lt;sub&gt;3&lt;/sub&gt;-Atrazine</strong></td>
<td>ng/mL</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>MW:215.68</td>
<td>pmol/µL</td>
<td>0.232</td>
<td>0.232</td>
<td>0.232</td>
<td>0.232</td>
<td>0.232</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration Unit</td>
<td>CS-1</td>
<td>CS-2</td>
<td>CS-3</td>
<td>CS-4</td>
<td>CS-5</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>ng/mL</td>
<td>N/A</td>
<td>1.56</td>
<td>15.63</td>
<td>30.88</td>
<td>61.00</td>
</tr>
<tr>
<td>MW: 151.16256</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>ng/mL</td>
<td>N/A</td>
<td>1.94</td>
<td>19.37</td>
<td>38.75</td>
<td>77.99</td>
</tr>
<tr>
<td>MW: 194.19</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td>pmol/µL</td>
<td>N/A</td>
<td>0.010</td>
<td>0.100</td>
<td>0.200</td>
<td>0.402</td>
</tr>
<tr>
<td>MW: 299.364</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>pmol/µL</td>
<td>N/A</td>
<td>1.77</td>
<td>17.75</td>
<td>35.50</td>
<td>71.00</td>
</tr>
<tr>
<td>MW: 176.22</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>ng/mL</td>
<td>0.75</td>
<td>7.50</td>
<td>75.02</td>
<td>150.04</td>
<td>300.08</td>
</tr>
<tr>
<td>MW: 734.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>pmol/µL</td>
<td>0.001</td>
<td>0.010</td>
<td>0.102</td>
<td>0.204</td>
<td>0.409</td>
</tr>
<tr>
<td>MW: 290.3</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Sulfamethazine</td>
<td>pmol/µL</td>
<td>0.28</td>
<td>2.80</td>
<td>28.00</td>
<td>56.01</td>
<td>112.02</td>
</tr>
<tr>
<td>MW: 278.36</td>
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<td></td>
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</tr>
<tr>
<td>Trimethoprim</td>
<td>ng/mL</td>
<td>0.31</td>
<td>3.07</td>
<td>30.75</td>
<td>61.50</td>
<td>119.99</td>
</tr>
<tr>
<td>MW: 385.8</td>
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<tr>
<td>Cotinine-D₃</td>
<td>ng/mL</td>
<td>0.18</td>
<td>1.77</td>
<td>17.70</td>
<td>35.41</td>
<td>70.81</td>
</tr>
<tr>
<td>MW: 176.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>ng/mL</td>
<td>0.42</td>
<td>4.19</td>
<td>41.94</td>
<td>83.89</td>
<td>167.78</td>
</tr>
<tr>
<td>MW: 309.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine-D₅</td>
<td>pmol/µL</td>
<td>0.001</td>
<td>0.010</td>
<td>0.106</td>
<td>0.212</td>
<td>0.413</td>
</tr>
<tr>
<td>MW: 290.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13C₃-Caffeine</td>
<td>ng/mL</td>
<td>0.20</td>
<td>1.97</td>
<td>19.70</td>
<td>39.40</td>
<td>78.79</td>
</tr>
<tr>
<td>MW: 194.19</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13C₃-Atrazine</td>
<td>ng/mL</td>
<td>369.89</td>
<td>369.89</td>
<td>369.89</td>
<td>369.89</td>
<td>369.89</td>
</tr>
</tbody>
</table>
3.3 Concentration in Water and Sludge

Sampling was conducted at the Northwest (NW--secondary treatment with UV) and Bustamante (Busta--secondary treatment) wastewater treatment plants. Influent and effluent composite samples were collected (Table 13) to obtain a baseline of PPCPs lost during treatment and those released back into the environment.

Table 13. Plant and Sampling date for Influent and Effluent

<table>
<thead>
<tr>
<th>Date</th>
<th>Amount</th>
<th>Northwest</th>
<th>Bustamante</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/12/2010</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>11/19/2010</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>12/10/2010</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>12/17/2010</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>1/7/2011</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>1/14/2011</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>2/11/2011</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>3/11/2011</td>
<td>2L/2L</td>
<td>Influent</td>
<td>Influent/Effluent</td>
</tr>
<tr>
<td>4/8/2011</td>
<td>2L/2L</td>
<td>Influent/Effluent</td>
<td>Influent/Effluent</td>
</tr>
</tbody>
</table>

After collection, the samples were filtered and pH adjusted to 2, and sample preparation was performed within a 48 hr period by filtration and SPE extraction. The extracts were stored in a freezer at 4°C prior to the analysis. Once ready for analysis samples were run through the LC-MS/MS each run lasting approximately 40 minutes. The retention times obtained from running the standards were used for the identification of the target analytes in actual samples. Concentrations for the identified samples were calculated using linear equations obtained from the calibration curves formed using the mixture of standards (Table 14).
Table 14. Formulas used for quantification

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Slope Equation Y-intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>y=6.22e+5x-1.80e+4</td>
</tr>
<tr>
<td>Caffeine</td>
<td>y=8.61e+5x-2.30e+4</td>
</tr>
<tr>
<td>Codeine</td>
<td>y=2.60e+4x-7.41e+2</td>
</tr>
<tr>
<td>Cotinine</td>
<td>y=2.09e+5x-6.92e+3</td>
</tr>
<tr>
<td>Cotinine</td>
<td>y=2.09e+5x-6.92e+3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>y=3.19e+4x-1.56e+2</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>y=5.78e+5x+2.58e+3</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>y=2e+6x-4.82e+4</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>y=1e+8x-5e+6</td>
</tr>
</tbody>
</table>

For analysis all peaks identified possessed a signal to noise ratio greater than three to be considered a signal and greater than ten to be measured for quantification. Figure 21 shows the chromatogram of an actual sample. More chromatograms for wastewater and sludge samples are included in Appendix D. The concentrations of PPCPs in the wastewater samples were determined and summarized in Table 15. The target analytes abundant consistently in both influent and effluent samples were acetaminophen, codeine, erythromycin and trimethoprim. A trend can also be seen that some degradation may be taking place from the drop in concentration of analytes when analyzing the water as it enters the treatment plant (influent) and as it is discharged (effluent). Soil absorption is a key factor which may bind PPCPs into complexes limiting their activity. \(^{(26)}\) PPCPs are found at such trace levels that the degree of ion suppression depends on the concentration of the analytes being monitored.
Table 15. Concentrations of Target Analytes found in water samples (ng/L). The values are averages of Influent and Effluent of wastewater treatment plants in the El Paso, TX region. *n=number of samples run

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Influent Concentration (ng/L) n=4</th>
<th>Effluent Concentration (ng/L) n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>0.24±(0.34)</td>
<td>0.30±(0.10)</td>
</tr>
<tr>
<td>Cotinine</td>
<td>ND</td>
<td>0.22±(0.11)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.60±(0.12)</td>
<td>0.60±(0.07)</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>ND</td>
<td>0.42±(0.12)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1.1±(0.51)</td>
<td>0.93±(0.26)</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.91±(0.19)</td>
<td>0.50±(0.02)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>ND</td>
<td>7.3e-3±(0.01)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1.9±(2.7)</td>
<td>3.0±(3.6)</td>
</tr>
</tbody>
</table>

Table 15 shows the total concentrations of the influents and effluents for both wastewater treatment plants. The total number of samples for the influent is 4 and 8 for the effluent. PPCPs were present in each of the samples analyzed. For those analytes indicated as not detected were either not present or were below the quantification limit of the study. The analytes found in both samples were acetaminophen, caffeine, trimethoprim, codeine and erythromycin. Trimethoprim and codeine showed some removal during the wastewater treatment process. However the analytes acetaminophen, caffeine and erythromycin showed little to no removal during this process. For instance a study on antibiotics such as erythromycin a large molecule used as an antibiotic, contains an amine functional group which may act as a site for reactivity which may alter its structure and activity to allow degradation. However factors (complex structure and steric hindrance) play a role in preventing such alterations from occurring the amine acts as a protecting group allowing the compound to withstand the treatment process. Caffeine

55
however, is highly soluble in water and a naturally occurring compound in plants.\textsuperscript{(69,70)} With this in mind caffeine may already be present in water before treated wastewater is discharged into receiving waters. Its high solubility makes it easy for this compound to form complexes with particulate matter present in wastewater and alter its structure to form stable cations allowing it to withstand treatment processes.\textsuperscript{(69,73)} Caffeine has also been observed in suppressing signals by attaching to particulates, this allows for co-elution of less volatile compounds during the clean-up procedure.\textsuperscript{(69,73)} During ESI sample introduction complete ionization will not occur and droplets will form at the ESI interface allowing solvent to enter the system masking the signal and suppressing others analyte signals.\textsuperscript{(69,73)} The PPCPs present are a estimate of the PPCPs detected by the method implemented in this study. However to better interpret the significance and better form a baseline of PPCPs present in the El Paso, Texas area further analysis must be performed on remaining wastewater samples and sludge samples to better understand the efficiency of removal in effluent and those present in sludge which may pose a risk to the environment.
Figure 21. A chromatogram of an Actual Sample. Peaks for targeted PPCPs were detected and identified based on the retention time and mass spectrum.
The analysis conducted in this study shows the presence of PPCPs in wastewater between the ranges of 1 fmol/µL to 10 fmol/µL (ppt range). Some PPCPs were not detected and that means that they might have been removed by wastewater treatment processes or attached to sludge. However some analytes detected had a signal to noise ratio greater than three but less than ten set for quantification and were not used in the total concentration calculations. Nonetheless, these numbers were used to further express the presence of PPCPs. This study shows the presence of many compounds found similarly throughout the US. Table 17 shows current literature focused on the detection of PPCPs in Wastewater Influent, Effluent and groundwater samples.

Sludge samples were collected for both wastewater treatment facilities over three months (Table 16). These samples were kept in a freezer at -81°C for preservation prior to the sample preparation, which was conducted within a 48 hr from the sampling. The extracts have been prepared and will be analyzed for PPCPs by the HPLC-MS/MS using the same analytical and quantification set up as that for the water samples. The analysis will be completed once the instrument becomes available.

**Table 16.** Plant and sampling date of Sludge

<table>
<thead>
<tr>
<th>Date</th>
<th>Amount</th>
<th>Northwest</th>
<th>Bustamante</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/11/2011</td>
<td>~100g</td>
<td>Sludge</td>
<td>Sludge</td>
</tr>
<tr>
<td>3/11/2011</td>
<td>~100g</td>
<td>Sludge</td>
<td>Sludge</td>
</tr>
<tr>
<td>4/8/2011</td>
<td>~100g</td>
<td>Sludge</td>
<td>Sludge</td>
</tr>
</tbody>
</table>
Table 17. Literature research of PPCPs in the United States.

<table>
<thead>
<tr>
<th>Location, State</th>
<th>Sample Matrix</th>
<th>Results (ng/L)</th>
<th>Authors</th>
<th>Journal/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States, Florida</td>
<td>Wastewater Treatment Plant and Hospital Effluent Seawater</td>
<td>Caffeine 5.7 – 68</td>
<td>Simrat P. Singh et al.(40)</td>
<td>Ecotoxicology (19) 338-350</td>
</tr>
</tbody>
</table>
| United States | Surface Waters | Acetaminophen:1.87-17.1  
Caffeine 2.15-32.7  
Trimethoprim: 0.12-5.51 | Hongxia, Li et al. (41) | Environmental Toxicology and Chemistry (29) 751-762 |
| United States, Ohio | Wastewater Treatment Plant (Influent/Effluent) | Sulfamethazine: 0.0269 Influent 26.65 Effluent  
Ciprofloxacin: 0.151 Influent 0.062 Effluent  
Cotinine: 0.606 Influent  
Caffeine: 10.1 Influent 0.0131 Effluent | Alison L. Spongberg, Jason D. Witter (42) | Science of the Total Environment (397) 148-157 |
| United States, Missouri | Surface and Groundwater | Caffeine and Acetaminophen: 224, 77.2,70 | Wang, Chuan et al. (43) | Water Research (45) 1818-1828 |
| United States, Tennessee | Surface and Groundwater | Caffeine: 628 ng/L Influent | Yu, Chang-Ping et al. (44) | Chemosphere (75) 1281-1286 |
| United States, New Mexico | Hospital and Dairy Effluent Wastewater Treatment Plant and Groundwater | Trimethoprim: 110-470 | Brown, Kathryn D. et al (45) | Science of the Total Environment (366) 772-783 |
| United States, El Paso Texas | Wastewater Treatment Plant (Influent/Effluent) | Influent/Effluent:Acetaminophen: 0.238/0.284  
Codiene:0.598/ .0653  
Erythromycin: 1.09/ 0.924  
Fluoxetine: 0.906/0.465  
Trimethoprim: 1.92/3.56 | Rodolfo Guerrero Jr. | University of Texas at El Paso Present Study |
All PPCPs analyzed in the environment show a similar trend of having the levels detected at ng/L (ppt) or pg/L (ppq) levels. Even at these trace amounts, studies have found the continual PPCP exposure has had impacts on aquatic life. A study done by Dr. Moon of the University of Ottawa used goldfish-carp cDNA array to test the effect of fluoxetine, results showed an interference with the regulation of certain critical genes (53), specifically, isotocin, a neuropeptide that modulates spawning and social behaviors. Fluoxetine has also showed the potential to reduce egg production and aromatase expression in zebrafish at environmentally relevant concentrations (ppt). On-going research is now being redirected to focus on the affects of these compounds at the trace level they are being discovered at in the environment. Multigenerational exposure now becomes a concern and what effects will they have over a long period of time. As can be seen from table 17 much of the analytes used for this study are present throughout the U.S. All concentrations quantified in this study were in trace amounts fluoxetine, trimethoprim and erythromycin having the highest of all analytes tested for. These results compare well to those reported in the literature in Louisiana (Table 17). The other analytes also were very similar to those found in the literature such as Caffeine, Acetaminophen and Cotinine. Much of the literature focuses on the discharge of water from wastewater facilities as a starting point to see how effective the process is at removing them from the environment. A study was done in Albuquerque, NM, in 2006 by Brown et al. in which antibiotics were tested for in hospital, residential, dairy effluent and the Rio Grande. Antibiotics have been known to cause genotoxic effects in aquatic organisms and development of antibiotic resistance in bacteria. 11 antibiotics were tested for, in 58% of the samples one antibiotic was present and 25% contained three or more. (45) The raw untreated wastewater contained sulfamethoxazole, trimethoprim (also detected
in this study) and ofloxacin ranging from 110 to 470 ng/L and were reduced by 20 to 70% in the treated effluent.  

Water samples analyzed upstream of the wastewater facility showed no detection of antibiotics and only sulfamethoxazole was present downstream of the wastewater facility. This study shows the presence of at least one antibiotic present after wastewater treatment in this region. The Rio Grande which runs down into the El Paso, Texas, area serves as a main source of surface water used for irrigation with the continual discharge by wastewater treatment facilities with the presence of PPCPs shows how they can move from their point of release and contaminate connecting water bodies and communities. A collective effort must be made to better understand the persistence and mobility of PPCPs once introduced into the environment. It should be noted that PPCP compounds are not completely removed and are released with the treated water as is seen in the literature and this study.

These emerging contaminants, PPCPs, are not just a localized situation they are and have been the focus of much research around the world. Table 18 shows the literature of studies done around the world of wastewater samples. Much of the results show a similar trend to those in the U.S. and the results presented in this study. The target analytes, such as Caffeine, Trimethoprim, Erythromycin and Acetaminophen, present in this study along with those found in the U.S. are similar to those found throughout the world. The concentrations found throughout the world seem to be much higher than those found in the U.S. which could be possibly due to the wastewater treatment process or the contribution of other companies such as hospital waste to the wastewater facilities tested. Even with these higher concentrations all analytes are within the ppt (ng/L) or ppb (μg/L) range. With continuing research and the trends, which have been outlined
in this study, showing the similarities in the US and throughout the world will help to provide better understanding and methods to help eliminate these compounds as well as the pathways and effects they can have once introduced into the environment.
<table>
<thead>
<tr>
<th>Location</th>
<th>Sample Matrix</th>
<th>Results (ng/L)</th>
<th>Journal/Volume</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.K. South Wales</td>
<td>Surface Water</td>
<td>Erythromycin: 617 WWTP</td>
<td>Analytical and Bioanalytical Chemistry (391) 1293-1308</td>
<td>Kasprzyk-Hordern, Barbara et al (47)</td>
</tr>
<tr>
<td>China</td>
<td>Wastewater Treatment Plant</td>
<td>Caffeine: 5860 Influent 108 Effluent</td>
<td>Environmental Science and Technology (45) 3341-3348</td>
<td>Qian Sui et al. (48)</td>
</tr>
<tr>
<td>Japan</td>
<td>Surface Water</td>
<td>Acetaminophen: 22</td>
<td>Water Science &amp; Technology (56) 12 133-140</td>
<td>N. Nakada et al. (49)</td>
</tr>
<tr>
<td>Canada</td>
<td>Wastewater Treatment Plant</td>
<td>Acetaminophen: 35</td>
<td>Science of the Total Environment (399) 50-65</td>
<td>D. R. Lapen et al. (50)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Wastewater Treatment Plant</td>
<td>Acetaminophen: 1800-30967</td>
<td>Water Science &amp; Technology (62) 2450-2458</td>
<td>Angela Yu-Chen Lin et al. (51)</td>
</tr>
<tr>
<td>Greece</td>
<td>Wastewater Treatment Plant</td>
<td>Caffeine: 74900 Influent 7900 Effluent</td>
<td>Journal of Hazardous Materials (179) 804-817</td>
<td>Christina I. Kosma et al. (52)</td>
</tr>
</tbody>
</table>

Table 18. Literature Research on PPCPs.
3.4 Estimated Removal

Table 19 shows the average estimated removal from both plants for influent and effluent samples collected. As shown in the table the compounds with the estimated highest removal from the Northwest plant are acetaminophen and codeine of approximately 50%. It was observed with available data for 27 PPCPs in the US, UK and Germany the percent removal of acetaminophen was 81%.\(^{(67)}\) For the Bustamante plant the highest estimated removal rate of the target analytes are trimethoprim and codeine of approximately 20 to 40%. The removal rate observed for data collected in the US, UK and Germany for trimethoprim and erythromycin, had a percent removal of 29% and 38%.\(^{(67)}\) The values presented here give the average percent removal of studies performed in the US, UK and Germany.\(^{(67)}\) The values obtained in this study are similar to those reported. Caffeine, trimethoprim and erythromycin show very little removal during the treatment process. However, this may be in part to the number of samples run in which more samples are needed for the statistical evaluation of these compounds removal. The treatment used for disinfection also plays a role in the efficient removal of PPCPs. Dr. Westerhoff of Arizona University conducted research to see the removal rate during different process of the wastewater treatment process. It was observed that chlorination over a 24 hr period could remove up to 90% of PPCPs present and less than 20%, results varied based on the functional groups present (hydroxyl, aromatic rings and carboxyl groups).\(^{(74)}\) Ultraviolet light disinfection requires 100 times higher dosage than that used for microbial growth which makes it highly unlikely to be effective in removal of PPCPs.\(^{(74)}\) However to get a better understanding of the efficiency of the wastewater treatment facilities and to form a clearer baseline the remaining wastewater samples and sludge must be analyzed. This shows that some PPCPs can
be partially removed during WWTP processes by a secondary treatment. It should be noted that the Northwest Wastewater Treatment Plant uses Ultraviolet light disinfection while the Bustamante Plant implements chlorination. The data obtained thus far are not sufficient enough to evaluate the removal rate of certain PPCPs by the treatment practice. Although not all PPCPs were removed during the WWTP processes it is also very likely that many PPCPs absorbed to sludge separated during the cleaning process. When time is allotted on the machine further work will be focused on the analysis of the remaining water samples as well as sludge samples which may contain larger concentrations of PPCPs, which may provide reason for the removal of other PPCPs not detected in the aqueous samples.

Table 19. Estimated Removal of PPCPs at both WWTPs, n=number of samples analyzed

<table>
<thead>
<tr>
<th>Compounds</th>
<th>NW Effluent n=4</th>
<th>NW Influent n=2</th>
<th>Estimated Removal</th>
<th>Busta Effluent n=4</th>
<th>Busta Influent n=2</th>
<th>Estimated Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codeine</td>
<td>0.477±(0.55)</td>
<td>1.04</td>
<td>54%</td>
<td>0.452±(0.52)</td>
<td>0.768</td>
<td>41%</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.227±(0.26)</td>
<td>0.476</td>
<td>52%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.620±(0.17)</td>
<td>0.684</td>
<td>9%</td>
<td>0.528±(0.74)</td>
<td>0.511</td>
<td>-3.20%</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.741±(0.86)</td>
<td>0.727</td>
<td>-1.90%</td>
<td>1.10±(0.74)</td>
<td>1.45</td>
<td>24%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>5.38±(10.31)</td>
<td>3.85</td>
<td>-40%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NW=Northwest Wastewater Treatment Plant  Busta=Bustamante Wastewater Treatment Plant
4 Conclusions

The method was modeled for the mixture of PPCPs after EPA method 1694 using nanospray technology and green chemistry concepts. This is aimed at forming a baseline for the PPCPs present in the El Paso, Texas, area. SPE, coupled with LTQ-MS/MS-nano-LC analysis, was applied to the analysis of PPCPs: acetaminophen, caffeine, codeine, cotinine, ciprofloxacin, erythromycin, fluoxetine, sulfamethazine and trimethoprim in aqueous and sludge wastewater samples. The optimized preparation method, which used two one liter samples to concentrate PPCP mixtures, used 12 mL of methanol for the elution of target analytes and an SRM method, specific to each analyte, 40 min per run to enhance detection sensitivity.

The Limit of Detection (LOD) for target analytes acetaminophen, cotinine, codeine and caffeine were found to be between 10 fmol/µL to 1 pmol/µL. Conversely, fluoxetine, trimethoprim, sulfamethazine, ciprofloxacin and erythromycin were between 1 fmol/µL to 1 pmol/µL. However, the method showed the ability to determine PPCPs in the ppq range. SPE is a valuable tool when analyzing trace compounds in such a complex matrices. This process proved to be reliable in removing most interferences. However, some signal suppression was still present when running samples making peak selection and quantification challenging. The estimated removal for some compounds for the samples ran, showed good removal of acetaminophen, codeine and trimethoprim.

All PPCPs are seen as a risk; however the effects they actually have depend on the function and properties of the chemical under investigation. Those of high priority are antibiotics which enhance bacterial resistance to treatments, antidepressants and chemotherapy
drugs which act as endocrine disruptors and anti-inflammatory drugs which can have inhibitory effects on cell growth.\textsuperscript{(68,67)} Caffeine a stimulant has also showed the potential to cause environmental effects, in which the \textit{Pseudomonas fluorescens} can bacteria use caffeine as a carbon source and produce ammonia. With naturally occurring caffeine and the added amounts from wastewater treatment facilities could have detrimental effects increasing the concentration of ammonia making the waters uninhabitable for aquatic life.\textsuperscript{(69)} This study showed the presence of PPCPs seen as high risk contaminants. However there are no exact figures on limits of exposure to these chemicals for humans.\textsuperscript{(70)} Much of the research done is on aquatic life much of the concentrations found in this study are below the limit of those found to cause effects. More research must be performed to better understand this large class of contaminants of emerging concern (CEC) to set safety criteria. Many challenges are still present when analyzing such a broad class of contaminants. More studies must go into better understanding the degradation and fate of PPCPs once introduced into the environment. Achieving high sensitivity, eliminating matrix effects, signal suppression, human health effects and optimization of the clean-up procedure are just a few.

Future work includes the analysis of fifty-four extract influent, effluent samples and six sludge extract samples. Machine time has been estimated to one week starting possibly December 10\textsuperscript{th}. 

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Appendix A

**Calibration Curves (Peak Intensity vs. Concentration) used for limit of detection**

### Acetaminophen

<table>
<thead>
<tr>
<th>Concentration (pmol/µL)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00E+00</td>
</tr>
<tr>
<td>2</td>
<td>5245.1</td>
</tr>
<tr>
<td>4</td>
<td>30000</td>
</tr>
<tr>
<td>6</td>
<td>45000</td>
</tr>
<tr>
<td>8</td>
<td>60000</td>
</tr>
<tr>
<td>10</td>
<td>75000</td>
</tr>
</tbody>
</table>

**Equation:**

\[ y = 5245.1x + 1644.7 \]

**R²:** 0.9484

### Caffeine

<table>
<thead>
<tr>
<th>Concentration (pmol/µL)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00E+00</td>
</tr>
<tr>
<td>5000</td>
<td>20000</td>
</tr>
<tr>
<td>10000</td>
<td>40000</td>
</tr>
<tr>
<td>15000</td>
<td>50000</td>
</tr>
<tr>
<td>20000</td>
<td>60000</td>
</tr>
<tr>
<td>25000</td>
<td>70000</td>
</tr>
<tr>
<td>30000</td>
<td>80000</td>
</tr>
</tbody>
</table>

**Equation:**

\[ y = 4086.7x + 537.6 \]

**R²:** 0.9733
Sulfamethazine

$y = 9317.1x + 251.03$

$R^2 = 0.9754$

Trimethoprim

$y = 101384x + 14153$

$R^2 = 0.9768$
Codiene

\[ y = 105.69x + 1.062 \]
\[ R^2 = 0.9899 \]

Fluoxetine

\[ y = 11630x + 2853 \]
\[ R^2 = 0.9526 \]
Ciprofloxacin

\[ y = 9907.2x + 403.85 \]

\[ R^2 = 0.9607 \]

Intensity

Concentration (pmol/µL)

Erythromycin

\[ y = 1168x - 113.87 \]

\[ R^2 = 0.995 \]
Calibration Curves (Peak Area vs. Concentration) with 95% confidence Intervals

**Acetaminophen**

\[ y = 622303x - 17054 \]

\[ R^2 = 0.9946 \]

**Cotinine**

\[ y = 209126x - 6920.8 \]

\[ R^2 = 0.992 \]
Caffeine

\[ y = 861191x - 23062 \]
\[ R^2 = 0.9947 \]

Sulfamethazine

\[ y = 2E+06x - 48222 \]
\[ R^2 = 0.9947 \]
Trimethoprim

$y = 1E+08x - 5E+06$

$R^2 = 0.9916$

Codiene

$y = 26078x - 741.09$

$R^2 = 0.9944$
Fluoxetine

\[ y = 578633x + 2588.4 \]

\[ R^2 = 0.9997 \]
Appendix B

Percent Solids for Sludge Samples

<table>
<thead>
<tr>
<th>WWTP Sludge</th>
<th>Date</th>
<th>Dry Weight</th>
<th>Wet Weight</th>
<th>% Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>2/7/2011</td>
<td>3.601</td>
<td>9.313</td>
<td>40</td>
</tr>
<tr>
<td>NW</td>
<td>3/7/2011</td>
<td>2.557</td>
<td>6.306</td>
<td>41</td>
</tr>
<tr>
<td>Busta.</td>
<td>2/7/2011</td>
<td>1.220</td>
<td>9.190</td>
<td>13</td>
</tr>
<tr>
<td>Busta.</td>
<td>3/7/2011</td>
<td>1.516</td>
<td>9.098</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WWTP Sludge</th>
<th>Date</th>
<th>Dry Weight</th>
<th>Wet Weight</th>
<th>% Solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>NW</td>
<td>2/7/2011</td>
<td>7.272</td>
<td>10.043</td>
<td>72</td>
</tr>
<tr>
<td>NW</td>
<td>3/7/2011</td>
<td>5.767</td>
<td>10.289</td>
<td>56</td>
</tr>
<tr>
<td>Busta.</td>
<td>2/7/2011</td>
<td>4.597</td>
<td>10.544</td>
<td>44</td>
</tr>
<tr>
<td>Busta.</td>
<td>3/7/2011</td>
<td>4.461</td>
<td>10.496</td>
<td>43</td>
</tr>
<tr>
<td>Busta.</td>
<td>4/4/2011</td>
<td>4.697</td>
<td>10.987</td>
<td>43</td>
</tr>
</tbody>
</table>
Appendix C

LTQ-MS/MS-nano LC Chromatograms (Peaks Areas Circled for selection)

Trimethoprim

RT: 0.00 - 40.19 SM: 13G

1 pmol/ul

NL: 1.51E5
TIC F: ITMS + c NSI
SRM ms2
291.00@cid21.00
[230.00-231.00] MS
RG2011060642

MA: 11983403
AA: 150252
AA: 28194

Codeine

RT: 11.23
MA: 23795

NL: 2.60E2
TIC F: ITMS + c NSI
SRM ms2
300.00@cid55.00
[152.00-153.00] MS
RG2011060642

Fluoxetine

RT: 29.74
AA: 580528

NL: 7.43E3
TIC F: ITMS + c NSI
SRM ms2
310.30@cid35.00
[148.00-149.00] MS
ICIS RG2011060642

Erythromycin

RT: 27.41
AA: 31821

NL: 2.65E2
TIC F: ITMS + c NSI
SRM ms2
734.40@cid50.00
[158.00-159.00] MS
ICIS RG2011060642
D₃-Cotinine

RT: 6.02
MA: 114459

1³-C₃-Caffeine

RT: 17.37
MA: 1905236

D₅-Fluoxetine

RT: 29.78
MA: 58653

1 pmol/ul

NL: 1.63E3
TIC F: ITMS + c NSI
SRM ms2
180.00@cid45.00
[79.40-80.40] MS
RG2011060642

NL: 2.45E4
TIC F: ITMS + c NSI
SRM ms2
198.00@cid35.00
[139.50-140.50] MS
RG2011060642

NL: 6.77E2
TIC F: ITMS + c NSI
SRM ms2
315.30@cid35.00
[152.50-153.50] MS
ICIS RG2011060642
0.01 pmol/ul

Trimethoprim

RT: 19.67
MA: 28594

Codeine

RT: 11.36
MA: 14.31

Fluoxetine

RT: 28.87
MA: 326

Erythromycin

NL: 3.91E2
TIC F: ITMS + c NSI
SRM ms2
291.00@cid21.00
[230.00-231.00]
MS RG2011060638

NL: 1.11
TIC F: ITMS + c NSI
SRM ms2
300.00@cid55.00
[152.00-153.00]
MS RG2011060638

NL: 4.58E1
TIC F: ITMS + c NSI
SRM ms2
310.30@cid35.00
[148.00-149.00]
MS RG2011060638

NL: 2.93
TIC F: ITMS + c NSI
SRM ms2
734.40@cid50.00
[158.00-159.00]
MS RG2011060638

NL: 4.58E1
TIC F: ITMS + c NSI
SRM ms2
310.30@cid35.00
[148.00-149.00]
MS RG2011060638

NL: 2.93
TIC F: ITMS + c NSI
SRM ms2
734.40@cid50.00
[158.00-159.00]
MS RG2011060638
Appendix D

Chromatograms for WWTP Samples

E:\LTQ Raw files\...\RG2011052652


Acetaminophen
TIC F: ITMS + C OSI SRM
m/z 2 152.20@cd25.00
[110.00-111.00] MS
RG2011052652
NL: 2.92E1
NL 1.78E1

Cotinine
TIC F: ITMS + C OSI SRM
m/z 2 177.00@cd46.00
[98.00-99.00] MS
RG2011052652
NL: 3.22E1

Caffeine
TIC F: ITMS + C OSI SRM
m/z 2 195.00@cd23.00
[138.00-139.00] MS
RG2011052652
NL: 2.49E1

Sulfamethazine
TIC F: ITMS + C OSI SRM
m/z 2 279.00@cd22.00
[156.00-157.00] MS
RG2011052652
Trimethoprim
- RT: 18.83
- AA: 10933
- SN: 38

Codeine
- RT: 23.17
- AA: 273
- SN: 31

Fluoxetine
- RT: 25.16
- AA: 829
- SN: 23

Erythromycin
- RT: 25.01
- AA: 10476
- SN: 200

Not Detected
- RT: 12.84
- AA: 14533
- SN: 24

- RT: 12.33
- AA: 459
- SN: 43

- RT: 17.26
- AA: 979
- SN: 15

- RT: 4.61
- AA: 493
- SN: 8

- RT: 6.10
- AA: 85.41
- SN: 17

- RT: 0.77
- MA: 223
- SN: 2

- RT: 18.37
- MA: 63259
- SN: 223

- RT: 16.37
- MA: 53259
- SN: 36

- RT: 26.34
- MA: 53259
- SN: 36

- RT: 19.62
- MA: 53259
- SN: 36

- RT: 19.03
- MA: 53259
- SN: 36

0.00 - 33.00 Sample: 13G

NL: 6.67E2
- TIC F: 'TMS + c NSI SRM m/z 2
- 301.00@3d21.00
- [230.00-231.00] MS ICIS
- RG2011052662

NL: 8.71
- TIC F: 'TMS + c NSI SRM m/z 2
- 300.00@3d55.00
- [152.00-153.00] MS ICIS
- RG2011052662

NL: 5.13E2
- TIC F: 'TMS + c NSI SRM m/z 2
- 310.30@3d55.00
- [149.00-149.00] MS ICIS
- RG2011052662

NL: 5.49E1
- TIC F: 'TMS + c NSI SRM m/z 2
- 734.40@3d50.00
- [188.00-159.00] MS ICIS
- RG2011052662
Trimethoprim
TIC: FTMS + c NSI SRM ms2
291.00 @ add 11.00
[230.00-231.00] MS ICIS
RG2011052864
NL: 1.51E3
TIC: FTMS + c NSI SRM ms2
300.00 @ add 55.00
[152.00-153.00] MS ICIS
RG2011052864
NL: 1.72E1
TIC: FTMS + c NSI SRM ms2
310.00 @ add 55.00
[149.00-149.00] MS ICIS
RG2011052864
NL: 1.92E1
TIC: FTMS + c NSI SRM ms2
734.40 @ add 50.00
[159.00-158.00] MS ICIS
RG2011052864
NL: 1.32E2
TIC: FTMS + c NSI SRM ms2

Codeine
Not Detected

Fluoxetine
Not Detected

Erythromycin
Not Detected
Trimethoprim: Not Detected

Codeine: Not Detected

Fluoxetine: Not Detected

Erythromycin: Not Detected

RT: 0.00 - 33.03  SM: 13G
Acetaminophen
RT: 7.12
MA: 2135
SN: 69

Cotinine
RT: 17.96
MA: 1059
SN: 73

Caffeine
RT: 17.96
MA: 1146
SN: 30

Sulfamethazine
RT: 16.32
MA: 502
SN: 26

Not Detected

Acetaminophen
NL: 4.21E1
TIC F: ITMS + cNSI SRM
m/z 2 152.20 @ cid 25.00
[110.00-111.00] MS
RG2011060814

Cotinine
NL: 1.24E1
TIC F: ITMS + cNSI SRM
m/z 2 177.00 @ cid 45.00
[98.00-99.00] MS
RG2011060814

Caffeine
NL: 3.79E1
TIC F: ITMS + cNSI SRM
m/z 2 195.00 @ cid 23.00
[138.00-139.00] MS
RG2011060814

Sulfamethazine
NL: 1.87E1
TIC F: ITMS + cNSI SRM
m/z 2 276.00 @ cid 22.00
[156.00-157.00] MS
RG2011060814
<table>
<thead>
<tr>
<th>Compound</th>
<th>RT</th>
<th>AA</th>
<th>MA</th>
<th>SN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimeprin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Trimethoprim**

- RT: 20.67
- AA: 221
- MA: 3491
- SN: 104

**Codeine**

- RT: 27.36
- AA: 207
- MA: 18.75
- SN: 115

**Fluoxetine**

- RT: 30.31
- AA: 3989
- MA: 7051
- SN: 15

**Erythromycin**

- Not Detected
Trimethoprim
Not Detected

Codeine
Not Detected

Fluoxetine
Not Detected

Erythromycin
Not Detected

RT: 6.00 - 33.13  SM: 13G

TIC F: ITMS + c NSISRM ms2
NL: 6.01E2
[291.00@6d21.00]
[230.00-231.00] MS: ICIS
RG2011052870

TIC F: ITMS + c NSISRM ms2
NL: 2.47E1
[300.00@6d55.00]
[162.00-163.00] MS: ICIS
RG2011052870

TIC F: ITMS + c NSISRM ms2
NL: 1.78E1
[310.30@6d35.00]
[140.00-149.00] MS
RG2011052870

TIC F: ITMS + c NSISRM ms2
NL: 4.15E-1
[734.40@6d50.00]
[158.00-159.00] MS
RG2011052870
References


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61. Maskau, K. and Zhou, J.L. Colloids as a sink for certain Pharmaceuticals in the Aquatic Environment. 17.4: Environmental science and pollution research international , 2010.


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Curriculum Vitae

Rodolfo Guerrero Jr. was born in El Paso, Texas. The oldest of four children born to Elva and Rodolfo Guerrero Sr., he graduated from Montwood High School, El Paso, Texas, in 2002. After graduating he entered the University of Texas at El Paso in the Fall of 2002 with the help of the Texas Grant and UTEP Smart Grant into the engineering program. After completing his first year he transferred into the chemistry department to pursue his Bachelors degree. During this time he joined Dr. Wen-Yee Lee’s lab group in the Spring 2008 and worked on a research project looking at the development of using bioluminescent bacteria as a detector of certain phthalates and presented preliminary results at the UTEP SACNAS chapter expo. He then graduated from the University of Texas at El Paso in May 2008 with his Bachelor’s of Science degree in Chemistry and received an internship with Cardinal Health during the summer. He then entered the chemistry graduate program in Fall 2008 to pursue his Master’s degree under Dr. Wen-Yee Lee. He took on the research project of detecting Pharmaceuticals and Personal Care Products (PPCPs) in wastewater influent and effluent by High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS). He presented his research at the SACNAS and ACS National Conferences in 2009. He also presented at the Border Water Quality Conference and placed third in the poster presentation competition in 2010. While in the program he was awarded the Thelma Morris Endowed Scholarship and Southwest Consortium for Environmental Research Policy (SCERP) Fellowship. During this time he also received a position as a research assistant under Dr. Igor C. Almeida.

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