ANALYTICAL METHOD DEVELOPMENT FOR MEASUREMENT OF

UNREGULATED ORGANIC CONTAMINANTS IN AQUEOUS SAMPLES USING

LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

by

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ABSTRACT OF THE DISSERTATION

Analytical Method Development for Measurement of Unregulated Organic
Contaminants in Aqueous Samples using Liquid Chromatography – Tandem Mass
Spectrometry
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Organic wastewater contaminants (OWCs) such as pharmaceuticals, hormones, and

perfluorinated compounds (PFCs) are of growing environmental and public health

concern. These OWCs were found in U.S. drinking water supplies according to

nationwide studies by the U.S. Geological Survey. Many OWCs are not, however,

regulated or routinely monitored in drinking water.

The objective of this dissertation was to develop and optimize analytical methods

for trace analysis of unregulated organic contaminants in drinking water sources.

Furthermore, household water treatment-processes were studied to measure the efficacy

of removal of these unregulated organic contaminants from drinking water samples.

Two liquid chromatography-ion trap mass spectrometry systems (LC-IT-MS/MS)

were compared for rapid, reliable and sensitive detection of the most abundant PFCs,

perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). An ultra

performance LC-linear IT-MS/MS achieved the lowest detection limits measured, 0.03pg

and 0.24pg for PFOA and PFOS respectively, which were approximately two orders of magnitude more sensitive than an LC-IT-MS/MS. With the increased sensitivity, the direct analysis of PFOA/S without solid phase extraction pre-concentration steps was also demonstrated. In addition, MS methods using Full Scan, Single Ion Monitoring, and MS/MS were compared and optimized for a sensitive analysis of PFCs.

A novel rapid method was created by switching polarity for the simultaneous analysis of twenty unregulated compounds, including pharmaceuticals. Sensitive method detection limits were achieved in the range of sub ng/L to hundreds ng/L for all target compounds. An optimized analytical method was applied to quantify low ng/L levels of these target compounds in field water samples from regions throughout New Jersey. Eight target compounds were measured below 1µg/L and two target compounds (i.e. metformin and estradiol) were measured slightly above 1µg/L in the field water samples.

Finally, granular activated carbon (GAC) and ion resin (Brita™) filtration, ozonation, and microwave heating were tested for efficacy at removing 20 target compounds in drinking water samples. The GAC/ion resin mechanism with adsorption properties demonstrated greater removal of the target compounds than the other two mechanisms. Even this water treatment-process only partially removed these target compounds with its mean removal of $\leq 66\%$.

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1.1 Background

1.1.1 United States Geological Survey (USGS) investigation

The United States Geological Survey (USGS) conducted the first nationwide investigation of the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants (OWCs) in U.S. water resources. The study demonstrated that 80% of 139 streams across 30 states had detectable concentrations of OWCs during a 1999 and 2000 sampling period.[1] Among the 95 OWCs selected, the majority do not have regulatory based allowable guidelines for concentrations in drinking water. Over 170 of the organic compounds with EPA drinking water standards or health advisories for levels in potable water supplies; no pharmaceuticals are currently included.[2] In this USGS study, five analytical methods employing gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) were created to measure concentrations of these OWCs. The GC-MS methods were used to detect semi volatile and volatile pollutants including pesticides. Measurement of drinking water contaminants initially quantified by USGS was shown using a solid phase microextraction (SPME) and GC-MS method.[3] In addition to GC methods, broad classes of non-volatile compounds were quantified using LC-MS methods.

1.1.2 Source Water Assessment Program (SWAP)

Under the Safe Drinking Water Act by the United States Environmental Protection Agency (US EPA), a source water assessment program (SWAP) was created to provide basic information about the drinking water in each public water system. The

three main source water assessment steps were outlined as follows: 1) delineate the

source water assessment area, 2) conduct an inventory of potential sources of

contamination and 3) determine the susceptibility of the water to contamination.[4] The

state SWAP guidance was documented in an EPA report: 816-R-97-009 on August,

1997.[5] More than 30 states providing the SWAP or similar programs included Arizona,

California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Idaho,

Illinois, Iowa, Kansas, Maine, Michigan, Minnesota, Montana, Nebraska, Nevada, New

Jersey, New Mexico, North Dakota, Ohio, Oklahoma, Rhode Island, South Carolina,

South Dakota, Tennessee, Washington, West Virginia, Wisconsin, and Wyoming. The

SWAP may differ by state since each program is adapted to a state's water resources and

drinking water priorities. Major states' SWAP plans and reports can be reviewed on-line

linked to an EPA website.[6]

1.1.3 Occurrence, fate and transport of OWCs

Groundwater is one of the major sources of water for processing to drinking water.

Approximately 40% of the nation's public water supplies are from groundwater and more

than 40 million people, mainly rural populations, supply their own drinking water via

domestic wells.[7] Another more recent USGS study documented the detection of OWCs

in 81% of the groundwater sites sampled across 18 states.[8] Generally, broad classes of

OWCs were detected in the groundwater samples: insect repellent, plasticizers and

detergent metabolites were most frequently detected classes of OWCs followed by

pharmaceuticals. The results also showed that the number of compounds detected

significantly decreased as a well's depth increased.[8] This suggested a potential for

groundwater contamination through leaching from landfills and other surface/leaching

phenomena.[9] In addition, wellheads were previously reported as the sources of organic contaminants due to shallow seals and gravel packs.[10]

The OWCs were generally detected more frequently in surface water samples than in groundwater. Numerous studies found contaminants such as surfactants, pharmaceuticals, steroids and other OWCs in untreated drinking water sources.[1, 8, 11] The five most frequently detected OWCs in surface water were cholesterol (59%, natural sterol), metolachlor (53%, herbicide), cotinine (51%, nicotine metabolite), ß-sitosterol (37%, natural plant sterol), and 1, 7-dimethylxanthine (27%, caffeine metabolite). However, the five most frequently detected OWCs in ground water included tetrachloroethylene (24%, solvent), carbamazepine (20%, pharmaceutical), bisphenol-A (24%,plasticizer), 1,7-dimethylxanthine (16%, caffeine metabolite), and tri (2-chloroethyl) phosphate (12%, fire retardant).[11]

This phenomenon has not been limited to the United States.[12, 13, 14] Several municipal wastewater and raw waters used for drinking water production in Western Europe were contaminated with various household and industrial chemicals, pharmaceuticals, and personal care products: benzotriazoles, benzothiazole-2-sulfonate, diclofenac and carbamazepine showed mean concentrations of 1-10µg/L.[14] In the greater Dublin area, 15 pharmaceutical compounds found in influent and effluent samples from three wastewater treatment plants (WWTP) were measured with salicylic acid and ibuprofen being the most abundant.[15] Similarly, 13 pharmaceutical compounds were found in WWTP influent and effluent from different locations in Spain and Croatia: acetaminophen, atenolol, mevastatin, trimethoprim, and ibuprofen at higher

concentrations of µg/L.[16] A secondary observation was that some OWCs were very seasonally dependent.[17]

The direct discharge of wastewater effluent was proposed to be one direct pathway of OWCs into surface water [13, 18] in addition to other environmental fate and transport processes (i.e. sorption and biodegradation).[19, 20] Kasprzyk-Hordern *et al.*[13] showed the impact of factors such as surrounding area, proximity to wastewater effluent and weather conditions, which can affect the concentrations of pharmaceuticals, and personal care products (PPCPs), endocrine disruptors and illicit drugs in surface water. The average daily load of PPCPs was calculated to be approximately 6 kg, which were discharged daily into rivers.[13] Sorption and bio-degradation followed by photo-degradation and hydrolysis were measured for the predominant fate processes for pharmaceuticals.[19] The neutral and ionic properties of the target particles drove the sorption process mechanisms of the pharmaceuticals.[19] A recent study [20] measured the loss of pharmaceuticals but did not find a major loss from sorption during the transport of estuarine surface water. Instead, microbial degradation was found to be the principal loss with rates that varied as the relative persistence of pharmaceuticals varied from one compound to another within a single water treatment system. The six most labile compounds included nicotine ($t_{1/2}$ = 0.68-9.7 days), acetaminophen ($t_{1/2}$ = 1.2-11 days), fluoxetine ($t_{1/2}$ = 5.9-9.8 days), diltiazem ($t_{1/2}$ = 5.5-36 days), nifedipine ($t_{1/2}$ = 5.7-6.3 days), and caffeine ($t_{1/2}$ always >40 days). However other pharmaceutical compounds including salbutamol, antipyrine, cotinine, sulfamethoxazole, carbamazepine, and trimethoprim were shown to be the least labile with their $t_{1/2}$ always greater than 40 days.[20] Kasprzyk-Hordern[13] also determined some PPCPs (e.g., erythromycine-H_2O,

codeine, carbamazepine, gabapentin and valsartan) were both ubiquitous and persistent in the aqueous environment. Relative degradation rates for those pharmaceuticals were still slower than other small biomolecules, such as glucose and amino acids. This suggested that many OWCs, especially pharmaceuticals and endocrine disrupting compounds were significantly more recalcitrant to microbial degradation than similarly sized small biomoleucules.[20]

Along with the biodegradation mechanism, photodegradation is another source of loss in the fate and transport mechanism of PPCPs in environment. Previous studies showed fast photodegradation for certain pharmaceuticals including: ranitidine, sulfamethoxazole, diclofenac, ofloxacin and propranolol with $t_{1/2}$ of 0.6, 2.4, 5.0, 10.6, 16.8 days, respectively.[21, 22] Other pharmaceuticals, cimetidine, carbamazepine and clofibric acid, however, showed strong resistance to photodegradation. The bio- and photo- degradation processes were suggested as key mechanisms for loss of the target OWCs, which should be considered when tracing organic contaminants from their source. In addition, it is also essential in the understanding removal studies that showed precursor compounds transformed to altered forms of degradation products.[23]

For the transport of OWCs to drinking water sources, a variety of animal and human uses as well as waste sources were identified as routes to water contamination. **Figure 1.1** was created to show environmental pathways for drinking water contamination from human and veterinary drugs based on previous studies. It is a composite derived from other published fate studies.[12, 19, 24] Several studies demonstrated that outdated medicines or their remains are being flushed down household drains along with drugs and their metabolites from excreted human waste. Bound and Voulvoulis[12]

suggested the significant pathways of pharmaceutical aquatic contamination are from disposal of domestic household waste. Similarly, Kümmerer[19] included animal husbandry and fruit production when they described the occurrence, fate, and transport of pharmaceuticals in the aquatic environment. It was also noted that pharmaceutical compounds disposed of as household waste may end up at landfill sites where they enter the landfill effluent, transport to soil and eventually cause groundwater contamination.[12, 19, 24] In a recent study, waste-indicator and pharmaceutical compounds were detected in leachate-contaminated ground water. The contaminants included acetaminophen, caffeine, cotinine, 1, 7-dimethylxanthine, fluoxetine, and ibuprofen found in four wells downgradient from a landfill in Indiana.[25] Kinney *et al.*[24] have measured maximum and minimum (detectable) concentrations of pharmaceuticals at the lowest depth of sampled soil suggesting interactions of soil components with pharmaceuticals during leaching. For veterinary pharmaceuticals, direct contamination of soil via manure and surface or ground water contamination by runoff from agricultural fields was thought to be more prevalent.[26, 27] Hirsch *et al.* showed however, that drinking water source contamination from veterinary applications was relatively less prevalent than was previously expected.[28]

1.1.4 Risk Assessment of target compounds in drinking water

It is difficult to perform risk assessments and determine health effects for many OWCs since the concentration of pharmaceuticals in drinking waters were very low (ng/L) compared to their levels in medical doses. A risk assessment of pharmaceuticals in both the U.S. and the European Union was not initially addressed under a marketing authorization[29, 30] but, unintended human exposure to pharmaceutical compounds, hormones and perfluorinated compounds caused alarm since many have been found in

drinking water supplies. The key scientific concerns in performing a toxicological risk assessment of adverse health effects were reviewed as follows.

Previously, environmental factors (e.g., fate, transport, and removal) were often lacking in risk assessments. Schulman et al.[31] showed a chemical-specific risk assessment on four pharmaceutical compounds: acetylsalicylic acid (analgesic), clofibrate (lipid regulator), cyclophosphamide (cytotoxic/anticancer) and indomethacin (anti-inflammatory). The levels measured for these pharmaceuticals in aquatic media were believed to be below "safe" limits.[31] Similarly, Jones et al.[32] estimated no significant risk in an aquatic environmental assessment of the top 25 English prescription pharmaceuticals using a model to predict environmental concentrations. Schwab's mathematical model also predicted no effect concentrations of pharmaceuticals using acceptable daily intakes based on US EPA's no observed adverse effect level (NOAEL) for active pharmaceutical ingredients.[33] The assumptions were made for low river flow and no depletion, which did not model metabolism, removal and degradation. However, factors such as sorption, metabolism, degradation and transformation of these organic contaminants were rather significant[20, 23] and should be considered in any practical model from environmental fate/transport. In addition, a majority of these risk assessment studies were done using their measured concentrations and detection frequencies were based on European data. Only a couple of studies of human health risk assessment from the U.S. using pharmaceuticals found in drinking water sources were performed.[33, 34] This area needs further work.

Perfluorinated compounds (PFCs) are used for many important manufacturing and industrial applications including paper production, textile manufacturing, leather

treatment, surfactant additives, coatings manufacture, and firefighting foams and equipment production. They were recently studied for their human risk assessment from drinking water contamination. The most abundant PFCs, PFOA and PFOS (PFOA/S) were ubiquitously found in human blood and wildlife throughout the world.[35] Notably, PFOA/S do not degrade in the environment, persist in the human body, and cause adverse health effects. PFOA has been declared as "a likely carcinogen" by a USEPA Science Advisory Board review panel.[2] Recently, the USEPA set a Provisional Short-Term Health Advisory level of 0.4 µg/L and 0.2 µg/L for PFOA and PFOS, respectively in drinking water (US EPA, 2009). In addition, a drinking water guidance level protective of lifetime exposure of 0.04 µg/L was advised based on a published US EPA risk assessment.[36]

One of the biggest concerns from risk assessment studies was the bio-accumulation and subsequent chronic health effects from OWCs for some pharmaceuticals and perfluorinated compounds that persist and do not easily degrade in the environment.[19, 35] The bioaccumulation is likely to cause a chronic poisoning from repeated exposure even at a trace level of toxic contaminant over a long period of time.[37] This is especially true for drinking water exposure since people drink water daily over their life time. Little is known, however, about the chronic effects of OWCs (e.g., pharmaceuticals) and their end points. Estrogenicity is one of the most evaluated effects, but no reports of life-cycle effects were generated for any compounds with the exception of ethynil-estradiol.[38, 39, 40, 41, 42] Estrogenicity studies demonstrated a high risk factor reported as EC_{50} value of 1ng/L using a molecular marker of endocrine disrupting chemicals (EDCs). Chronic effects of the non-steroidal anti-inflammatory drugs,

acetylsalicylic acid and diclofenac have also been reported: Renal lesions were observed at 5 µg/L in humans[43] and 1µg/L for sub-cellular effects.[44] An exposure to β-blockers (propranolol) showed reduced reproduction in *Ceriodaphnia dubia* at 250 μg/L and in *Hyalella azteca* at 100 μg/L.[45] In addition, β blockers consist of selective and non-selective blockers; however, the specific receptors can act as non-selective blocking receptors, where the toxicity and risk assessment are of concern.[46] Overall, each risk assessment was mainly dependent on the amount of substance consumed, degradation, and toxicity (acute and/or chronic).[23] In addition, ecotoxicological effects from bio-accumulation were also reported.[23, 47]

One of the biggest challenges in the risk assessments for daily consumption of these OWCs was that they generally occur as mixtures of compounds, with a median of four compounds and a maximum of 31 compounds per site.[11] Previously, a study of mixtures of drinking water contaminants was recommended using VOCs, pesticides and nitrates in the United States stream water.[48] However, the current emerging unregulated organic contaminants are not yet considered in mixture effects and have to be assessed as a mixture for adverse health effects from drinking water contamination.[48, 49] Risk assessments and evaluations of toxicological effects of compound mixtures are difficult to perform; compounds can easily degrade or transform into compounds that can be more toxic than their precursor.[23, 50] A previous study showed increased estrogenicities measured from the concentrations of estrogenic compounds in water samples, which suggests the presence of unknown estrogenic compounds and/or additive effects of mixtures at low concentrations.[51] Similarly Hernando *et al*. showed toxic effects even at low concentration levels (ng/L or µg/L) of pharmaceutical compounds and/or their

metabolites including antibiotics and steroids.[52] Therefore, mixture effects as well as the accumulation of degradation compounds make an overall risk assessment difficult based solely on studies that report only measured concentrations of target analytes.

1.2 Target Compounds of Interest

More and more OWCs have been recognized as new, emerging contaminants with environmental impact.[53] For drinking waters, uses including pharmaceuticals, antibiotics, steroid hormones and PFCs have been identified as in need of characterization as unregulated drinking water contaminants. **Table 1.1** shows a list of twenty target compounds as well as their chemical structure and nomenclature.

1.2.1 Prescription and non-prescription drugs

Prescription and non-prescription drugs were selected based on a list of OWCs by USGS. Notably, the antidiabetic drug, metformin had the worldwide highest production number and was found in concentrations in the range of several 100 ng/L in sewage and surface waters in Germany.[54] Beta-blockers and anti-ulcer agents were among other therapeutic classes that have been frequently found in wastewater and surface water. Atenolol, metoprolol, propranolol, sotalol and ranitidine were the previously measured beta blockers and ulcer treatment drugs in influent and effluent wastewater.[46] Beta (β) blockers bind to β-adrenergic receptors and block activation by physiological agonists so they are used for treatment of hypertension, angina and other disorders of the cardiovascular system. The selective blocking receptor albuterol was chosen as a beta-2 adrenergic agent according to USGS selection.[1] The most frequently detected anti-ulcer drugs, cimetidine and ranitidine were also selected for this study. Their degradation products were also examined since the histamine H2-receptor antagonists were

susceptible to photochemical degradation.[21] β-blockers and anti-ulcer drugs were

generally challenging analytically in their analysis due to their amine functionalities and

basic sites on the molecules.[46] The non-steroidal anti-inflammatory drug, ibuprofen,

which inhabits synthesis and release of prostaglandins via COX, was found in the

environment as one of the more prevalent drugs.[23] It was also selected for this work.

Overall, 14 of the selected compounds were prescription and non-prescription drugs,

chosen based on the USGS study. These drugs included a broad therapeutic class of

antidiabetic agent (Glucophage™), analgesic (Tylenol™) Beta2 Adrenergic agent, central

nerve stimulant, antiulcer (Zantac™), anticoagulants, antidepressant,

antianginal/antihypertensive (calcium channel blocker), and lipid regulating agent

(cholesterol lowering). The detailed list of target compounds with their therapeutic

classes was provided in **Table 1.1**.

1.2.2 Steroid hormones

Hormones first became a focus of pharmaceuticals in the environment during the

1970s; however, scientific interest and public awareness grew in the mid nineties with

interest in endocrine disrupting chemicals (EDCs).[19] EDCs can be of either natural or

synthetic origin and have the ability to interfere with the normal functioning of the

endocrine system.[55, 56] Estradiol was widely studied because of its prevalence in drinking

water sources at the lower ng/L-range.[57, 58] In addition, steroid hormones, testosterone

and progesterone were selected based on USGS findings.

1.2.3 Antibiotics

Another important group that has frequently been measured is antibiotics. In

addition to growth hormones, antibiotics were often discovered in the run-off from

livestock facilities.[46] The concentrations in surface water and effluent from STPs were commonly found in ng/L-µg/L ranges. An increasing concern with antibiotics was the bacterial resistance to antibiotics and disinfectants that started to be detected in waste, surface, and ground water as well as sediment and soils.[19] Segura *et al.*[59]expressed concerns due to their potential contribution to the spread of antibiotic resistance in bacteria evaluated as potential environmental concerns. For this work, antibiotic chlortetracycline was selected for a further investigation in drinking water samples.

1.2.4 Perfluorinated compounds (PFCs)

Perfluorinated compounds (PFCs) are used for many important manufacturing and industrial applications including paper production, textile manufacturing, leather treatment, surfactant additives, coatings manufacture, and firefighting foams and equipment production. Most notably, perfluorooctanoic acid (PFOA) has been used as a processing aid in the manufacture of Teflon. According to a previous study done in NJ public drinking water systems, 65% of the systems found PFOA at concentrations ranging from 0.005 to 0.039µg/L.[36] Higher PFOA concentrations ranging 1.78 to 4.3 and from 0.4 to 3.9 µg/L were found in Little Hocking, Ohio and Lubeck, West Virginia, respectively.[60] For this work, PFOA and perfluorooctanesulfonic acid (PFOS) were selected as the most abundant PFCs with potential adverse health effects.

1.3 Analytical Methodology

An increasing number of studies and laboratories have started to analyze pharmaceuticals, antibiotics, steroid hormones and perfluorinated compounds found in environmental samples. However, it is difficult to compare and/or evaluate their concentrations across the globe without standard analytical methods. For instance, it was

difficult to determine whether the improvement in sensitivity and reliability is due to different analytical techniques or true differences in measurements. In this work, the current analytical methodologies for trace analysis of pharmaceuticals, hormones and PFCs were reviewed for potential variability and improvement.

1.3.1 Sample preparation

Environmental water concentrations of organic wastewater contaminants (OWCs) mainly, pharmaceuticals, antibiotics, steroid hormones, and PFCs were typically found at trace levels of ng/L-µg/L.[61] These levels of target compounds often require extraction and concentration prior to instrumental analysis. Solid phase extraction (SPE) is one of the most widely used sample preparation techniques for environmental aqueous samples. Although liquid-liquid extraction (LLE) is traditionally used, SPE was determined to be a preferable alternative to LLE because both clean-up and extraction are performed simultaneously. Principally in SPE, an aqueous sample is passed through an SPE sorbent and based on the analyte's affinity for the sorbent will either be retained or passed through the sorbent. SPE cartridges packed with reversed phase material (alkyl-modified silica and polymer based) were often used to extract pharmaceutical compounds.[62] In this work, Yoon *et al*.[63] examined several different types of reversed-phase SPE cartridges demonstrating good recoveries for PFOA and PFOS. A mixed mode material with reverse phase properties was also used as the optimal SPE sorbent for the broad range of pharmaceuticals and other OWCs.[46, 62, 64, 65] A mixed mode- reverse phase SPE sorbent (Oasis HLB by Waters, Milford, MA) showed the highest recoveries even without pH adjustment.[16, 66, 67] In a few studies, a mixed cation-exchange sorbent (Oasis MCX) was selected to be used at acidic pH (1.5-2) for extraction of acidic, basic and neutral

compounds since the cation exchanger binds the basic compounds in the ionized form

and the reversed phase can retain acidic and neutral compounds.[64, 68] Oasis MCX

sorbents showed overall less extraction efficiency than HLB sorbents and often required a

pH adjustment. [61] Solid phase microextraction (SPME) was also a good alternative to

SPE in analysis of environmental water samples. SPME is based on the partition

equilibrium of the analyte between the samples and a sorbent and has the benefit of being

a solvent-free and one-step extraction technique.[69] In previous work, an SPME method

was optimized to increase the extractable number of drinking water contaminants and

reduce sample preparation steps, time, and cost as well as sample volume.[3] However, no

great success was reported for optimal detections of target OWCs including

pharmaceuticals with SPME, suggesting SPME procedures are still limited in scope of

method manipulation because there is a limited choice of sorbent coatings on the market

for selectivity of various analyte properties.[70]

1.3.2 Previous analytical methods: GC-MS

Previously, a gas chromatography-mass spectrometry (GC-MS) was used to

determine semi-volatile organic compounds in various environmental samples (e.g. soil,

air, water) in an enhancement of EPA method 8270. The GC-MS method was also able to

detect pesticides including organochlorine pesticides, organophosphorus pesticides, and

nitrogen containing pesticides as well as polynuclear aromatic hydrocarbons (PAHs) and

polychlorinated biphenyls (PCBs).[3, 71] Few earlier studies used GC-MS methods to

determine pharmaceuticals and personal care products (PPCPs) with applicability,

sensitivity and cost-effectiveness in environmental laboratories.[65, 70] For analysis of

PPCPs, DB5, DB5 MS or HP5 MS GC columns (Agilent Technology, Palo Alto, CA,

USA) of a 30 m x 0.25 mm x 0.25 μm size was generally used in GC. Helium was used

as a carrier gas and 1-3 μL extracts were injected into GC using split/splitless injector.

GC temperature was generally programmed in the range of 50-300 ºC with a typical run

time of 30-45 min.[65, 72] However, these GC-MS methods were not directly applicable to

non-volatile (e.g., majority of pharmaceuticals) and thermally liable compounds (e.g.

certain perfluorinated compounds), which required derivatization prior to GC analysis.[70]

Figure 1.2 was created based on previous reviews by Pietrograde and Basaglia[70] and

Fatta *et al.*, [64] which described typical analytical procedures for analysis of OWCs mainly

pharmaceuticals in aqueous samples. For the detection of these broad ranges of target

compounds, the derivatization technique seemed time consuming and insufficient due to

various physical and chemical properties of these compounds that may be affected. A

previous study showed side-reactions that were observed under different derivatization

conditions.[73]

1.3.3 Current analytical methods: LC-MS

Despite the merit of GC procedures, liquid chromatography (LC) showed more

widespread applicability.   It has been one of the most reliable methods to analyze

pharmaceuticals, personal care products and other organic compounds from

environmental matrices.[61, 62, 65] LC separations are achieved based on mobile phase

solvents and stationary phase column with the necessary analytes' retention.   In order to

improve separation, various characteristics of the LC column's properties, and the

composition for aqueous and organic mobile phase, including their pH/buffering

conditions were developed and optimized. Recently, ultra performance LC (UPLC) was

introduced for separating organic pollutants with its use of a high pressure system with a

sub-2 μm particle size column. The UPLC methods offered improvements in sensitivity and low volume samples in analysis of pharmaceutical residue in water samples.[74, 75, 76] Based on PFOA/S analysis, UPLC methods showed more than 2 fold- improved sensitivity over conventional HPLC methods.[63]

LC procedures were often coupled with various conventional detectors such as UV, fluorescence and mass spectrometry (MS) for qualitative and quantitative analysis. Camacho-Muñoz *et al*.[61] showed the simultaneous determination of 16 of the most common pharmaceutical compounds in influent and effluent wastewater and surface water using HPLC with diode array and fluorescence detectors. However, UV and fluorescence methods still require a secondary detector (e.g., MS) for accurate determination of target analytes as well as for confirmation purposes.[77] On the other hand, a LC-MS with an atmospheric pressure ionization source has gained great popularity because of its applicability and compatibility for the broad characteristics of pharmaceuticals and other OWCs.[64, 65, 78]

Electrospray (ESI) ionization has been widely used as an interface between an LC and an MS. Another "soft" ionization technique, atmospheric pressure chemical ionization (APCI) was previously studied for pharmaceuticals although relatively few analyses were done with APCI as compared to ESI.[79] While APCI as the ion source is believed to be less sensitive to matrix effects, Zhao and Metcalf[80] showed a signal enhancement in neutral pharmaceuticals using APCI suggesting it may still be required to eliminate and/or compensate for matrix effects. The decision between positive or negative mode for the selection of ESI or APCI is generally made by their chemical properties (e.g. acidic/basic) while amphoteric compounds and nitrogen and oxygen

containing compounds were typically ionized effectively in either mode. The eluant

composition and extracted sample matrix may also affect the final decision of whether

ESI or APCI in positive or negative mode is to be used.[79] Depending upon the ionization

mode/condition, fragmentation patterns may be different due to the functional elements

of a molecule that stabilize a positive or a negative charge. Switching between positive

and negative ion mode within one analytical run was performed with an ESI interface

showing improvements for the multi-residue (class) analysis.[81, 82, 83] Recently, a method

for determination of five pharmaceuticals with ESI using polarity (+/-) switching was

introduced.[84] The switching polarity was therefore examined for simultaneous

determination of 20 OWCs in this work.

An ion trap-MS technique has also been optimized with its unique ability to

acquire full-scan mass spectra using MS^2 and MS^n for target OWCs. The sensitivities in

different IT-MS methods (i.e. full scan, selected ion monitoring and MS^2) were compared

in the analysis of PFOA and PFOS.[63] A single-stage quadrupole MS was not generally

considered adequate for the detection of trace levels of OWCs.[79] A triple-quadrupole and

ion-trap mass spectrometers were preferred for sensitive drinking water analysis. More

sensitive and reliable analysis for pharmaceuticals and other OWCs are currently done

using a quadrupole-linear ion trap MS and time-of- flight.[85] With the advent of a new

linear IT instrument (LTQ, Thermo Fisher), an enhanced instrumental detection

performance was compared with a previous IT instrument (LCQ, Thermo Fisher).[63] In IT

methods, MS^2 data using data-depending scanning (DDS) was advantageous especially

with the identification of unknown degradation products found in some pharmaceutical

compounds. The usage of DDS in LC-MS^2 produced "clean" product ion mass spectra

without prior knowledge of the precursor ion.[86] However, a potential drawback of DDS was a loss of sensitivity in the quantification analysis because non-targeted MS/MS spectra were competing with each other. In this work, details of LC, ESI and MS conditions were addressed for target OWCs in environmental water samples according to their own physical and chemical properties as well as experimental trial and error. Also the potential degradation products of pharmaceuticals (e.g. cimetidine, ranitidine) were identified using DDS.

1.4 Background Contaminations and Matrix Effects

The current analytical methods using SPE and LC-MS/MS are continuously being optimized to lower the detection limits of target analytes.[87] The perfluorinated compounds (PFOA and PFOS) require detection at trace levels since these compounds were often found in low part per trillion concentrations in drinking water samples [36, 88, 89, 90, 91, 92, 93, 94]. PFOA and PFOS were also detected in blank water samples for these potential reasons: 1) PFOA and PFOS are persistent and abundant in many industrial and consumer products including laboratory materials made up with PTFE.[95, 96] 2) They are major by-products and/or end-products of perfluorinated compounds and resist degradation via oxidation, hydrolysis, or reduction under biotic and abiotic condition[95, 96] 3) Lower detection limits allow detection both in environmental and laboratory contaminants. A previous study also showed the detection of procedural and instrumental contamination from PFOA and PFOS.[97] The sources of these background contaminants were identified as coming from: reagent solvents, SPE cartridges, liners, and tubing and HPLC instrument parts. The optimized methods however avoid background contaminations with PFOA/S by eliminating the SPE steps.[63]

The matrix effects are another challenge. They may affect ionization performance and result in an erroneous quantification by LC-MS. Co-extracted materials or even co-eluted target compounds that interfered with the ionization of a target analyte were often observed.[79] Recommendations for reducing matrix effects included using various extraction protocols with selective conditions, but are not always possible due to the broad range of target analytes with different physicochemical properties.[64] Other recommendations included using a lower flow rate, but at a loss of extraction efficiency. [98, 99]

1.5 Water Treatment Process for Target OWC (Pharmaceuticals) Removal

Unfortunately, the majority of OWCs are not effectively eliminated or decreased by current wastewater treatment prior to becoming drinking water supply source.[11] Particularly, OWCs in surface- and groundwater are transported and delivered to our domestic tap water without complete removals.[100] Great efforts at removing OWCs are being done at stages of wastewater treatment plants but the elimination of these OWCs prior to becoming drinking water are not yet achieved.[18, 57, 72]

Two pharmaceuticals; ibuprofen and diclofenac with the highest and lowest percent removal of 92% $\pm$ 8% and 26% $\pm$ 17%, respectively, via several wastewater treatment plants in Finland [101] were reported. Previously, aerobic treatment using activated sludge showed the most favorable wastewater treatment for some pharmaceuticals and personal care products (PPCPs) mainly anti-inflammatories and antibiotics.[57] The overall removal efficiencies of anti-inflammatory and the antibiotic sulfamethoxazole were reported at 40-65 % and 60%, respectively.[57] This study showed that the pre-treatment and primary sedimentation steps were not effective removal

methods for the PPCPs.[57]   Instead, degradation and adsorption of contaminants onto activated sludge were suggested as two main mechanisms for contaminant removal.[57] Similarly, removal of tetracyclines (antibiotics) was previously reported at a high of 80-85% removal by different activated sludges.[102] Ozonation, however, did not affect the removal efficiencies of PPCPs during anaerobic digestion in sludge.[103] Overall, removal studies showed a general agreement with removal of PPCPs (i.e., antibiotics and anti-inflammatories) achieved more effectively with activated sludge or/and oxidation ditches than with other treatments including biological filters (e.g., trickling filter bed) or reed beds.[18, 72]

More recent removal studies proposed advanced oxidation processes (AOPs) for the removal of residual pharmaceuticals from aqueous systems.[104] Yang *et al*. introduced a microwave enhanced Fenton- process to treat high concentration pharmaceutical wastewater.[105] Similarly, a degradation mechanism with mild solar photo-Fenton and TiO_2 was studied for OWCs including pharmaceuticals at low concentrations.[106] Photo-Fenton was by far more effective than TiO_2 for degrading acetaminophen, antipyrine, atrazine, caffeine, diclofenac, isoproturon, progesterone, sulfamethoxazole, and triclosan. However, the drawback of the photo-Fenton was observed with a formation of radical scavengers such as carbonate species (CO_3^{2-} and HCO_3^-) that compete with organic contaminants for hydroxyl radical reactions and decrease the degradation efficiencies. Although there are fewer numbers of drinking water treatment steps than of wastewater treatment, Ternes *et al*. investigated filtration with granular activated carbon (GAC) and ozonation (0.5-3 mg/L) for the major elimination of pharmaceuticals in treatment of a drinking water.[107]

Potential removal methods for target OWCs and their adaptations toward domestic household systems were studied in this work.

1.5.1 Activated carbon/Brita™ filtration

Granular activated carbon (GAC) filtration was primarily studied as one of the most effective system in removing pharmaceuticals.[107] GAC adsorbs many organic pollutants in which concentrations of adsorbed contaminants equilibrate with concentrations in influent in the liquid-phase.[108] The packed-bed granular activated carbon (GAC) was previously recognized as a "Best Available Technology" for treating numerous organic pollutants by U.S. EPA. Westerhoff *et al*.[108] showed substantial removal of endocrine-disruptors, and pharmaceuticals and personal care products (EDC/PPCPs) using powder activated carbon (PAC) and/or ozone systems. Octanol-water partition coefficients served as a reasonable indicator for removal. The exception was also reported with EDC/PPCPs in protonated or deprotonated forms at tested pH conditions as well as heterocyclic or aromatic nitrogen containing compounds (caffeine, pentoxifylline).[108] Brita™ is a well known household water filtration system using by activated carbon and ion exchange resin. The water filter system in Brita™ is comprised of a cartridge with a mix of a weakly acidic exchange resin and a silverized granular activated charcoal.[109] Based on manufacture recommendations and previous studies, it reduced lead, copper, mercury, cadmium, and toxicity from metals, and chlorine, which also improved water's taste and odor.[109, 110] Overall, Brita™ filtration was selected as a good candidate to test for the reduction/removal of OWCs in domestic household systems.

1.5.2 Oxidation process

While biological wastewater treatment showed insufficient removal of pharmaceuticals, advanced oxidation processes (AOPs) were on the other hand, recommended to treat pharmaceutical wastewater samples.[105] One of the most widely distributed AOPs was photolysis that uses the interaction between artificial or natural light and the target molecules.[104] Unfortunately, PFOA and PFOS have the strongest carbon-fluoride bonds in organic chemistry and theoretically were not subject to photolysis.[35, 96] Fenton's oxidation or Fenton-like reactions that mainly are used for chemical oxidation demand (COD) removal, and UV_{254} photolysis was previously suggested to remove pharmaceuticals in surface water and industrial effluents.[104, 105] One of drawbacks of this treatment included a need for dissolved ions to be recovered from the treated solution, requiring an additional procedure.[106] Ozonation on the other hand, was another AOP that was a good candidate to treat OWCs. Fundamentally, ozone is a strong oxidant process that either decomposes in water to form stronger oxidizing agents than ozone (i.e. hydroxyl radical) or reacts selectively with certain functional groups of target molecules through an electrophilic mechanism.[104, 111, 112, 113] Based on these mechanisms, EDC/PPCPs showed transformation to oxidative byproducts. Steroids containing phenolic moieties (e.g. estradiol) were oxidized more efficiently with ozone than those without aromatic or phenolic moieties.[108] The major removal mechanism for two pharmaceuticals, gemfibrozil and ibuprofen, was previously observed with ozonation.[108] Therefore ozonation was another mechanism for treating the target OWCs of pharmaceuticals, antibiotics, steroid hormones and PFCs.

1.5.3 Microwave water-process

A potential alternative and affordable treatment process for degrading OWCs is the household microwave oven. Microwave ovens use microwaves for dielectric heating. Microwaves have been increasing used in organic synthesis due to the innovative heating mechanism compared with conventional heating.[114] Unlike conventional heating, dielectric heating (microwave) does not rely on heat-transfer, rather the electrical field exists in the body of the sample surface, which allows energy to be rapidly transferred beyond the samples surface.[105] Microwave ovens were suggested for enhancing the degradation efficiency of high concentration pharmaceutical wastewater.[105] The microwave coupled AOP (MW/H_2O_2-AOP) treatment was also suggested with enhanced efficiency.[115] Notably, the usage of microwave ovens in domestic households were very high as the total number of consumer ovens in the U.S. was well over 100 million and the world total was estimated to be over 250 million.[116] The household microwave oven is therefore examined as a portable, effective and economic method for the potential removals of precursor OWCs by degradation efficacy.

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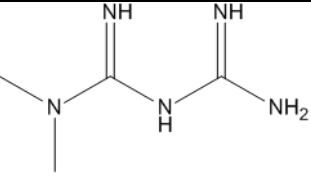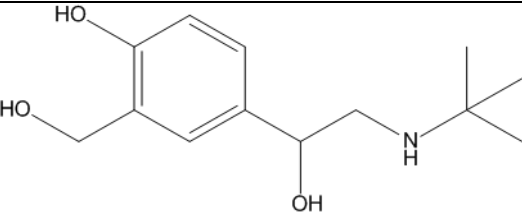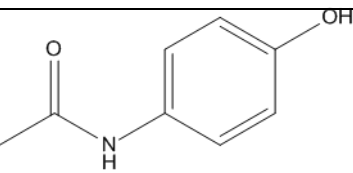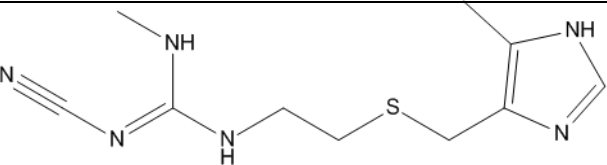
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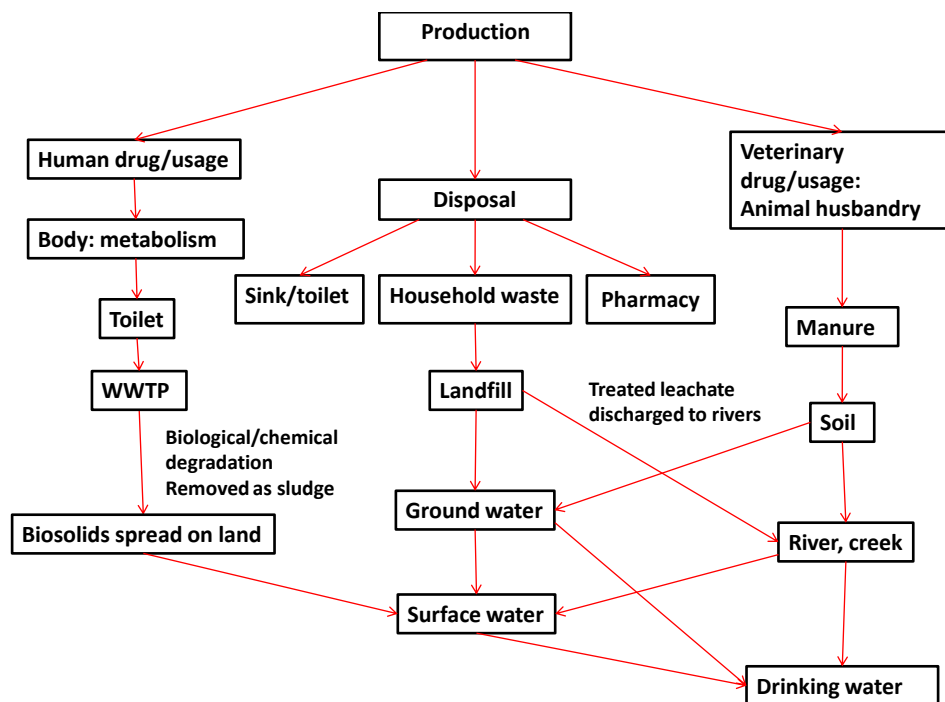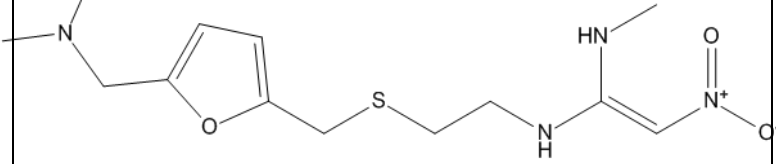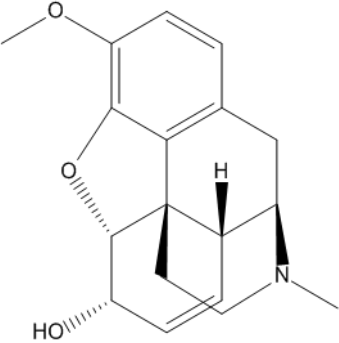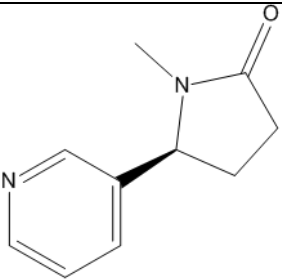
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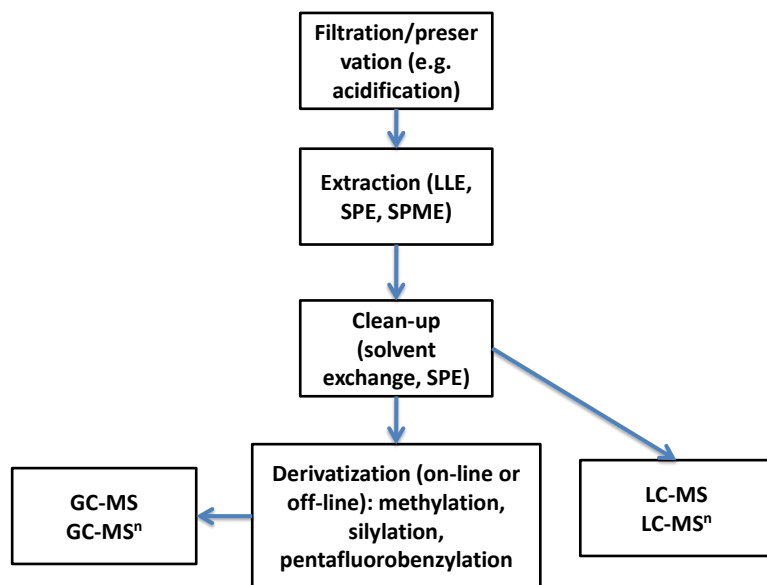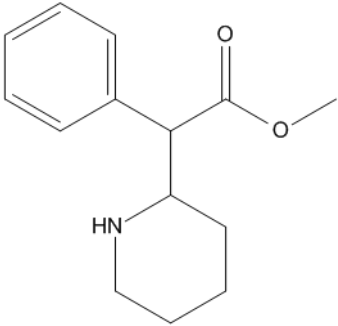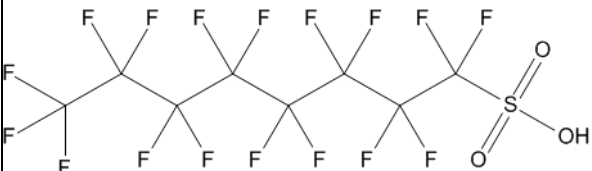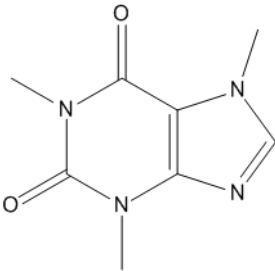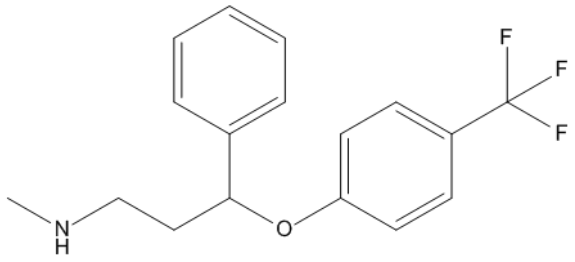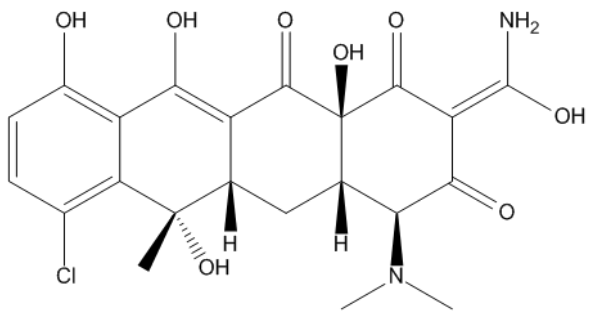
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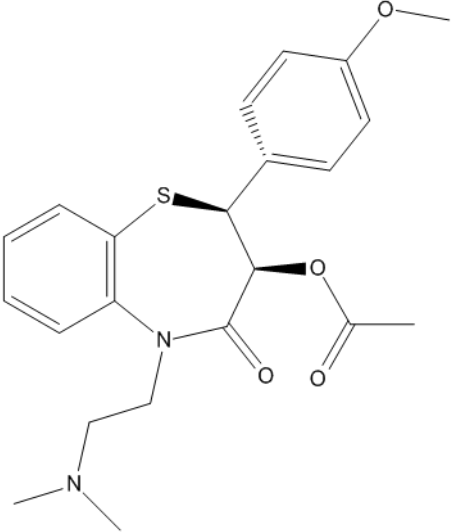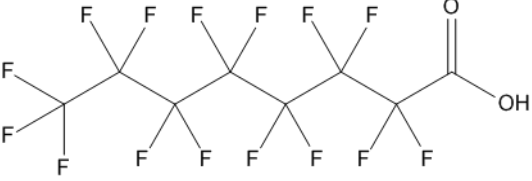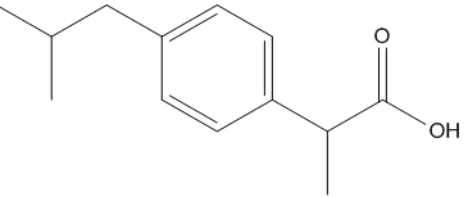
Target analytes	Classification	Mol. Mass (g/mol)	Chemical formula	Chemical structure
Metformin	Antidiabetic agent (e.g.Glucophage)	129.16	$C_4H_{11}N_5$	
Albuterol	Beta2 Adrenergic agent	239.31	$C_{13}H_{21}NO_3$	
Acetaminophen	Analgesic (Tylenol)	151.17	$C_8H_9NO_2$	
Cimetidine	Gastrointestinal agent	252.34	$C_{10}H_{16}N_6S$	
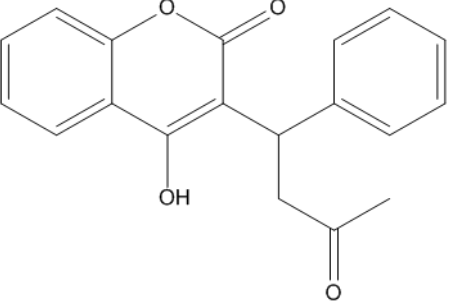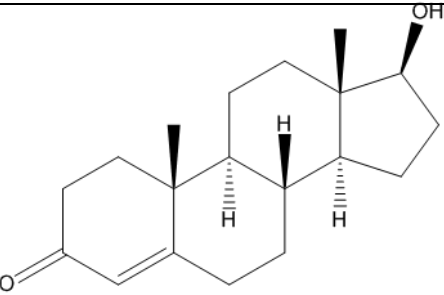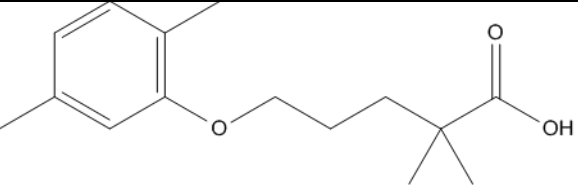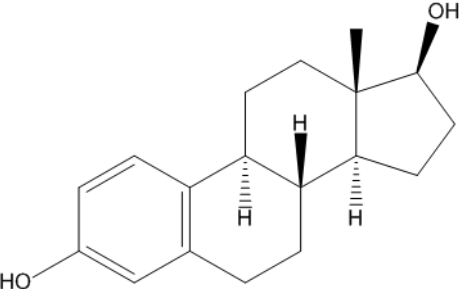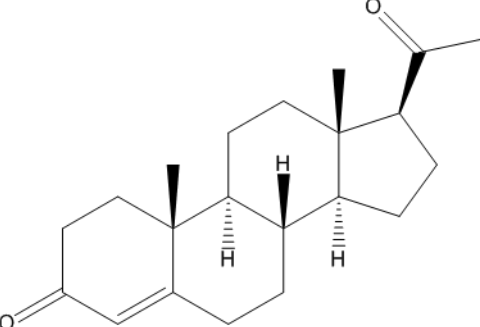
Ranitidine	Antiulcer (Zantac)	314.41	$C_{13}H_{22}N_4O_3S$	
Codeine	Analgesic	299.36	$C_{18}H_{21}NO_3$	
L-Cotinine	Central Nervous System stimulant (a metabolite of nicotine)	176.22	$C_{10}H_{12}N_2O$	

Methylphenidate	CNS Agent	233.31	$C_{14}H_{19}NO_2$	
PFOS	Perfluorinated chemical (fluorosurfactant)	500.13	$C_8HF_{17}O_3S$	
Caffeine	CNS stimulant	194.19	$C_8H_{10}N_4O_2$	

Fluoxetine	Antidepressant	309.33	$C_{17}H_{18}F_3NO$	
Chlortetracycline (CTC-HCl)	Antibiotic	515.35*	$C_{22}H_{23}ClN_2O_8$	

Cis-Diltiazem	Antianginal; antihypertensive (calcium channel blocker)	414.52	$C_{22}H_{26}N_2O_4S$	
PFOA	Perfluorinated chemical (fluoropolymer)	414.07	$C_8HF_{15}O_2$	
Ibuprofen	Anti-inflammatory (e.g. Advil)	206.28	$C_{13}H_{18}O_2$	

Warfarin	Anticoagulant	308.33	$C_{19}H_{16}O_4$	
Testosterone	Steroid hormone	288.42	$C_{19}H_{28}O_2$	
Gemfibrozil	Lipid regulating agent (cholesterol lowering)	250.34	$C_{15}H_{22}O_3$	

Estradiol	Steroid hormone; Endocrine and metabolic agent	272.38	$C_{18}H_{24}O_2$	
Progesterone	Steroid hormone	314.46	$C_{21}H_{30}O_2$	

Chapter 2 - A Comparison of Two Optimized Liquid-Chromatography-Ion Trap Mass Spectrometry Methods for Quantification of Perfluorooctanoic acid and Perfluorooctanesulfonic acid, Towards a Direct Analysis Method for Field Water Samples

2.1 Abstract

An analytical method has been developed for direct determination of the two most prevalent perfluorinated compounds (PFCs): perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS).   As a comparison to current methods a solid phase extraction (SPE) and high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS) was optimized for sensitivity in application to drinking water samples.   The optimized extraction protocol demonstrated high recoveries for both PFOA ($104\pm2\%$) and PFOS ($100\pm9\%$) in water samples (1L). The optimized limits of detection (LOD) of 3.1 and 9.1 pg (injected) for PFOA and PFOS, respectively, were still not sensitive enough for a direct determination for many water samples. An ultra performance LC- linear ion trap MS method was tested for rapid, reliable, and sensitive direct detection of these target analytes. The UPLC-MS/MS (LTQ) achieved the required LOD measuring 0.03pg and 0.24pg for PFOA and PFOS, respectively, which were 103 and 38 fold more sensitive than the comparable HPLC-MS/MS (LCQ) method. While the SPE-HPLC-MS/MS method provided greater sensitivity than some comparable methods, the increased sensitivity using the UPLC-MS/MS method allowed for direct analysis of PFOA and PFOS without sample pre-concentration. In addition the direct determination method minimized the background contamination issue. Background correction is recommended when using solid phase extraction because target analytes were found in many laboratory blanks. They were not found with the UPLC-MS/MS

method.   The optimized LTQ method was used to quantify low ng/L levels of PFOA and

PFOS in thirteen NJ water samples. Raw and treated water samples were analyzed and

determined for PFOA/S contamination during water treatment using the SPE-LTQ-

MS/MS method PFOA and PFOS were reduced after treatments, but not completely

removed even after a final stage of chlorination. The direct injection method was also

used on multiple drinking water samples demonstrating its utility for PFOA measurement.

In addition, a storm water sample from NJ was quantified for PFOA and PFOS and levels

of 66.6 ng/L and 724 ng/L, respectively, were found using the direct analysis method.

Overall, field water sample analysis using the direct LTQ method showed its sensitivity

and applicability to measure PFOA/S in water samples without sample preparation steps.

2.2 Introduction

Perfluorinated compounds (PFCs) are used for many important manufacturing

and industrial applications including paper production, textile manufacturing, leather

treatment, surfactant additives, coatings manufacture, and firefighting foams and

equipment production. Most notably, perfluorooctanoic acid (PFOA) has been used as a

processing aid in the manufacture of Teflon.   PFOA is found in the blood of people and

wildlife throughout the world.[1] This is of concern because PFOA does not degrade in the

environment, persists in the human body, and causes adverse health effects. PFOA has

been declared as "a likely carcinogen" by the United States Environmental Protection

Agency (US EPA) Science Advisory Board review panel.[2] On January 15, 2009, the

USEPA set a Provisional Short-Term Health Advisory level of 0.4 µg/L in drinking

water.[3] At the same time, USEPA set a Provisional Short-Term Health Advisory level of

0.2 µg/L for and perfluorooctanesulfonic acid (PFOS).[3] In New Jersey (NJ), a drinking

water guidance level protective of lifetime exposure of 0.04 micrograms per liter (µg/L)
was established based on a published USEPA risk assessment.[4] It is anticipated that
analytical methods that can detect very low levels of PFOA, PFOS and other
perfluorinated compounds will be needed to monitor concentrations of these analytes at
the new drinking water standard levels.

Earlier techniques for determination of PFCs included combustion methods in
which organic fluorine was converted to soluble fluoride and measured as total content of
fluoride. Similarly, neutron activation, X-ray fluorescence, 19F NMR and attenuated
total reflected-Fourier transformation infrared spectroscopy were also previously used to
determine the total fluorine content or the amount of certain PFCs in water samples.[5]
However, these techniques were non specific, without the capability of separating
different classes of PFCs, and did not have the sensitivity and selectivity required for
"natural" water samples for PFC analysis.[5-12]

Another well-utilized methodology for determining environmental contaminants,
gas chromatography-mass spectrometry (GC-MS) was also used to detect a wide range of
PFCs.[5-13] Certain classes of fluorinated alkylated substances and perfluorinated
surfactants including PFOS, however, do not form stable volatile derivatives and were
not detectable using GC/MS methods.[5,6,13] Moreover, a major drawback of GC methods
was the derivatization step required prior to GC injection.

Recently, high performance liquid chromatography (HPLC) techniques have been
developed to be applicable to the analysis of all classes of PFCs without a derivatization
step, with better sensitivity and selectivity than GC methods.[5,6] LC methods reduce
sample preparation steps and have flexibility with several conventional detectors

applicable for analysis of PFCs. Some of these widely used fluorescence and UV

detectors were affordable and applicable with LC for PFC analysis. However,

florescence and UV detectors were less advantageous than MS because, they often

require the addition of chromophores for PFC detection and accurate/precise

measurements are often lacking without a secondary detector for confirmation and

quantification purposes. Compared to these conventional detectors, LC-electrospray

(ESI)-MS and LC-tandem MS were determined to be the most suitable and sensitive

analytical techniques and therefore used to focus on PFOA and PFOS analysis.[5, 6]

For this study, extensive optimizations of LC-ESI-MS methods were performed

using an HPLC-MS (LCQ) and an UPLC-MS (LTQ). By performing these optimizations,

the sensitivity of both analytical methods was compared for LC conditions and limits of

detection/quantification. PFOA and PFOS are often present (if detected) at very low

concentrations, at the sub-part per trillion levels (ng/L) in drinking water samples[10,12]

therefore, trace level analysis of these targeted analytes is essential. One of the biggest

challenges in PFOA and PFOS trace level analysis was blank contamination with target

analytes because many laboratory products contain PFCs. The potential sources of blank

contamination were identified and eliminated during optimization steps. The majority of

PFC methods were previously developed using single or triple quadrupole (QqQ) MS

techniques.[5, 11, 12, 14-16, 19] This study showed the comparable sensitivity to QqQ

experiments[11, 12, 14, 25, 29] using two ion-trap MS techniques. Moreover, the optimized

sensitivity of this method was improved to ultra trace, parts per quadrillion (ppq) levels.

Overall, this method is simple, rapid, and flexible, and can be further refined to detect

PFCs in various environmental aqueous matrices. The sensitivity of this optimized

UPLC-MS/MS (LTQ) method will allow for a direct measurement of both PFCs in drinking water samples avoiding pre-concentration steps and removing the potential of background contamination from the SPE step. Finally, various types of raw, treated, tap and storm water samples from NJ sources were analyzed for PFOA and PFOS using two ion trap MS techniques (LCQ and LTQ).

2.3 Experimental

2.3.1 Standards and reagents

Perfluorooctanoic acid standard (98.6%, Cat #: PFOA-001S; AccuStandard®, New Haven, CT), and perfluorooctanesulfonic acid standard in methanol (98.4%, Cat#: PFOS-001S; AccuStandard®, New Haven, CT) were diluted directly from purchased stock. Deionized water was produced from a Millipore workstation (model #: Quantum® EX, Billerica, MA) and used directly for laboratory blanks, spiked samples and SPE solvents. HPLC-grade water, methanol and acetonitrile (Burdick and Jackson High Purity Solvents, West Chester, PA) were purchased from VWR and used as LC elution solvents. All HPLC mobile phase solvents were filtered and degassed through 47 mm diameter, 0.45 μm pore size nylon filter membranes (Osmonics, Minnetonka, MN) prior to use. Five molar ammonium acetate (0.2 μm filtered) from Ambion Inc. (Austin, TX) or 0.1% formic acid (98%) from Sigma-Aldrich (St. Louis, MO) was added to the LC mobile phase. All chemicals and solvents were used without further purification.

2.3.2 SPE Sample preparation

Prior to SPE steps, all spiked and field samples were collected using 1L amber glass bottles (I-CHEM certified™, Rockwood, IN) and were stored at 4˚C no longer than a maximum of 2 weeks before the sample analysis as suggested in EPA method 537. For

pre-concentration and sample cleanup, analytes were isolated by continuous flow-solid-phase extraction (CF-SPE) using superclean ENVI-18 columns (1.0 g sorbent, Supelco Inc., Bellefonte, PA). This SPE column was selected after an optimization study using different reversed phase SPE columns. All CF-SPE procedures were performed using a Visiprep^{TM} DL disposable liner-SPE vacuum manifold (Supelco Inc., Bellefonte, PA). Up to twelve SPE columns were each connected to individual disposable liners (Supelco Inc., Bellefonte, PA) in the SPE manifold. The manifold was directly linked to a vacuum supply with tubing. The columns were initially conditioned with 5 mL of methanol followed by 5 mL of deionized water and were not allowed to dry after conditioning. The water samples were loaded onto these conditioned columns at a flow rate of 4 mL/min. Once all of the water from the sample bottles was loaded onto the SPE columns, the columns were completely dried under a vacuum stream for approximately 30 min. The analytes were then eluted off the column using 6 mL of methanol under a gentle vacuum. Finally, the solvent was evaporated and the extracted eluant was pre-concentrated under a gentle vacuum in the Visiprep manifold, until a final volume of approximately 1 mL, was achieved.

2.3.3 HPLC-MS (LCQ)

The HPLC used for analyte separation was a Waters Alliance 2690 dual-syringe solvent delivery system with an automatic sampler (Waters, Milford, MA). A 30 μL aliquot of sample was injected through the autosampler onto an Envirosep PP column (Phenomenex) 12.5 cm x 2.0 mm, 5 μm. The mobile phase had a linear gradient with 10 mM aqueous ammonium acetate solution as component A and 100% methanol as component B. The organic gradient started at 60% B for 2 min, changed to 95% B in 8

min, held for 1 min, then back to 60% B in 7 min and held (equilibrated) at 60% B for 2 min. The total analysis time was 20 min. The injection volume was 30 µL and LC flow rate was 200 µL/min.

The HPLC was coupled to an LCQ classic (Thermo Fisher Scientific, San Jose, CA). PFOA and PFOS were ionized using an electrospray interface in negative ion mode, detected in the ion trap mass spectrometry (ESI-ITMS) and quantified using an optimized extracted ion chromatogram (EIC) MS program. Distilled N_2 (99.999% purity grade) was used for collision, auxiliary and sheath gases. The capillary temperature was 250ºC and the sheath gas flow rate was 1.335 L/min. The spray and capillary voltages were 5kV and -4 V, respectively. The tube lens offset was 35V.

Instrumental control and data acquisition for the ESI-ITMS were done using Xcalibur software (Thermo Fisher Scientific, San Jose, CA). PFOA/S standards of 1 µg/mL concentration were directly infused to tune the instrument on the precursor ion and determine the product ions of interest. Qualitative determination of the analytes was done using their chromatographic retention times and their precursor MS ions of $[M-H]^{-}$ of m/z 413 and 499 for PFOA and PFOS, respectively. Identification of target analytes was confirmed by both their product ions (369 m/z and 419 m/z for PFOA and PFOS, respectively) and fragmentation patterns. Peak area, peak height, and signal-to-noise ratio (S/N) were evaluated for optimal chromatographic response. Peak area was chosen for its accurate and precise quantification for these targeted analytes. Full Scan (FS), selected ion monitoring (SIM), and tandem MS (MS/MS) of extracted ion chromatography (EIC)-MS modes were compared to determine the more sensitive and selective detection method. For the FS method, the mass range was set for 100 to 550

m/z ; scan time 3 μs/scan; maximum injection time of 200 msec.; automatic gain control

(AGC) value of $1x10^7$; typical activation energy (Q) of 0.25 eV and activation time of 30

msec. For the SIM method, the isolation width was set for 3 m/z; scan time of 5 μs/cans;

maximum injection time of 200 msec.; AGC value of $2x10^7$; typical activation energy (Q)

of 0.25 eV and activation time of 30 msec. For the MS/MS method, the MS/MS scan

range was 367.4 to 370.4 m/z for PFOA and 497.7 to 500.7 m/z for PFOS. For

determination of PFOS, a MS/MS condition was optimized for the isolation of precursor

ion with enhanced sensitivity. The collision energy (% CE) was optimized for the

maximum total ion counts (TICs) determined with PFOA and PFOS fragments: PFOA

was monitored at 20% CE (413>369) and PFOS was monitored at 10% CE (m/z =

499>499). The other MS conditions remained the same as previously described. The

PFOA and PFOS standards consisted of a mixture of linear and branched isomers;[9, 21]

however, these isomers were not separated chromatographically. Instead PFOA and

PFOS were represented as a single peak to maximize analyte response.

2.3.4 UPLC-MS/MS (LTQ)

The target analytes (i.e., PFOA and PFOS) were separated on a Hypersil GOLD

column (2.1 x 50 mm, 1.9 μm; Thermo Scientific, San Jose, CA) using a binary mobile

phase system of water and acetonitrile (ACN) with 0.1% formic acid at flow rate of 100

μL/min. Twenty five μL aliquots of water samples were injected onto an Accela UPLC

system for direct LTQ measurement, while five μL of the SPE extracts were injected onto

the same UPLC system.   This system was configured with a dual-piston, quaternary, low

pressure mixing pump with a built-in vacuum degasser and pulse damper (Thermo Fisher

Scientific). A UPLC gradient program was performed as follows: 60% ACN for 1

minute, then increased to 95% in 6 min., held at 95% for 1 min, then decreased to 60% in 1 min and held (equilibrated) at 60% for 1min. The total UPLC gradient run was 10 min and the column oven temperature was held at a constant 30˚C.

The UPLC was interfaced to an electrospray ionization (ESI) source operating in negative mode then into an LTQ XL Mass Spectrometer (Thermo Fisher Scientific). The MS instrument was tuned with the PFOA standard (100 µg/L) using T direct infusion. The PFOA standard was used since it had less signal intensity than the PFOS. Two tune files were created using m/z precursor ion, (413 m/z) and its m/z product ion (369 m/z) for the FS and SIM methods and the MS/MS method, respectively. The spray voltage and current were set at 5kV and 5 µA, respectively with a capillary voltage of -25V. The capillary temperature was set at 275 ˚C, with a tube lens offset of -125V. PFOA and PFOS were analyzed using the MS/MS optimized earlier in HPLC-LCQ Classic-MS.

2.3.5 Blanks, quality control and calibration

In order to monitor for potential laboratory contamination, water blanks prepared using deionized water system were extracted and analyzed along with the field water samples and a laboratory spike. Methanol blanks were run between samples to monitor for instrumental contamination and any carry-over. PFOA and PFOS spiked water samples were prepared at 1, 10, and 100 ng/L levels to match the anticipated levels in aqueous field samples. These spiked water samples were extracted using the same procedure as the field water samples to check for any bias and determine the precision of this analytical method. All calibration curves were not forced through zero but maintained an $r^2 > 95$ %. Chromatographic peak area, height and S/N were used to determine the target analytes. Peak area was used to measure the optimal signal

intensities for quantification, which provided the most reliable response among the chromatographic response choices (i.e. signal-to-noise ratio and peak height). For calibration, PFOA and PFOS standards were initially checked for linearity from 50 ng/L to 1000 µg/L concentrations. A linear relationship ($r^2 \geq 0.97$) was established over the range of 50 ng/L to 50µg/L. In order to accurately measure the true levels of PFOA and PFOS in the field water samples, a second curve would be needed for concentrations above 50 µg/L.

2.3.6 Field water samples

The raw and treated water samples were collected by personnel from the NJ Department of Environmental Protection (DEP) in June and October 2008. Storm and tap water samples were collected by personnel from Rutgers University in January 2008 and October 2009, respectively. Raw and treated water samples collected from Fairlawn (FL) and Merchantville-Pennsauken (MP) were analyzed using the SPE-UPLC-MS/MS method described in this paper. A storm water sample from Kearny Marsh (KM) and tap water samples from Plainsboro (PB), Highland Park (HP) and Spotswood (SW) were analyzed using both the SPE-HPLC-MS/MS and direct UPLC-MS/MS methods described in this paper.

2.4 Results and Discussion

2.4.1 Optimization of SPE columns

Six different reversed phase SPE columns were selected based on previous PFC analysis.[5, 7-12, 14, 16-18] The PFOA and PFOS standards spiked in deionized water (n≥3) were used to measure extraction efficiencies of these columns (**Figure 2.1**). These results showed a wide range of 74 to 119 % recoveries for PFOA and PFOS. The

variation of extraction efficiencies for PFOA and PFOS may have occurred because of the interaction/affinity between the analytes and the stationary phase of the SPE sorbent, which generally depends upon the properties and mass of SPE sorbent as well as its pore size and volume.

The SPE recovery on these reversed phase SPE columns was good overall with a minimum of 74 % recovery for target analytes. However, the primary challenge was from a potential background contamination with the target analytes, which was likely to be responsible for the recoveries greater than 100%. Similarly, the background contamination of target analytes has been described in previous work.[7-11, 14, 19, 20] The sources of contamination were identified as PFOA/S used as reactants in the manufacture of many laboratory and instrumental materials including SPE liners, frits and the reagent solvents. In Weremiuk *et al.,* the blank concentrations of PFOA were reported ranged from 0.18 to 0.22 ng/L in deionized, Millipore-filtered, and tap water for a 500 mL water sample and PFOS was lower at a level of 0.05 ng/L.[9] Furthermore, the nature of the target analytes as anionic forms of perfluoroalkylcarboxylates and sulfonates (i.e., PFOA and PFOS) are very stable forms in water as well as in polar organic solvents. In this work, the blank contamination level using deionized water samples measured was as high as 1ng/L for PFOA.

PFC analysis using SPE methods is typically done using HLB columns. However, these results show that a wide selection of non-HLB columns is acceptable and that these non-HLB columns have greater extraction efficiency and are more economical. Overall, it was determined that the reversed phased SPE columns tested were all suitable for PFOA and PFOS analysis. Generally these cartridges had high recoveries, mean of 109

± 9 % ($\geq 96\%$) and 93 ± 9 % ($\geq 74\%$) for PFOA and PFOS across all SPE vendors studied. The ENVI 18 column was the cartridge of choice with good precision, recoveries near 100% (104 ± 2 % and 100 ± 9 % for PFOA and PFOS, respectively) and slightly lower background than the other vendors. Blank subtraction may be important with this method especially for samples at very low-concentration (<1 ng/L).

2.4.2 HPLC-MS optimizations

The reversed phase analytical columns generally demonstrated good recovery for analysis of PFCs.[7-12, 14-18, 20-22] Different types of the reversed phase analytical columns were examined in this work: RP C-8 (Zorax®), RP Amide (Discovery®), RP C-18 (Discovery®) and Envirosep PP (Phenomenex®). The RP C-18 column showed better peak resolution than the RP C-8. Trifunctional C-18 based Envirosep PP gave optimal separation based on peak shape and retention time (RT). Using the Envirosep PP column, the RTs of the target analytes were less than 4 min, while they eluted at 27 min with the RP C-18 column using the same flow rate.

The flow rate was optimized from flow rates of: 100, 150, and 200 µL/min. The mean total ion counts (TIC) for the target analytes were measured using SIM mode and it was determined that a slight improvement could be observed at the highest flow rate of 200 µL/min (1.4 fold increase) compared to the lowest flow rate of 100 µL/min.

The mobile phase composition was also an ammonium acetate buffer solution optimized in aqueous phase with methanol as an organic phase. The ammonium acetate salt was believed to be a good buffering agent and pH control was a key to suppression of adverse ionic interactions that can occur between silanols and these analytes. It was also a good ionizing agent, which helps to enhance the negative ESI detection as an electron

acceptor. **Figure 2.2** shows the chromatographic intensities of the PFOA at different mobile phase compositions of aqueous ammonium acetate buffer (2 mM) and methanol. The higher composition of the aqueous phase (90% A) with more ammonium acetate solution created the highest intensities among 90%, 70%, 50% and 40% aqueous (A). This result clearly demonstrated ammonium acetate in water may enhance ESI ionization efficiency. The ammonium acetate concentration and HPLC gradient were therefore adjusted to optimize ESI sensitivity and reduce analysis time. Among concentrations of 0, 2, 5, and 10 mM ammonium acetate, the 10 mM concentration was selected for producing the greatest signal intensities even at a lower initial aqueous composition of 40%A. At 40%A the most rapid elution of target analytes was achieved. The 10mM concentration of ammonium acetate with higher organic phase composition showed a relatively fast HPLC analysis profile with a greater ESI sensitivity for the target analytes. In addition, the HPLC gradient from 60 to 95% B gave an enhanced peak separation with a reduced total analysis time of 20 min. The current PFOA and PFOS method showed overall an agreement with previous work for the optimized HPLC conditions (e.g., HPLC column, elution solvent and run time).[23-27] The chromatographic separation of target analytes was not easily achieved due to their similar chemical structures and properties. Unfortunately, ammonium acetate salt caused a clogging problem so that a washing step with 100% water was required in this method.

2.4.3 UPLC-MS/MS adaptations

The method was also optimized using the UPLC system, which has not previously been used for PFC analysis. Several LC conditions were modified and resulted in substantial improvements in the method's high throughput and sensitivity. The HPLC

conditions with ammonium acetate in aqueous solution, showed increased ESI signals, but it generally required a continuous washing procedure to reduce the potential clogging problem in an HPLC system.   UPLC systems are very susceptible to clogging so the ammonium acetate (10 mM) was changed to aqueous formic acid for the UPLC method. The formic acid was a good alternative buffer because it made the target analytes available for apolar interactions with the stationary phase without clogging the HPLC system.   Furthermore, small decreases in pH levels from the addition of formic acid (i.e. 0.1% and 0.2%) in the aqueous phase did not affect the response factor for these target analytes. For UPLC, the formic acid (0.1%) was preferred because of a greater linearity and reproducibility, as the sensitivity did not suffer in the method. The flow rate was also modified in this UPLC method to100 µL/min as greater signal intensity was achieved without a loss of peak shape.

A UPLC type column with a particle size of 1.9 µm was used in place of the HPLC 5 µm. This UPLC method used less mobile phase solvent and reduced the total analysis time from 20 to 10 min. The sample volume was decreased by at least three times ($\leq$10 µL), which simultaneously reduced peak fronting and splitting effects. The method's high throughput allowed twice as many water samples to be analyzed in the same analysis time as compared to the previous HPLC method.

Blank contamination with PFCs was also described in previous studies.[8, 10, 14, 20] Flaherty et al. [20] demonstrated a potential source of contamination from the instrument itself. The injected sample of PFOA was separated from the contaminant PFOA using Hypercarb filters (Keystone Scientific). The authors suggested the HPLC components made of PTFE were possible contributors. Multiple authors also observed polar solvents

such as methanol contained these contaminants. The optimized UPLC method did not

suffer from instrument blank contamination. This was attributed to the use of ACN and

formic acid solution in water instead of methanol and ammonium acetate. It may also

have been due to a difference in some of the fittings/materials (i.e., stainless steel) used in

the UPLC instrumentation. This was verified by using both methanol and water blanks,

which were run after each sample and showed no PFOA/S peaks. The MS parameters

used for the HPLC separation were maintained in the UPLC/MS method as the analyte

elution order and MS fragmentation patterns were consistent across both separation

methods.

2.4.4 MS optimizations by FS, SIM and MS/MS

The MS methods were optimized using LCQ conditions for sensitive PFOA/S

analysis. Previously, the PFOA/S determination was often done using MS/MS

techniques; however, MS comparison and optimization were not clearly shown in

previous PFOA/S analysis. The optimization was performed to compare and validate for

the sensitive/selective determination of target analytes.   PFOA and PFOS calibration

curves were generated under different MS conditions including Full Scan (FS), Single

Ion Monitoring (SIM), and Tandem Mass Spectrometry (MS/MS) as shown in **Table 2.1**.

They were compared for their linear fit for PFOA/S using r^2. All MS methods showed a

good linear relationship $r^2 \geq 0.97$. The linear relationship ($r^2 = 0.97$) in MS/MS was

slightly lower than the r^2 (0.99) of SIM and FS due to the slightly higher variation with

the isolation of the precursor ion in MS/MS mode which has been previously described.[28]

The sensitivity for each MS method was then compared using the response factor (slope

of calibration) while, all HPLC- MS conditions remained the same. Among these MS

methods, the response factor for PFOA was the highest using the FS method followed by SIM and MS/MS methods. On the other hand, the response factor of the PFOS was the highest using MS/MS method followed by SIM and FS methods (**Figure 2.3**). The MS/MS method for PFOS was optimized using two different precursor/product programs. The conditions were at low (10%) and high (55%) collision energies. At high collision energy MS/MS, the product ion of PFOS was isolated while at low collision energy MS/MS, the precursor ion was further isolated for quantification. Both MS/MS methods reduced potential interferences and isolated ions of interest. The lower collision energy however for the precursor ion showed greater sensitivity than higher collision energy of MS/MS product ion by more than 2 orders of magnitude as well as maintained a good linearity ($r^2 = 0.97$). The MS/MS method for PFOS determination was optimized for the isolation method of precursor ion at a low collision energy based on this.

2.4.5 Sensitivity using LOD, LOQ and MDL

The sensitivity of the analytical method was measured using the limit of detection (LOD), the limit of quantification (LOQ) and method detection limit (MDL), for each MS method (i.e., FS, SIM and MS/MS) and the adopted UPLC-MS/MS method. The LOD was estimated using the IUPAC definition as the lowest concentration or mass of analyte that the analytical process can reliably detect: $LOD = k_D S_B/m$ where m is the slope of the calibration curve obtained via linear regression; k_D is the numerical factor chosen according to the 99% confidence level, and S_B is the standard deviation of blank measures.[30-33] The LOQ is not defined in IUPAC a publication[30-33]; however, it has been used to provide supplemental statistical separation of the blank measurement and true analyte signal distribution: $LOQ = k_Q S_B/m$.[34] For this work, the LOD and LOQ were

reported as the mass (*not* concentration) of analyte injected, because the chromatographic signal depends on the amounts as absolute quantities.[35] The S_B was calculated based on replicate measures (≥ 7) at the lowest level of analyte that the instrument can detect for a given procedure. The $k_D = 3$ for the LOD and $k_Q = 10$ for LOQ were used based on the IUPAC and ACS definitions.[30-34, 36-37]

Finally, MDLs in concentrations of PFOA/S were determined based on 1L water samples with the SPE pre-concentration. The LOD, LOQ and MDL of all three MS methods (FS, SIM, and MS/MS) and the adopted UPLC-MS/MS method were compared in **Table 2.2**. The LOD of PFOA was the lowest (3.1 pg) for the MS/MS method, which was smaller than for the FS (12.3 pg) and SIM (20.5 pg) methods, by factors of 4 and 7, respectively. This was because of a drastic reduction in noise levels in the MS/MS method, which created consistent signals, lowered S_B values and LOD. Similarly, the LOD for PFOS using the MS/MS method was lower. However, the LOD using the SIM method (8.1 pg) was only slightly lower than either the MS/MS method (9.1 pg) or the FS method (11.3 pg). The response factor (m) for the MS/MS method was the highest but it had the largest S_B, lowering the sensitivity of the MS/MS method. Again, the LOD is dependent upon both analytical response factor (m) and S_B as mentioned in the IUPAC definition.

While the UPLC-MS/MS method showed a similar sensitivity to previous methods for PFOA/S analysis, the sensitivity of the proposed UPLC-MS/MS method was compared with the HPLC-MS/MS method. Comparison between the HPLC-MS/MS method and UPLC-MS/MS method for both PFOA and PFOS detection/quantification showed that the UPLC-MS/MS method had substantially improved sensitivity. The

UPLC-MS/MS method had an LOD of 0.03 pg and 0.24 pg for PFOA and PFOS, respectively, which were 103 and 38 fold more sensitive than the HPLC-MS/MS method (**Table 2.2**). This LOD of the UPLC-MS/MS method was also close to the concentration that required no pre-concentration step to measure the concentration of PFOA/S in field water samples. Similarly, LOQ as a more reliable measure of the target analyte signal distinguished from background was also calculated for this work (**Table 2.2**). Overall, the MDL of these values corresponded to approximately 6 pg/L and 48 pg/L of PFOA and PFOS, respectively, which were far more sensitive than for previously available more sensitive methods.[23, 25, 29]

2.4.6 NJ Water sample analysis

PFOA and PFOS were detected and quantified in field water samples directly using the optimized UPLC-MS/MS method. PFOA and PFOS have been found at very low levels in drinking water samples collected in NJ.[3] It is therefore critical to define the specific lower limit of quantification, which accurately represents the true concentration of the target analytes in water samples. The standard calibration procedure was used rather than a labeled internal standard spike method because the trace impurities of the unlabelled analyte that were previously detected in the mass-labeled standard solution. This was a potential source of contamination of the target analytes that was eliminated in this study. Although only a small concentration ($\leq$1%) of the non labeled analyte was supposed to be present in the labeled PFOA; it has been suggested that the very low target analyte concentrations (ng/L) in the sample are comparable in magnitude to this small impurity.[22] Variation from sample to sample may occur because of its chemical properties.[8] Boulanger et al. had hypothesized that PFOSulfinate undergoes a hydrolysis-

like reaction in tap water where oxygen may react and generate greater PFOS levels then were present before treatment.[8] Matrix effects from the sample media were less of a concern in these drinking water samples so, it was not critical to use the internal standards like labeled PFOA which reduced the overall cost of the method significantly. Previously, water blanks were determined to contain target analytes above the LOD,[25] which were similarly observed in the HPLC-MS/MS method. Neither PFOA nor PFOS was detected in any of laboratory water (procedural) or methanol (instrumental) blanks. The water blanks run concurrently with field water samples showed no analyte contamination using the UPLC-MS/MS method.

For validation of direct UPLC-MS/MS method without the SPE sample preparation, PB, HP, SW, KM water samples were analyzed using both SPE-HPLC-MS/MS method and direct UPLC-MS/MS method (**Table 2.3**). For tap water of PB, HP and SW samples, only PFOA was presented measurable levels therefore, used to compare between two methods. In SPE-HPLC-MS/MS method, concentrations of PFOA were 12.6, 16.6 and 9.7 ng/L for PB, HP and SW samples, respectively. In direct UPLC-MS/MS method, concentrations of PFOA were 16.3, 28.0 and 12.2 ng/L for PB, HP and SW samples, respectively. Additionally, both PFOA and PFOS were identified and quantified in a storm water sample (KM) in both methods. The KM sample showed PFOA measuring 26.5 and 66.6 ng/L and PFOS measuring 718.5 µg/L and 723.6 ng/L in SPE-HPLC-MS/MS method and direct UPLC-MS/MS method, respectively.

Both methods, SPE-HPLC-MS/MS and direct UPLC-MS/MS gave similar measurement of PFOA and PFOS in NJ tap and storm water samples. One exception for PFOA in a storm water sample had the highest difference (2.5 fold), possibly from

analyte loss.   These results also showed an agreement but with a slightly higher measurement of PFCs using direct UPLC-MS/MS compared to the SPE-UPLC-MS/MS method.   This suggests a potential loss during SPE sample preparation for the HPLC-MS/MS method and may be another reason to avoid SPE if possible.   In addition, the UPLC-MS/MS method generated less signal background levels then the HPLC-MS/MS method. As a result, the integration of target peaks requiring more background subtraction may result in lower concentrations of analytes using the HPLC-MS/MS method. The direct UPLC-MS/MS method was validated against typical SPE-HPLC-MS/MS method using various types of field water samples. Overall, the difference of two methods was under a 95% limit of agreement with the confidence interval about mean difference of 48.92 and -20.03 using Bland-Altman analysis.   These results showed that UPLC-MS/MS method is far more sensitive than existing methods including the optimized HPLC-MS/MS method, which it allowed the direct analysis of PFOA and PFOS in field water samples.

Finally the UPLC-MS/MS method was also combined with SPE and applied to raw and treated water samples from two water systems in NJ (**Table 2.4**) to test the sensitivity of the method combination. In the FL and MP samples, PFOA and PFOS were found at levels from 3.9 to 46.2 ng/L and from 1.7 to 42.2 ng/L, respectively.   The lowest PFOA level detected in MP finished water was 3.9 ng/L, and the lowest PFOS level was detected in the same water sample, measured at 1.7 ng/L. Raw water samples generally contained more PFOA and PFOS than treated drinking water. Somewhat unexpectedly the finished water samples used for UPLC-MS/MS analysis had higher

levels of both than other tap water samples noted in **Table 2.4**. Based on these samples, some but not all of PFOA and PFOS were removed after treatment.

2.5 Conclusions

Due to trace levels of PFOA and PFOS being frequently detected in drinking water supplies including treated water collected throughout NJ, it was necessary to develop reliable methods that can quantify very low concentrations (ng/L). A sensitive method was optimized based on a SPE with reversed-phase chromatography and tandem mass spectrometry. An HPLC-MS/MS method was one of more sensitive but improved with the substitution of UPLC-MS/MS. This more sensitive method allowed direct measurement of PFOA and PFOS in water samples. Optimized conditions for PFOA and PFOS analysis were developed for this UPLC-MS/MS method, with sensitivity (~0.25pg injected) and fast analysis time (<10min). This work also compared and optimized HPLC-MS methods (i.e. FS, SIM, MS/MS) for PFOA and PFOS analysis. The UPLC-MS/MS showed two to three orders of magnitude more sensitivity than the HPLC-MS/MS for PFOA and PFOS analysis. This UPLC-MS/MS method was also sensitive enough to make a direct measurement of the PFOA and PFOS in field water samples significantly reducing time, analysis cost and potential sources of contamination or/and loss of target analytes during sample preparation steps. Three tap water samples were measured from 9.7 to 28.0 ng/L levels of PFOA using both HPLC-MS/MS and the direct UPLC-MS/MS method. Nine raw and treated water samples collected from NJ drinking water supplies were quantified using SPE-UPLC/MS/MS with a range of 1.7 to 46.2 ng/L for PFOA and PFOS. Based on these results, raw water samples were more likely to have detectable PFOA and PFOS concentrations than treated water samples. Finally, a

storm water sample had the highest levels of PFOA and PFOS, at 66.6 ng/L and 724 ng/L, respectively using this direct UPLC-MS/MS method demonstrating the ruggedness of the method. Overall, this UPLC-MS/MS method was validated against the HPLC-MS/MS method with a Bland-Altman analysis. Adding the UPLC created a method with high sensitivity that permits a direct analysis of PFOA and PFOS in drinking water samples.

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Figure 2.1 Extraction efficiencies (+%RSD) using popular commercial SPE cartridges
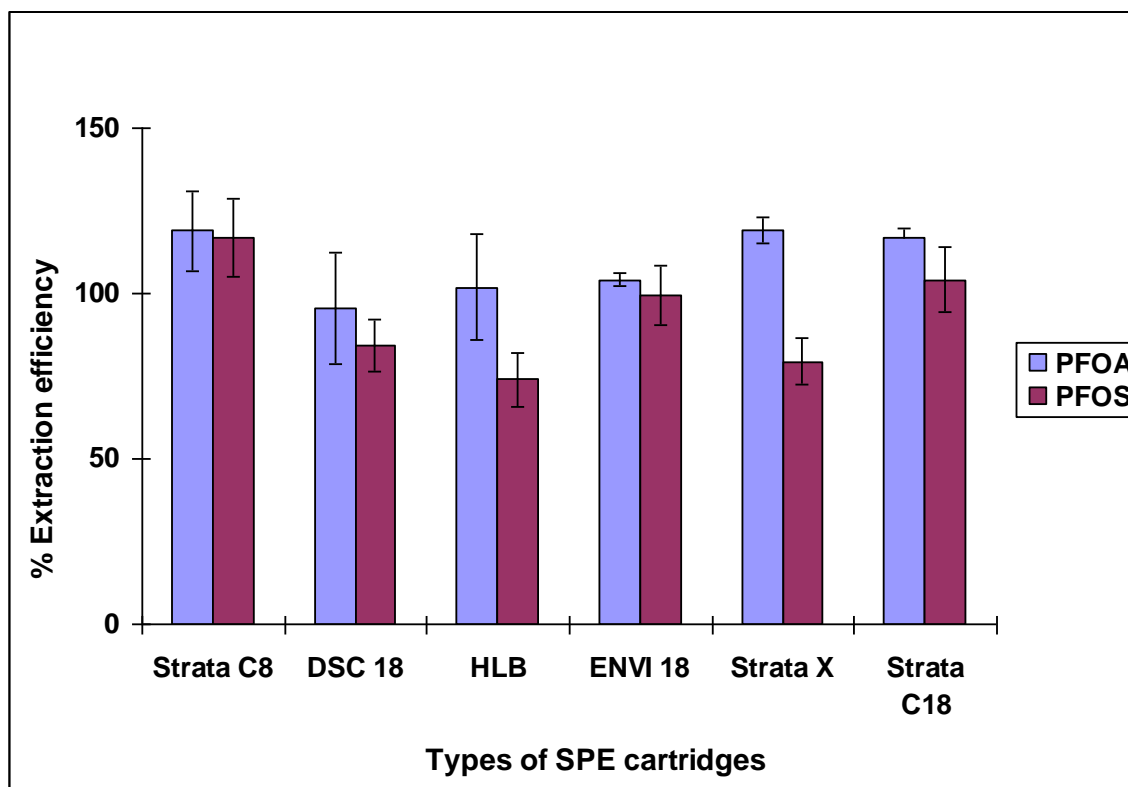
Figure 2.2 Extracted ion chromatogram demonstrating an increase in signal intensity with increased ammonium acetate (2mM) content
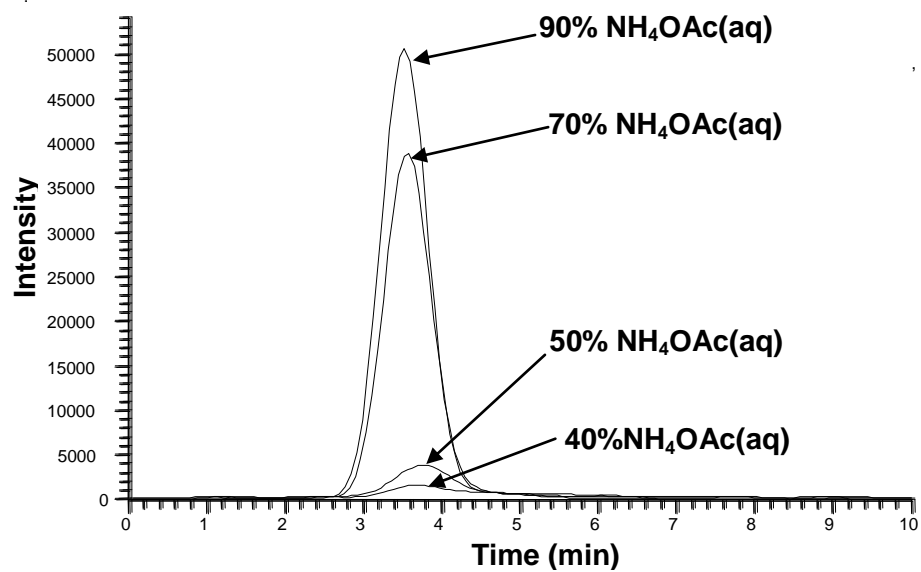
Figure 2.3 Comparison of PFOS response factors for different MS methods for concentration of 15 to 3000 pg
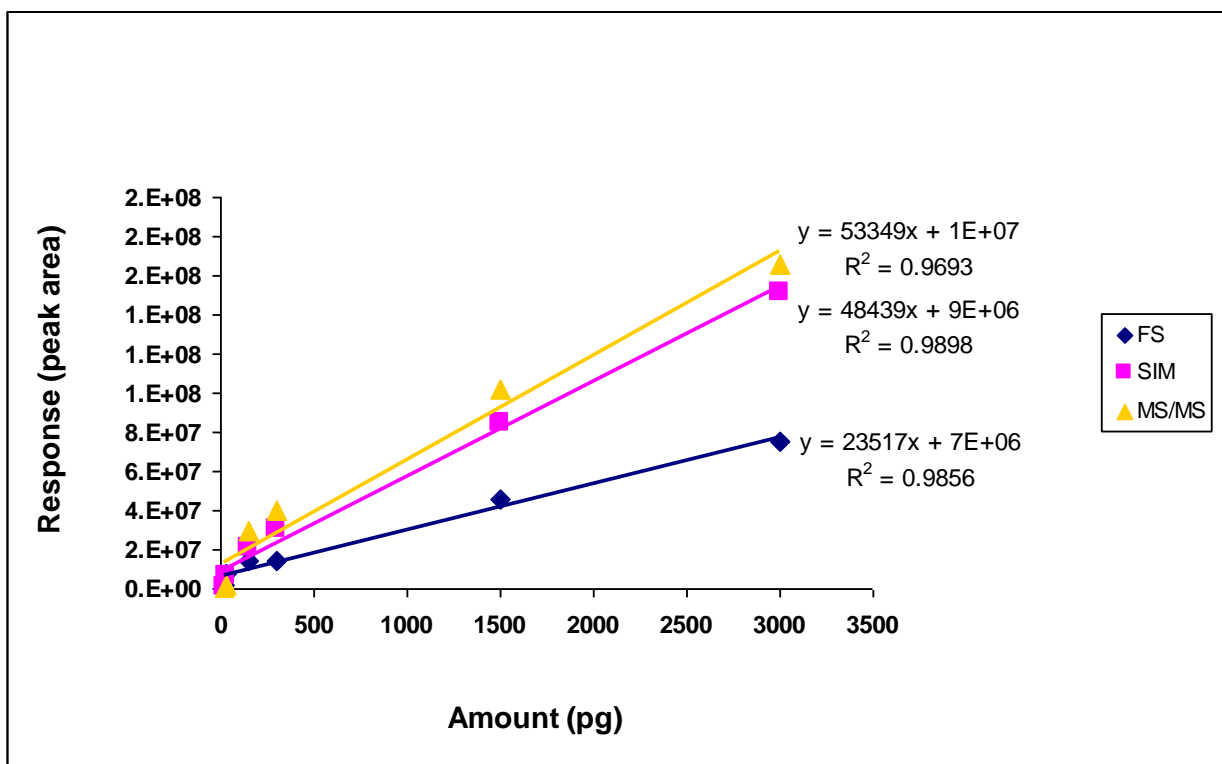
Table 2.1 PFOA and PFOS calibration for FS, SIM, MS/MS method

Linear Regression	PFOA			PFOS			
(y=mx+i)	FS	SIM	MS/MS	FS	SIM	MS/MS[a]	MS/MS[b]
m	8601	6460	6127	23517	48439	473	53349
i	72698	28802	-104540	6732930	8948243	34350	12309856
R^2	0.9996	0.9994	0.999	0.9856	0.9898	0.9925	0.9693

[a] MS/MS with high collision energy (55% CE): precursor ion $\xrightarrow{MS/MS}$ product ion

[b] Optimized MS/MS with low collision energy (10% CE): precursor ion $\xrightarrow{MS/MS}$ precursor ion

Table 2.2 Limit of detection (LOD), limit of quantification (LOQ) and method detection limit (MDL) for PFOA and PFOS

Sensitivity	*LOD (pg)*		*LOQ (pg)*		*MDL^a (pg/L)*	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
HPLC-FS	12.3	11.3	41	37.6	410	377
HPLC-SIM	20.5	8.1	68.4	27	683	270
HPLC-MS/MS	3.1	9.1	10.4	30.3	103	303
UPLC-MS/MS	0.03	0.24	0.1	0.80	6	48

[a] MDL in water sample (1L)

Table 2.3 Method comparison using field water samples

Sample location	Sample Type	Direct UPLC-MS/MS		SPE-HPLC-MS/MS	
		PFOA	PFOS	PFOA	PFOS
KM	Storm water	66.6	723.6	26.5	718.5
PB	Tap water	16.3	0.0	12.6	0.0
HP	Tap water	28.0	0.0	16.6	0.0
SW	Tap water	12.2	0.0	9.7	0.0

Table 2.4 Field Water Samples using SPE-UPLC-MS/MS

Sampling Site	Sample Type	PFOA (ng/L)	PFOS (ng/L)
FL	Finished-Air Stripper/Chlorination)	19.5	30.0
	Raw Well#2	35.9	12.2
	Raw Well#7	31.0	18.6
	Raw Well#9	25.5	15.8
	Raw Well#17	46.2	25.3
	Raw Combined Wells	41.9	42.2
MP	Raw Water	4.8	2.6
	Finished-Air Stripper	3.9	1.7
	Finished-Air Stripper/Chlorination	4.2	2.0

Chapter 3 - Method Development for Measurement of 20 Unregulated Compounds (Pharmaceuticals, Hormones, Perfluorinated Compounds) in Drinking Water Samples using LC-MS/MS in a rapid switching of ESI modes

3.1 Abstract

Prescription and non-prescription pharmaceuticals, antibiotics, steroid hormones, and perflurorinated compounds (PFCs) are continuously present but, not regularly monitored in drinking water sources. In this work, a liquid chromatography–tandem mass spectrometry (LC-MS/MS) screening method was developed for twenty emerging unregulated compounds in raw and treated drinking water. Various therapeutic classes of pharmaceuticals, antibiotics, steroid hormones were mainly selected based on their frequency of occurrence in a previous nationwide United States Geological Survey (USGS) investigation. The two most abundant PFCs perfluorooctanoic acid and perfluorooctanesulfonic acid were also monitored based on their adverse health effects, as they were determined to be "likely carcinogens" by EPA. The analytical method has focused on the method's robustness and high throughput required for a drinking water analysis. A solid phase extraction (SPE) sample preparation and LC-MS/MS methods were developed and optimized. A mixed mode-polymeric SPE sorbent (Phenomenex, Torrance, CA) extraction was performed with a combination of acid and base modified solvent elution strategy. The mean percent recovery of 20 target compounds was 89% except for cimetidine. The LC-MS/MS method utilizes a novel technique of a rapid switching between positive and negative ESI modes in one LC-MS/MS analysis. Although this ionization switching technique could decrease the sensitivity, the response showed an enhanced selectivity and sensitivity for the 20 target compounds. The method detection limits of 20 target compounds ranged from 0.2 to 3.6 x10^2 ng/L with a median

MDL value of 1.2 ng/L for the overall method sensitivity, which is adequate to detect and identify these environmental contaminants at ambient levels. The method was applied to various field samples of tap, well and storm water and matrix effects were not encountered. There were detectable levels of eleven target compounds with a mean frequency of 80%. The highest concentration of target compound detected was the steroid hormone estradiol, followed by antidiabetic metformin in these field water samples. Overall, the maximum concentration of other measured target compounds (acetaminophen, albuterol, *L*-cotinine, cimetidine, ranitidine, caffeine, PFOA and PFOS) was below 1µg/L.

3.2 Introduction

In the 21st century a continuously growing human population with a limited supply of freshwater based on supply and demand, drinking water resources may still be one of the most vital environmental issues. The U.S. Geological Survey conducted the first nationwide investigation of the occurrence of pharmaceuticals, hormones, and other organic wastewater contaminants (OWCs) in U.S. water resources.[1] The study result showed 80% of 139 streams across 30 states had detectable quantities of OWCs during 1999 and 2000.[1] Most of the 95 OWCs studied did not have standard guidelines for allowable quantities in drinking water. For this study, a sensitive analytical method was developed to measure the concentrations of some of these unregulated compounds in drinking water samples. A method measuring 20 pharmaceuticals, antibiotics, steroid hormones and perfluorinated compounds (PFCs) was created with analytes selected based on the frequency of their detection and human health concerns.[1, 2]

Previous studies similarly included some of the same prescription pharmaceuticals based on data estimating the number of prescription written per year.[3, 4] The most frequently found nonprescription compounds of acetaminophen, caffeine, and cotinine in surface- and ground- water samples[3] were included in the list of target compounds. In addition, the most abundant PFCs, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), were also included because of their prevalence in many NJ water systems[5] and because of this classification as a "likely carcinogen".[6] Estradiol and other steroid hormones are prevalent and have been growing concerns as endocrine disrupting chemicals (EDCs), which have the ability to interfere with normal functioning of the endocrine system.[7, 8] Overall, 20 compounds from various therapeutic classes of prescription and non-prescription pharmaceuticals, antibiotic, steroid hormones, and PFCs chosen as new, emerging contaminants in drinking water.[9] A list of the target compounds, their use, chemical formulas, and structures was included in ***Chapter 1, Table 1.1***.

With a broad range of 20 target analytes of different physiochemical properties, development of the analytical method was challenging and requiring compromised conditions to provide selectivity and sensitivity required for drinking water samples. This work focused on the development/optimization of a single liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that simultaneously separated and quantified these 20 of the most prevalent unregulated OWCs. The optimized method used rapid switching between positive and negative ESI modes during the LC-MS/MS analysis. The sensitivity of the method ranged from sub ng/L to hundreds of ng/L for all target analytes in drinking water samples.

3.3 Experimental

3.3.1 Materials

Pharmaceutical standards of acetaminophen, albuterol, caffeine, cimetidine, codeine, *L*-cotinine, *cis*-diltiazem, fluoxetine, gemfibrozil, ibuprofen, metformin, ranitidine, methylphenidate, testosterone, and warfarin in methanol were all purchased from Alltech Associates, Inc. (Deerfield, IL). Chlortetracycline (CTC-HCl), estradiol and progesterone were purchased in powder form from Sigma-Aldrich (St. Louis, MO) and dissolved in methanol. Perfluorooctanoic acid (PFOA, purity 98.6%) and perfluorooctanesulfonic acid (PFOS, purity 98.4%) were obtained from AccuStandard, Inc. (New Haven, CT). Deionized water was produced by a Millipore workstation (Quantum® EX) and used for laboratory blanks, spiked samples, and SPE solvent. HPLC-grade water and methanol (Burdick and Jackson High Purity Solvents) were purchased from VWR and used as HPLC solvents. All HPLC mobile phase solvents were filtered and degassed through 47 mm diameter, 0.45µm pore size nylon filter membranes (Osmonics, USA) prior to use. Five molar ammonium acetate (0.2 µm filtered) from Ambion Inc. (Austin, TX) and glacial acetic acid (>99.7 %) from EM Science Inc. (Gibbstown, NJ) were diluted in HPLC water and used for HPLC solvents.

3.3.2 Solid-phase extraction

Prior to the SPE steps, all water samples were collected using 1L amber glass bottles from I-CHEM certified™ and stored at 4°C no longer than a maximum of 2 days before sample preparation. For pre-concentration and sample clean-up, a mixture of target analytes were isolated by continuous flow- solid-phase extraction (CF-SPE) using a reversed phased polymer, Strata™ X mixed mode cartridges (200 mg, 6 mL;

Phenomenex, Torrance, CA). All CF-SPE procedures were performed under a SPE vacuum manifold using a Visiprep™ DL disposable liner (Supelco Inc. Bellefonte PA). Up to twelve SPE columns were connected to individual disposable liners in the SPE manifold that was directly linked to a vacuum supply by tubing. The columns were initially conditioned with 5 mL methanol followed by 5 mL of deionized water. After conditioning, the columns were not allowed to dry. Water samples (1 L) were loaded onto these conditioned columns at a flow rate of approximately 4 mL/min. Once all of the water from the sample bottles was loaded onto the SPE columns, the columns were completely dried under a vacuum stream for approximately 30 min. The elution solvent system was optimized and the optimal elution solvent steps were as follows: 2 x 3 mL of 100% methanol followed by 2 x 2mL of methanol acidified with acetic acid (pH 3.8) used to collect acidic and neutral compounds. Then basic analytes were eluted off the column with 2 x 2 mL of 5% NH_4OH in MeOH (*v: v,* pH 8). The solvent was evaporated and the extracted eluant was pre-concentrated under a gentle vacuum in the Visprep manifold until a final volume of approximately 200 μL, was achieved. Finally, 800 μL of HPLC graded water was added to make a final volume of 1 mL prior to HPLC analysis.

3.3.3 Liquid chromatography (LC)

LC analysis was performed using a Thermo Separation Products (TSP) P4000 Quaternary gradient HPLC pump system with a TSP AS3000 autosampler. Separation was done on a reversed phase Synergi Polar C-18 analytical column (Phenomenex) of 4-μm particle size, 150-mm length, 2.1-mm i.d. with a quaternary solvent system: 100% water (mobile phase A), 100% methanol (mobile phase B), 10 mM ammonium acetate (mobile phase C) and 0.87 M acetic acid (mobile phase D). Initially, mixtures of

methanol, acetonitrile and water with formic acid as well as ammonium acetate buffer

solution were examined in varying ratios of mobile phase compositions. This quaternary

solvent composition was determined to be optimal with an HPLC gradient from 10% B

(initial condition) for 1min., increased linearly to 95% B at 35 min., changed to 98% B at

37 min., maintained at 98% B for 4 min, decreased back to 10% B in 2 min., and re-

equilibrated at 10% B for 2 min while mobile phase C and D remained constant at 1%

throughout the run.   The flow rates were changed from 200 μl/min (up to 95% B) to 180

μl/min (at 98%B) then back to 200 μl/min (at equilibration). The total analysis time was

45 min and the injected volume was 25 μL.

3.3.4 Mass spectrometry (MS)

Positive ion mode: MS analysis was performed using a Thermo Finnigan Deca

LCQ-Ion Trap Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped

with an electrospray interface operated in positive and negative modes. An ESI mode

(positive vs. negative) for all analytes was optimized with respect to signal intensity prior

to the development of the method using polarity switching. The tune files were created

using LCQ Tune Plus software (Thermo Fisher Scientific) using a mixture of 20

standards in methanol at 1μg/mL. The typical operation conditions for the analysis were

as follows: ion spray voltage, 5000V; capillary voltage, 19V; tube lens offset, 50V;

multipole 1 offset, -7V; lens voltage, -28V; multipole 2 offset, -9.5V; multipole RF

amplitude, 400V; entrance lens, -40V; capillary temperature, 180°C; sheath gas flow,

0.96 L/min (65arb). Distilled N_2 (99.999% purity grade) served as collision, auxillary

and sheath gases.

Negative ion mode: All conditions are the same as above except: capillary voltage, -4V; tube lens offset, -60V; multipole 1 offset, 9V; lens voltage, 20V; multipole 2 offset, 12.5V; multipole RF amplitude, 400V; entrance lens, 90V; capillary temperature, 180°C; sheath gas flow, 0.96 L/min (65arb). Distilled N_2 (99.999% purity grade) served as collision, auxillary and sheath gases

Instrument control, data acquisition and evaluation were done with Xcalibur software (Thermo Fisher Scientific). Selected reaction monitoring (SRM) experiments were carried out to obtain a maximum sensitivity/selectivity for detection of each target analyte. Due to the broad range of compounds of interest, compromised parameters were selected to benefit the majority of analytes. The values of the typical SRM parameters in this method were as follows: % collision energy (% CE), 35%; isolation width, 3m/z; scan time, 2μscans; maximum injection time, 400 milliseconds; automatic gain control (AGC) value, $2x10^7$; typical activation energy (Q) and time, 0.25 and 30 msec. The MS/MS transitions for each analyte were viewed in MS/MS full scan mode prior to determination of the SRM condition for each target analyte. The most abundant SRM transition for each target analyte was selected for quantification. The precursor and product ions and their fragmentation patterns (i.e. relative ratios) as well as the % CEs for all target analytes were optimized and these results will be discussed in *Results and Discussion* (*section 3.4.2.3*).

3.3.5 Validation and application of the method

The % recovery and precision (%RSD) of the SPE method were carried out using a mixture of the 20 target compounds spiked in triplicate at 200 ng/L. Calibration curves were created using at least five concentration levels from 1μg/L to 200 μg/L. Intergraded

peak areas using SRM transitions of the target analytes were used to construct these

calibration curves. The method detection limit (MDL) was calculated for both the

analytical method (without a pre-concentration step) and in drinking water samples (1L)

with an SPE pre-concentration. They were calculated using replicates (n=7) of the lowest

concentration of each analyte. The three main confirmation criteria applied to each target

analyte in field water samples were 1) their retention times/order of elution, 2) accurate

mass, and 3) fragmentation pattern. The optimized method was applied to tap, well (raw

and treated) and storm water samples from domestic households and towns in New Jersey.

All samples were analyzed for the presence of the 20 target analytes on the same day to

avoid interday variation.

3.3.6 Statistical analysis

Analysis of variance (ANOVA) was performed to check for factorial

combinations of elution methods using three solvents (acidified MeOH, Acetone and

dichloromethane) with all target compounds. Cimetidine was not included because it is

not maintained as an analyte and therefore its properties could not be effectively

measured. This statistical test was performed under *ANOVA: Two-Factor with*

Replication in Excel™ software. Triplicates per samples were used with alpha (α) =0.05.

ANOVA was performed to check for significant difference in elution conditions using

acidic MeOH and basic MeOH ($CH_3COOH\text{-}NH_4OH$ vs. $CH_3COOH\text{-}DryNH4OH$ vs.

$CH_3COOH\text{-}NH_4OAc$ vs. $CH_3COOH\text{-}DryNH_4OAc$). This test was also performed under

ANOVA: Single-Factor in Excel™ software. The null hypothesis was that all of the

extraction efficiency means of four groups (elution conditions) were equal and alpha (α)

=0.05 was used for its statistical criteria.

3. 4 Results and Discussion

3.4.1 SPE Sample preparation

The conventional sample preparation method, solid phase extraction (SPE), was used mainly to clean-up and pre-concentrate the target compounds in environmental aqueous samples. The SPE method was preferred over a liquid-liquid extraction, solid phase microextraction, liquid-phase microextraction and lipophilization because it demonstrated better extraction efficiencies with a number of target compounds as well as higher precision.[10, 11] The simple SPE method was optimized for a higher recovery of the 20 target compounds. The four key areas for optimization of this method were as follows: 1) simple and effective SPE using the mixed mode SPE sorbent; 2) comparison/optimization of current elution solvents: MeOH (acidified), DCM, and Acetone; 3) simultaneous extraction of acidic, basic and neutral compounds using acetic acids, NH_4OAc, NH_4OH additives; 4) optimal sample diluents of water/methanol (4:1=v:v). In previously reported methods, pH was often adjusted to preserve environmental water samples (e.g. river and surface water) at a low (or high) pH prior to SPE steps and to avoid potential microbial degradation and loss. [12, 13] Some pharmaceuticals including acetaminophen, fluoxetine, testosterone, and progesterone showed a high recovery from SPE at pH 2.[12, 14] Martínez Bueno et al.[15], however, showed a higher pH level of 8 to be optimal for the majority of pharmaceuticals and related contaminants, while Conley et al. [16] found pH 7 to be optimal. In addition, the phenolic compounds and steroids (estradiol) showed no effect on extraction recovery between 4.15 and 7.96, but a further increase in pH caused a reduction in the extraction efficiency.[17] It was therefore difficult to determine one pH level that would satisfy the

broad range of acidic to basic target compounds.   Instead the pH adjustment was applied during the elution step using acetic acid and ammonium hydroxide or ammonium acetate solutions since the pKa of different compounds (acidic, neutral, and basic) can be used to isolate each separately within their optimal pH environment. This is discussed further below.

1) Simple and effective SPE method

Using a mixed mode-reversed phase SPE sorbent gave greater recoveries for various pharmaceuticals, as was also shown in previous work.[18, 19, 20] However Strata-X (Phenomenex) was preferred over commonly used Oasis HLB (Waters) as a good alternative and inexpensive SPE cartridge of choice considering only few studies showed its applicability in pharmaceuticals analysis.[13] The Strata X cartridges with a modified styrene divinylbenzene polymeric surface had hydrophilic, hydrophobic and pi-pi retention mechanisms, which allowed screening a broad range of acidic, basic and neutral compounds.   In addition, a strong cation-exchange mixed mode polymeric cartridge (Strata-X-C by Phenomenex and Oasis MCX by Waters) previously showed a high recovery of pharmaceuticals after derivization and/or pH adjustment prior to SPE steps.[14, 21, 22, 23] SPE procedures using the mixed mode-polymeric phase SPE sorbent were simple and quick, eliminating the derivatization or pH adjustment required prior to and/or during SPE steps. This SPE method still showed good recoveries for both polar and non-polar compounds as well as aromatic compounds with increasing selectivity and sensitivity. The extraction efficiencies are discussed below.

2) Comparison/optimization of current elution solvents: MeOH (acidified), DCM, and acetone

Methanol as an elution solvent has primarily been used because it gave good

extraction efficiency with minimal elution volume in SPE for various pharmaceuticals.[13,

15] In order to increase the number of target compounds to be extracted in the current SPE

method, more than one elution solvent was used. Three of the more effective elution

solvents used in previous studies,[4, 13, 24, 25] acidified methanol, dichloromethane, and

acetone were compared for enhanced extraction efficiencies of these target analytes and

Figure 3.1 shows the % recoveries relative to molecular weight (MW) of the 20 analytes

from smallest to largest. Acidified methanol was prepared by adding acetic acid to a pH

3.8 to improve the extraction efficiency. This phenomenon has also been reported in the

literature by Ramirez *et al.*[4] and Cahill *et al.*[3], which showed enhanced extraction

efficiencies of a greater number of pharmaceuticals at the acidic condition of pH 3.8 to 4.

In this result, acidified methanol as an elution solvent gave the highest extraction

efficiencies while DCM gave the lowest. The p-value for compounds (sample) using the

replicate data was less than alpha (3.4e-81<.05), so we reject the null hypothesis that the

means of extraction efficiencies of compounds are the same. The p-value for elution

methods (columns) was less that alpha (2.9e-6<.05), so we reject the null hypothesis that

the means of extraction efficiencies of elution methods (acidified MeOH, Acetone,

DCM) are the same. In addition, p-value for the combination (interaction) of compounds

and extraction methods was less than alpha (1.7e-13<.05), so we reject the null

hypothesis and can say that the effectiveness of the compound extraction efficiency is not

the same for the methods (acidified MeOH, Acetone, and DCM). Overall, the null

hypothesis did not hold that means are the same based on ANOVA (see **Table 3.1**).

All % recoveries of target compounds except for cimetidine and ranitidine (<20%)

exceeded 60% in acidified MeOH. Poor recoveries for cimetidine and ranitidine were also reported in previous studies by Cahill *et al.*[3] Therefore recovery methods for these two pharmaceutical compounds (beta 2 histamine (H2) receptor antagonists) were further developed in the following optimization.

3) Simultaneous extraction of acidic, basic and neutral compounds using acetic acid, NH_4OAc, NH_4OH additives

While using an acidic elution solvent produced higher recoveries for the majority of target analytes, basic elution solvents such as ammonium acetate (NH_4OAc) and ammonium hydroxide (NH_4OH) were studied to improve percent recoveries of basic compounds mainly cimetidine (pKa 7.1) and ranitidine (pKa 8.2). The percent recoveries using NH_4OH as an elution solvent were generally higher than NH_4OAc when a list of target compounds was compared side by side (see **Table 3.2**). More noticeably, ≥ 89 % recoveries of ranitidine (≤ 4%RSD) were observed using basic elution solvents of both, NH_4OH and NH_4OAc solution. Unfortunately, % recoveries of cimetidine did not improved drastically and the highest % recovery was at 50 ± 11 % using NH_4OH solution. In addition, chlortetracycline (CTC) was not effectively extracted in all elution conditions except for NH_4OH extraction after an elution of acidified methanol ($MeOH^+$). In addition, no replicate analysis of CTC was reported due to a systemic error during LC-MS acquisition. Overall, no drastic loss of measured percent recoveries for the majority of target compounds was observed when acidic solvent elution was used followed by basic solvent elution. Stability of target compounds was checked with basic solvent extracts of both, NH_4OH and NH_4OAc solution by evaporating them to dryness vs. a 200

µL final solvent extract volume. As shown in **Table 3.2**, increased percent recoveries of

target compounds including metformin, *L*-cotinine, ibuprofen, gemfibrozil, cimetidine,

codeine, warfarin, fluoxetine, ranitidine, progesterone, PFOA, and *cis*-dilitizem were

observed in 200 µL solvent extracts. These showed losses of target compounds when a

sample extract was evaporated to dryness, suggesting the 200 µL solvent extraction was

preferred. Since differences of mean extraction efficiencies are statistically significant

with the ANOVA test resulting F = 3.80 and the critical F = 2.73 at a critical value (α) of

0.05 (see **Table 3.3**), the mean extraction efficiency of $CH_3COOH\text{-}NH_4OH$ (89%) was

therefore higher than that of either $CH_3COOH\text{-}DryNH_4OH$ (81%), $CH_3COOH\text{-}NH_4OAc$

(66%) or $CH_3COOH\text{-}DryNH_4OAc$ (63%). Based on these results, the SPE elution

solvent system was re-optimized for acidic elution with Acidified MeOH followed by

basic methanol using NH_4OH with higher recoveries (>70%) for all target compounds

except for cimetidine (50 ±11 %).   .

4) Optimal sample diluents of water/methanol (4:1=*v: v*)

Prior to HPLC injection, the sample extracts were diluted in solvent matrix

comparable to each initial mobile phase composition. Different percentages of MeOH (i.e.

100, 50, 20 % MeOH) were tested during this optimization. **Figure 3.2** shows the

chromatographic behavior of the earlier eluting compounds of cimetidine (top) and

codeine (bottom) that were greatly affected by the composition of sample diluents. Poor

peak separation and resolution occurred when the sample diluents were substantially less

polar than its initial mobile phase composition (i.e. 90% aqueous). The optimized

diluents were prepared in 80% aqueous and 20% methanol (*v: v*) prior to HPLC injection.

3.4.2 HPLC-MS/MS method

The HPLC-MS method was optimized to identify and quantify the mixture of 20 target analytes. Firstly, the method was required to adequately separate the 20 target analytes simultaneously in one LC-MS/MS run. The method conditions including HPLC, ESI and MS parameters were optimized for these target analytes. The identification of each target analyte was done using the chromatographic retention time (RT), the MS of target ions and the fragmentation pattern. Good separation was a key in the method's success. Greater chromatographic resolution allowed for improved signal intensities of these target analytes because enough dwell time was spent on each transition without signal degradation. Some compounds including ranitidine and progesterone have equivalent precursor ions (i.e., 315 m/z) but are separated chromatographically. HPLC-MS conditions were optimized as follows: 1) selection of HPLC columns, 2) characteristics of mobile phase, 3) development/optimization of selective reaction monitoring (SRM) transitions for all target analytes, and 4) development of ESI-ITMS method for rapid polarity switching.

3.4.2.1 Selection of HPLC column

Based on previous studies reversed phase C-18 columns were the most frequently used HPLC column for these target analytes. Initially, conventional silica based C-18 analytical columns (i.e., Luna (2) C18, Phenomenex) were tested and demonstrated poor separation for metformin, ranitidine, albuterol, cimetidine, codeine, and *L*-cotidine: all were eluting at approximately the same retention time (≈2min). Similarly, C-16 and C-8 analytical columns also showed poor resolution for these compounds. Various factors

including the composition of the mobile phase and pH levels were also examined; however, no significant improvement in the chromatographic separation was obtained primarily due to their similar physicochemical properties. Based on these results, typical hydrophobic interactions between these target analytes and alkyl groups on the stationary phase of C-18, C-16 and C-8 columns were not exclusive enough for adequate isolation and separation of target analytes. Instead polar and π-π interactions between a phenyl group and the aromatic rings of the analytes were most favorable in the chromatograph separation for the majority of target compounds.   The pentafluorophenylpropyl phase (HS F5) column and the ether linked phenyl phase column with polar endcapping (Synergi Polar-RP) were examined and compared for the isolation and separation of these analytes. As a result, the Synergi Polar-RP column showed substantially less co-elution between target compounds and the optimal separation/isolation due to its effectiveness in polarity and aromatic selectivity.

3.4.2.2 Mobile phase optimization

The interaction of mobile phases and LC gradients were optimized to improve LC separation and detection sensitivity for the LC-MS method.   Methanol was preferred over acetonitrile as an elution solvent (organic phase) because it promoted π-π interactions between the aromatic rings in these analytes and the phenyl column.[26] A wide gradient from 10% to <100% methanol gave a good chromatographic separation due to the wide range of polarity in the target compounds. Ranitidine and progesterone, having the same precursor MS [315 m/z] were successfully isolated based on their RTs and SRM transition as shown in **Figure 3.3.** Initially, formic acid (0.1%) was added to the aqueous solvent to help retention between the column's stationary phase and analytes.

It is a good ionizing agent acting as an electron donor. Formic acid with enhanced

ionization efficiency was previously observed in ESI for both positive and negative

modes.[4, 27] However, it introduced a limitation on the measuring both positively and

negatively charged analytes. With formic acid, the acidic pharmaceutical compounds,

ibuprofen and gemfibrozil, were not easily ionized in negative mode and were more

effectively ionized in positive mode, described as "wrong-way-round ionization".[28]

Previously, the choices of mobile-phase additives were studied for ionization

efficiency in LC-MS.[28] The basic solution of triethylamine is often preferred in negative

ionization mode however, it was believed to suppress ionization of other compounds in

positive mode. On the other hand, trifluoroacetic acid as an ion-pairing agent may be

useful in positive ion mode but may completely suppress ionization in the negative ion

mode. With increasing pH, ammonium hydroxide was often suitable however, not in

negative ionization mode. The selection of mobile phase additives was important in

ionization efficiency but challenging when trying to enhance both positive and negative

ionization modes for protonated and deprotonated ions.

Finally, two types of additives of ammonium acetate (10mM NH_4OAc) and acetic

acid (0.87M) were introduced and showed the most enhancements for both positively and

negatively charged analytes. The ammonium acetate facilitated the analytes'

deprotonation and facilitated reproducible retention times with lower signal suppression

than other buffers.[29] On the other hand, acetic acid enhanced other analytes' protonation

and still increased negative ESI response.[30] Particularly, acetic acid improved

chromatographic peak resolution more than formic acid and other acids (i.e., formic acid

(1%, v/v), phosphoric acid (5%, v/v)), which was also observed by C. Nebot et al.[24] With

a quaternary solvent system, a HPLC gradient condition was optimized to use both additives of NH_4OAc and acetic acid to simultaneously extract positively and negatively charged analytes in one run. This will also greatly promote the rapid polarity switching, which will be discussed in *section 3.4.2.4*. As a result, all negatively charged analytes gemfibrozil, ibuprofen, PFOA, PFOS, and chlortetracycline were successfully detected together with the other 15 positively charged analytes in one method.

3.4.2.3 Development/optimization of MS/MS method

Using the aforementioned HPLC optimization, 20 target analytes were chromatographically separated and identified using their MS/MS transitions for confirmation. **Table 3.4** provided the optimized ESI-MS/MS conditions with precursor and product ions for each target analyte. The mean and median collision energy (%) were 36 and 35, respectively for these target analytes. The isolation width was optimized to $\pm$ 1.5 m/z for each quantification ion. For ibuprofen and PFOS, the precursor ion was further isolated using MS/MS with $\leq 10\%$ collision energy due to its unstable fragmentation pattern at any collision energy > 10%. Previous work has shown the major product ions of 161 m/z and 80m/z for ibuprofen and PFOS, respectively.[4, 24, 31] The ratios and signal intensities of fragments were slightly varied for compounds (e.g. codeine) with their potential stereoisomer. However, the most abundant product ions in the MS/MS transition remained identical and were used for quantification purposes. This intensive mass analysis was done to improve both selectively and sensitively in detection of target ions. Initially, multiple single ion monitoring (SIM) scans were used for each target ion to determine their retention times. Once all target ions were located on the chromatogram, their product ions were viewed using full scan type MS/MS to determine

their fragmentation pattern. Finally, the most abundant product ions were chosen for a selected reaction monitoring (SRM) scan. The total ion counts (TICs) were generally lower in SRM compared to either full scan or SIM. The relative response factors (intergraded area/mass), however was increased extensively by reducing noise levels and therefore background detected in SRM, which drastically improved the sensitivity of the method. **Figure 3.3** shows a direct comparison of SIM (top) and SRM (middle and bottom) methods for progesterone and ranitidine compounds with the equivalent precursor ions [315 m/z]. This gave an enhanced peak resolution and signal intensities for these analytes using the SRM method. The SRM transitions confirmed the target analytes by their fragmentation pattern when compared to a direct infusion of the standard and were necessary for the analytes with the equivalent precursor ion. This SRM transition was also a good validation tool for identifying a presence of shifted and/or carry-over peaks by inadequate HPLC conditions. In addition, the degradation products of cimetidine and ranitidine were observed using a Data Dependent scan of MS/MS created for their own SRM transitions for the quantitative analysis

3.4.2.4 Development of ESI-ITMS method for rapid polarity switching

Rapid switching between positive and negative ESI modes was developed to simultaneously detect both groups of positively and negatively charged analytes. The variety of target analytes necessitated this detection method. The requirement for both proton donor and acceptor (i.e., acetic acid and ammonium acetate) of ionizing agents was achieved during the HPLC optimization. ITMS conditions using a scan time of 2 μs/scan and maximum injection time of 400 milliseconds provided enough dwell times for each scan event. The compromised conditions chosen provided enough sensitivity

for all of these target analytes. The use of multiple segments (periods) and scan events was one of the keys in improving signal intensities.   The use of more than six segments was chosen based on the order of LC elution and the separation of compounds. The greater number of segments enhanced the selectivity since each segment had its own scan events for target analytes.   Each segment consisting of 2-4 target analytes also had its own ESI-ITMS tune file created to be optimal for the individual segment. According to the manufacture's recommendation, a tune file was tuned in both positive and negative ESI modes so as to optimally switch between positively and negatively charged ions within a segment. In addition, the sensitivity was enhanced when each segment was tuned using target analytes (m/z) giving slightly lower signal intensities than the greatest intensity at the same concentration of the mixed standards. As a result, the detection of target compounds with naturally lower signals than others was improved and achieved the compromised MS condition for all 20 target compounds. Overall, more segments with fewer scan events improved the signal intensities of target analytes by creating enough dwell time for each transition since there is less competition with smaller number of analytes. One optimized LC-MS/MS method with many segments was successfully applied to simultaneously detect 20 target analytes as shown in **Figure 3.4**

3.4.3 Method performance evaluation

3.4.3.1 Method detection limits (MDLs)

The MDL for 20 pharmaceuticals, hormones, and perfluorinated compounds was determined for each compound according to the IUPAC definition as the lowest concentration or mass of analyte that the analytical process can reliably detect.

The analytical DL using HPLC-MS/MS and the MDL for water samples (1 L) with SPE

clean-up and pre-concentration were measured for all target compounds as shown in

Table 3.5. The analytical DL was measured based on the performance of each target

compound in the HPLC-MS method. The MDL in water samples (1L) was also

measured using % recovery with an SPE method.[24] The MDLs for these target analytes

broadly ranged from 0.2 ng/L to 3.6×10^2 ng/L, ranitidine and methylphenidate to

chlortetracycline, respectively. As shown in **Table 3.5**, the MDLs varied from one

compound to another due to the breadth of selection of target compounds and the

compromised method used to analyze in one method.   The median MDL was 1.2 ng/L.

Overall, this result still showed comparable and/or lower MDLs for target analytes.[1, 3]

This method was successful at detecting low levels of this diverse group of analytes:

pharmaceutical classes of antidiabetic, beta2 adrenergic, analgesics, gastrointestinal,

antidepressant, anticoagulant, antianginal, central nerve stimulant, and antibiotics, as well

as steroid hormones and perfluorinated compounds (i.e. PFOA and PFOS) in actual

drinking water samples.

Finally, this positive and negative interchangeable ionization method, previously

believed to decrease the sensitivity[24], actually showed an enhanced sensitivity and

selectivity for these 20 analytes. This work therefore provided a robust and high

throughput analytical method that simultaneously measured 20 currently unregulated

compounds commonly found in drinking water supplies.

3.4.3.2 Field water samples from New Jersey

The method was validated using tap, well (raw and treated) and storm water

samples from domestic households and towns in New Jersey. The majority of field water

samples contained a mixture of several target compounds (% frequency): acetaminophen (42%), albuterol (58%), caffeine (92%), cimetidine (75% in precursor and 100% in degradation), *L*- cotinine (100%), estradiol (100%), metformin (100%), ranitidine (100%), PFOA (100%), and PFOS (17%). The mean percent frequency from these detected compounds was 80%. The concentrations of these detected compounds varied from one sample to another as shown in **Table 3.6**.

As expected, storm water samples of K1 and K2 had the highest summation of concentrations ($\Sigma\mu g/L$), 5.1 µg/L and 7.1 µg/L, respectively as well as higher concentrations of target compounds including caffeine (195 ng/L), PFOS (676 ng/L), PFOA (61ng/L) and estradiol (647 ng/L) among all water samples. Well water samples were generally least contaminated as the concentrations of all target analytes were below 1 µg/L except for the level of estradiol in one sample (R2 Well). The concentration of metformin was, however, the highest in tap water samples suggesting high intake/use of the antidiabetic drug. Overall, the steroid hormone, estradiol was mainly the highest concentration quantified in these field water samples and ranged from 234 ng/L to 6147 ng/L followed by metformin with a concentration range from 189 ng/L to 1334 ng/L. All other compounds detected from these field water samples were far below 1µg/L (**Table 3.6**).

In addition, a degradation product of cimetidine was measured in ranges of 421 ng/L to 994 ng/L in all field water samples, which were higher than the concentrations of its precursor by approximately two orders of magnitude. This phenomenon was previously reported in the literature by Perez *et al*.[32] who noted that the level of precursor (iopromide) dropped and its primary degradation product increased in concentration.

Buth *et al.*[33] have also proposed degradation products of cimetidine indicating that it undergoes significant transformation during wastewater disinfection (i.e. Chlorination).

3.5 Conclusions

Rapid sequential positive negative ion mode switching LC-MS/MS was used to isolate and quantify 20 emerging unregulated compounds in raw and finished drinking water from several sources. Superior sensitivity and selectivity were achieved for these analytes using multiple MS segment. This was previously believed to lower the sensitivity of the method[24] however, the technique showed enhanced sensitivity for all 20 target compounds. The overall method sensitivity was also improved by optimization of isolation, enrichment, and instrumental analysis steps with a median and mean MDL value of 1.2 ng/L and 22.7 ng/L, respectively.   The mean MDL was significantly higher than the median due to the inclusion of a couple of target compounds that were difficult to analyze and quantify.   These compounds were PFOS and chlortetracycline with MDL values of 2.7×10^1 ng/L and 3.6×10^2 ng/L, respectively, which were often analyzed in the ESI negative method and/or by an atmospheric pressure chemical ionization technique. The ability to measure such a broad array of compound classes is facilitated by the rapid switching between positive and negative detection modes. It is analytically significant because it increases the utility of many LC/MS based methods while reducing the number of methods that need to be run on a single sample. This method has robustness and a high sample throughput rate, which is required for drinking water analysis of unregulated compounds.

3.6 References

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Figure 3.1 Percent recoveries using acidified methanol (MeOH$^+$), dichloromethane (DCM), and acetone elution solvents
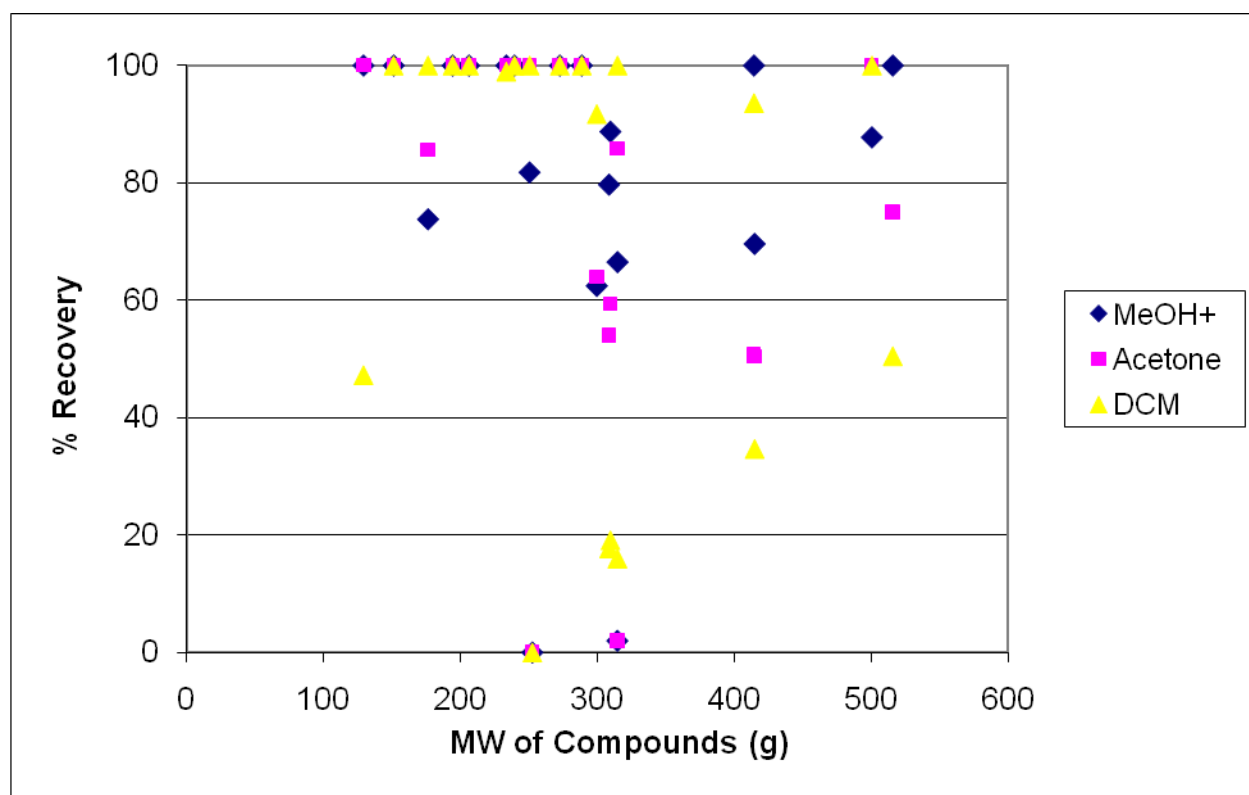
Figure 3.2 Comparison in different compositions of sample diluent: cimetidine (top) and codeine (bottom)
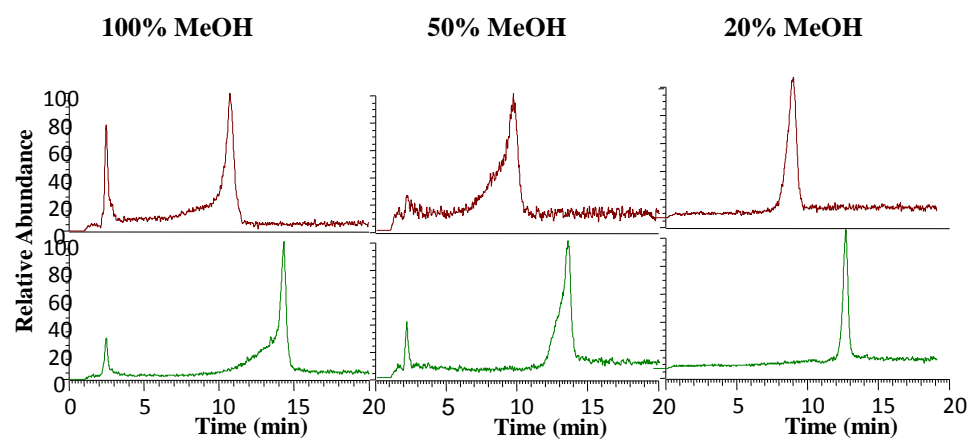
Figure 3.3 Single ion monitoring (top) vs. selected reaction monitoring (middle/bottom) using equivalent precursor ions of 315 m/z (ranitidine and progesterone)
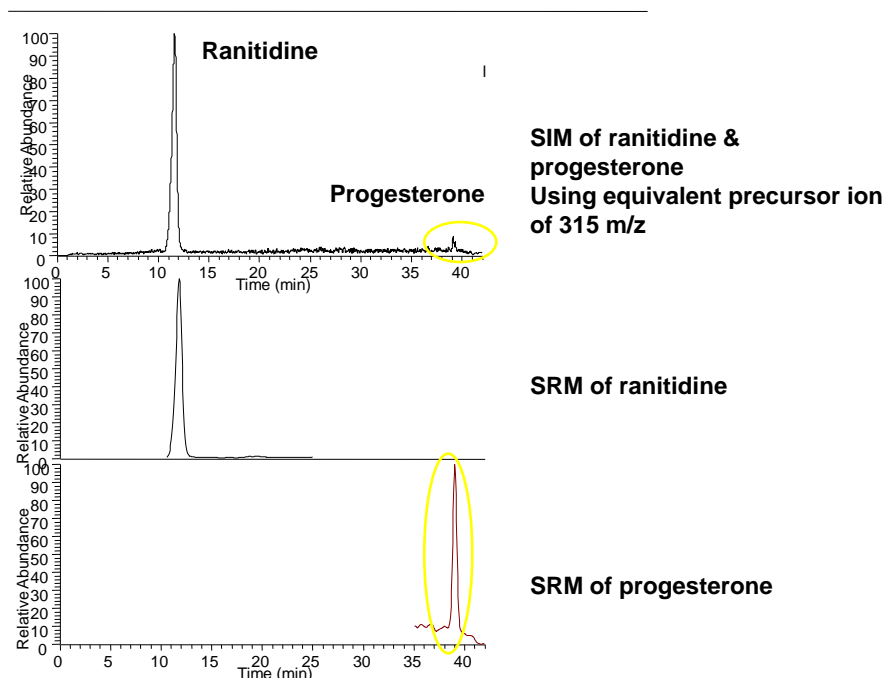
Figure 3.4 Target ion traces of 20 standard mixture in ESI +/- Modes
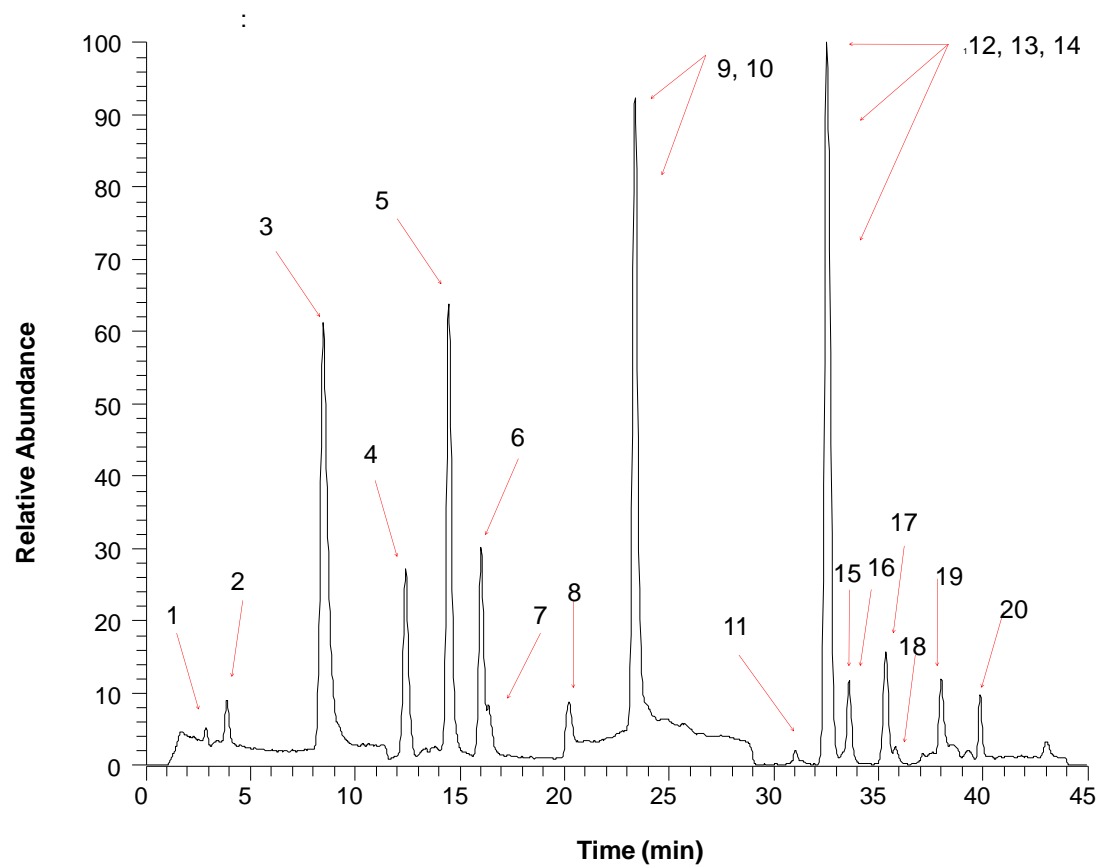
*Each peak was identified with its error & number corresponding to the order of elution (number) in **Table 3.4**.

Table 3.1 Analysis of variance (ANOVA) for factorial combinations of extraction efficiency

ANOVA					$\alpha=0.05$	
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample[a]	3.86E+17	18	2.14E+16	234.6041	3.44065E-81	1.695025
Columns[b]	2.6E+15	2	1.3E+15	14.26272	2.96287E-06	3.075853
Interaction[c]	1.94E+16	36	5.38E+14	5.886816	1.78691E-13	1.520729
Within	1.04E+16	114	9.13E+13			
Total	4.18E+17	170				

[a]Sample=compounds
[b]Columns=methods
[c]Compounds x methods

Table 3.2 Comparison of different elution conditions: %Recovery (%RSD; n=3)

Target analytes	MW (g)	NH$_4$OAc (200μL)	NH$_4$OAc (Dryness)	NH$_4$OH (200μL)	NH$_4$OH (Dryness)
Metformin	129.17	20 (37)	12 (34)	97 (14)	32 (26)
Acetaminophen	151.17	91 (12)	98 (2)	116 (12)	116 (2)
L-Cotinine	176.22	46 (11)	39 (6)	103 (10)	88 (16)
Caffeine	194.19	98 (5)	96 (8)	95 (5)	107 (9)
Ibuprofen	206.10	61 (2)	50 (11)	84 (12)	65 (14)
Methylphenidate	233.31	77 (11)	91 (8)	86 (6)	97 (11)
Albuterol	239.31	30 (7)	34 (14)	86 (6)	96 (4)
Gemfibrozil	250.34	63 (4)	43 (23)	81 (8)	45 (4)
Cimetidine[a]	252.34	16 (4)	15 (5)	50 (11)	47 (10)
Estradiol	272.39	125 (14)	108 (9)	107 (8)	134 (9)
Testosterone	288.42	89 (9)	76 (1)	99 (4)	98 (3)
Codeine	299.37	47 (13)	48 (13)	80 (7)	57 (6)
Warfarin	308.33	44 (9)	62 (8)	86 (3)	82 (19)
Fluoxetine	309.33	49 (15)	37 (12)	71(3)	47 (3)
Ranitidine[a]	314.41	96 (9)	89 (2)	93 (16)	89 (4)
Progesterone	314.47	65 (8)	63 (6)	78 (9)	66 (4)
PFOA	414.07	128 (4)	107 (12)	105 (7)	102 (11)
Cis-Diltiazem	414.52	54 (3)	48 (10)	93 (5)	47 (6)
PFOS	500.13	63 (34)	90 (17)	91 (30)	126 (19)
Chlortetracycline	515.35	n/a	n/a	81[b]	n/a

[a] Degradation products were included for quantification analysis

[b] No replicates

Table 3.3 Analysis of variance (ANOVA) for elution conditions (groups)

SUMMARY

Groups	Count	Sum	Average	Variance
$CH_3COOH-NH_4OAc$	19	1262	66.42105	1008.591
$CH_3COOH-DryNH_4OAc$	19	1206	63.47368	924.8187
$CH_3COOH-NH_4OH$	20	1782	89.1	208.8316
$CH_3COOH-DryNH_4OH$	19	1541	81.10526	904.5439

ANOVA $\alpha=0.05$

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8603.717	3	2867.906	3.80297	0.013639	2.730019
Within Groups	55050.96	73	754.1227			
Total	63654.68	76				

Table 3.4 ESI-MS/MS conditions: Precursor & product ions (SRM transition) for 20 target compounds

Order of elution	Target analytes	Precursor ion (ESI mode)	Product ions in MS/MS (intensity)	%CE
1	Acetaminophen	152 (+)	110.2 (100%), 88.9 (18%), 134.8/132.9 (20%/18%)	35%
2	Metformin	130 (+)	60.1 (100%), 85.2 (18%),113 (10%)	35%
3	Albuterol	240 (+)	222.1 (100%), 102.3 (10%), 166.01 (10%)	35%
4	L-Cotinine	177 (+)	146.3 (100%), 80.2 (59%), 98.1 (42%)	38%
5	Cimetidine	253 (+)	159.0 (100%), 117.2 (36%), 211.0 (22%), 172.0 (12%)	35%
6	Ranitidine	315 (+)	270.0 (100%), 176.0 (65%), 224.0 (35%), 124.2 (20%)	35%
7	Codeine	300 (+)	215.2 100%), 243.1 (66%), 282.2 (40%), 225.2 (38%)	37%
8	Methylphenidate	234 (+)	84.2 (100%)	35%
9	PFOS	499 (-)	499.1 (100%)	10%
10	Caffeine	195 (+)	138.1 (100%), 171.0 (70%),177.0 (35%), 132.9 (24%)	35%
11	Fluoxetine	310 (+)	147.9 (90%), 228 (40%)	35%
12	PFOA	413 (-)	369.0 (100%)	35%
13	Cis-Diltiazem	416 (+)	178.2 (100%), 370.1 (48%), 310.2 (18%), 415.4 (60%)	40%
14	Ibuprofen	205 (-)	205.1 (100%)	0%
15	Warfarin	309 (+)	163.2 (100%), 251.2 (26%), 147.2 (10%)	35%
16	Chlortetracycline	514 (-)	378.9 (100%), 446.8 (15%)	35%
17	Testosterone	289 (+)	253.2 (100%), 271.2 (86%), 97.2 (56%), 109.1 (34%)	35%
18	Gemfibrozil	249 (-)	121.3 (100%)	40%
19	Estradiol*	273 (+)	258.9 (100%), 255.2 (16%)/258.9 (100%=3.58e5), 272.4 (64%), 255.2 (20%)	45%
20	Progesterone	315 (+)	297.2 (100%), 279.2 (44%), 314.5 (40%)	40%

Table 3.5 Analytical Detection Limits (DL) and Method detection limits (MDL)

Compounds	Classification	DL (ng/mL)	MDL[a] (ng/L)
Metformin	Antidiabetic agent(e.g.Glucophage)	2.1	2.2
Acetaminophen	Analgesics(e.g. Tylenol)	1.2	1
Albuterol	Beta2 Adrenergic agent	0.7	0.8
L-Cotinine	CNS stimulant (a metabolite of nicotine)	1.4	1.4
Cimetidine	Gastrointestinal agents	0.4	0.8
Ranitidine	Antiulcer(e.g. Zantac)	0.2	0.2
Codeine	Analgesics	2.2	2.7
Caffeine	Central Nervous System(CNS) stimulant	2.5	2.6
Perfluorooctanesulfonic acid	Perfluorinated chemical (flurosurfactant)	2.4×10^1	2.7×10^1
Perfluorooctanoic acid	Perfluorinated chemical (fluoropolymer)	0.4	0.4
Methylphenidate	CNS Agents	0.2	0.2
Chlortetracycline	Antibiotic	2.9×10^2	3.6×10^2
Ibuprofen	Anti-inflammatory (e.g. Advil)	1.7×10^1	2.0×10^1
Warfarin	Anticoagulants	0.3	0.3
Fluoxetine	Antidepressant	0.6	0.8
cis-Diltiazem	Antianginal, antihypertensive (calcium channel blocker)	0.6	0.6
Testosterone	Hormone	2.4	2.4
Gemfibrozil	Lipid regulating agent (cholesterol lowering)	0.8	1
Estradiol	Endocrine and Metabolic Agents	2.1×10^1	2.0×10^1
Progesterone	Hormone	5.4	6.9

[a]MDL in water sample (1L)

MDL= T(n-1,1-α=0.99) (S) / m; Where, MDL= the method detection limit; T(n-1,1-α=0.99)= the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom; S = standard deviation of replicate analyses; m = the slope of the calibration curve obtained via linear regression

Table 3.6 Field water samples from New Jersey (1)

Sample Type	S Tap	P Tap	H Tap	S Tap	W Well	R Well	
Sample site	Monmouth	Middlesex	Middlesex	Middlesex	Burlington	Atlantic	Frequency of Detection (%)
Acetaminophen	11	14	9	10	<MDL	<MDL	42
Metformin	1,212	1,334	988	1,055	232	189	100
Albuterol	3	2	2	<MDL	1	<DL	58
L-Cotinine	34	21	39	49	16	8	100
Cimetidine	64	24	<DL	<DL	17	15	75
Degradation	884	400	994	788	492	474	100
Ranitidine	3	3	3	3	3	4	100
Caffeine	17	19	33	40	23	17	92
PFOS	<MDL	<MDL	<MDL	<MDL	337	<MDL	17
PFOA	2	12	16	9	1	1	100
Estradiol	467	2,609	1,288	1,461	275	244	100
Σ (ng/L)	2,696	4,438	3,373	3,415	1,396	951	

[a] Degradation of cimetidine

Table 3.6 Continued: Field water samples from New Jersey (2)

Sample Type	T Well	R1 Well	R2 Well	T Well	K1 Storm	K2 Storm	
Sample site	Atlantic	Camden	Camden	Ocean	Hudson	Hudson	Frequency of Detection (%)
Acetaminophen	<MDL	<MDL	10	<MDL	<MDL	<MDL	42
Metformin	286	315	288	219	405	293	100
Albuterol	1	3	<MDL	<MDL	1	<MDL	58
L-Cotinine	7	12	17	16	16	18	100
Cimetidine	56	23	61	<DL	25	29	75
Degradation	511	438	603	665	421	469	100
Ranitidine	2	2	3	3	3	2	100
Caffeine	16	25	21	<MDL	195	82	92
PFOS	<MDL	<MDL	<DL	<MDL	676	<MDL	17
PFOA	54	7	14	7	25	61	100
Estradiol	234	823	1861	189	3,421	6,147	100
Σ (ng/L)	1,166	1,650	2,877	1,099	5,186	7,102	

[a] Degradation of cimetidine

Chapter 4 - Household drinking water treatment processes for removal of 20 unregulated organic contaminants: Brita™ filtration (GAC/ion resin), ozonation and microwave heating system

4.1 Abstract

Among twenty emerging unregulated compounds, eleven including pharmaceuticals, steroid hormones and perfluorinated compounds, were frequently (80%) detected in drinking water samples analyzed in a previous study.[1] The concentrations of these compounds varied from single ng/Ls to single µg/Ls in these drinking water samples.[1] These results suggested a need for further water treatment processes prior to ingesting the water. In this work, common household water treatment processes were studied for their efficiencies of removing these emerging unregulated compounds in drinking water. A commercial Brita™ filter (granular activated carbon/ion exchange resin), ozonator and microwave oven were selected based on their general utilization for the household drinking water process. Efficiencies of these water decontamination processes were characterized based on the physicochemical properties of the target compounds: molecular weight (MW), octanol-water partition coefficient (log Kow) and acid dissociation constant (pKa). The percent removal of 20 target compounds varied from one compound to another and one treatment-process to another. Some of the highly removed/eliminated compounds included gemfibrozil ($100 \pm 0\%$), *cis*-diltiazem ($99 \pm 0\%$), albuterol ($100 \pm 0\%$), and fluoxetine ($97 \pm 1\%$) by one or more water processes tested. However, the least removable compound ($\leq 5\%$) in all water processes was a degradation product (of cimetidine). This demonstrated the significance of degradation/transformation products of pharmaceuticals in drinking water contamination, thus requiring a further investigation. With overall mean percent removal for the 20

target compounds, the Brita™ filtration (<66%) was most effective followed by ozonator (<54%) and microwave oven (<36%). Overall, these unregulated compounds were partially removed using these household technologies.

4.2 Introduction

Organic wastewater contaminants (OWCs) including prescription and non-prescription drugs, their metabolites, flame retardants and other OWCs are being continually detected in United States drinking water sources.[2] However, the majority of these OWCs are not regularly monitored in drinking water. It was reported that many pharmaceuticals and other OWCs were not only detected in raw water supplies of treatment plants but in finished water.[3] The detection of these compounds showed that they are not completely removed through conventional water treatment processes. Some pharmaceutical compounds and other OWCs have previously been reported to persist and survive subsequent water treatment.[4]

A previous study reported a mixture of at least 11 and as many as 17 OWCs in samples of finished water.[3] The detection of mixed OWCs caused concern for potential toxicological effects since the drinking water criteria are mainly based on the toxicity of individual compounds and not mixtures. Recently, degradation and/or transformation products of pharmaceuticals in drinking water sources were observed suggesting that they were less biodegradable than their precursors in treated drinking water and wastewater.[5] Similarly, previous studies also showed that transformed products are even more toxic than their precursors.[6,7]

In this study, household drinking water treatment techniques were characterized for their ability to remove OWCs from potable water. Three commercial household water

processing techniques were examined for efficiency of removing or decomposing twenty

unregulated compounds (mainly pharmaceuticals) using a previously created method.[1]

Firstly, a mixed bed system of granular activated carbon (GAC) and ion resin was tested

in a commercial Brita™ filter. It used a sorption/adsorption mechanism with

hydrophobic and non-polar properties. Previously, GAC was demonstrated to be very

effective in removal of pharmaceuticals and endocrine disruptors.[8, 9] Secondly, ozonation

was tested using a portable ozonator. This was also shown to be effective in eliminating

pharmaceuticals, mainly polar pharmaceuticals.[8,10] It was also used as an effective pre-

treatment during anaerobic treatment of sludge for removal of pharmaceuticals.[11]

However a cost analysis created concern when ozone treatment was used for wastewater

plants.[10] Lastly, a dielectric heating and thermal degradation processes by absorption of

wave energy was tested using a household microwave oven. Previously, a microwave-

enhanced advanced oxidation process was demonstrated for sewage sludge treatment.[12, 13]

Moreover, the use of microwave ovens in domestic households is very high. The total

number of consumer ovens in the U.S. was well over 100 million and the world's total

was estimated to be over 250 million.[23] A common household application of boiling and

/or heating water using a microwave oven system was studied for potential removal or

degradation of pharmaceuticals in drinking water.

Finally, a relationship between target compounds and their removal by the

aforementioned household water treatment techniques was modeled based on the target

compounds' physicochemical properties of molecular weight (MW), octanol-water

partition coefficient (log Kow) and acid dissociation constant (pKa). MW as a physical

property; log Kow as a hydrophobic and hydrophilic (polar) property; and pKa as a

chemical (acidic) property of target compounds, were selected to characterize their behavior in adsorption (physical), ozonation (chemical) and degradation (thermal) mechanisms. In our hypothesis, the physical nature of a compound including MW and log Kow may be indicators of its behavior in activated carbon adsorbent mixed with ion-exchange resins in a Brita™ filter.   For chemical ozonation, polar compounds that are more reactive with ozone were reviewed for their removal. Lastly, not much was known about the microwave heating process with thermal and dielectric degradation of OWCs. Therefore this work focused on degradation of precursors as well as their potential relationship with physicochemical properties of target compounds.

4.3 Experimental

4.3.1 Materials and specifications

Three commercial household water treatment systems were used in this study. Brita™ filter was manufactured by Brita company, Oakland, CA, and purchased at local retail outlets. Brita cartridges (96 g adsorbent) consisted of cation exchange resins with some coal-based granular activated carbon, used in pitcher filtration systems.[14]

The ozone generator (model: OZX-300AT) was manufactured by and purchased from Shanghai ENALY M&E Ltd, Shanghai, China. Ozone output was adjustable with a maximum ozone output of 200 mg/hr without connection to an air dryer and 500 mg/hr with connection to an air dryer. Internal air pump output was 1-2 L/min; wattage of 10W; pump pressure of 17Kpa. The ozone generating method was using corona discharge (ozone tube). The gas resource was ambient air with an air inlet/outlet diameter of 6.5 mm. A power source was operated with AC110-120V. The ozonator size was 215 mm x 150 mm x 60 mm with a net weight of 800 g.

A household microwave oven (model: R-220BW) manufactured by SHARP company, Mahwah, NJ, was used in this study. A power source of AC 120V; frequency of 60 Hz; and rated current of 9.5A were used. Output (cooking) power was 700 watt under the IEC-705 test procedure; operating frequency was typically 2450 MHz. The microwave distribution was a turntable with a stirring fan. Both 1L and 500 mL of amber glass bottles (I-CHEM[TM]) purchased from VWR were used to contain both treated and untreated water samples. Tap water from the Environmental Occupational Health Science Institute (EOHSI), Piscataway, NJ, was collected mornings in July and August, 2009 and spiked with the 20 target compounds at concentrations of 200 ng/L and 2 µg/L. The 20 target compounds, including pharmaceuticals, antibiotics, steroid hormones, and PFCs, were all purchased and diluted as described in previous paper.[1]

4.3.2 Sampling: water treatment process

The sampling protocol was as follows:

1. Sample first thing in the morning (i.e., after overnight standing of the water in the plumbing system).

2. Flush the 1L amber glass bottle with water from the cold tap several times.

3. Fill six 1L amber glass bottles with cold household tap water.

4. A half bottle of water (500 mL) was transferred into a 500 mL amber glass bottle for a treatment.

5. All water samples (500 mL) were spiked with 200 ng/L or 2 µg/L levels of the 20 mixed standards in triplicates.

6. Half of each water sample (500 mL) was treated/processed with A, B or C as follows:

A. Prior to use the silverized granular activated carbon (charcoal) and ion exchange resin of a Brita ^{TM} filter it was soaked in cold water for 30 min following the manufacture's recommendations. Then 500mL of the water sample was slowly poured into the filter system. Once, all the water has completely filtered through the Brita filter, it was poured back into the amber glass bottle prior to the extraction.

B. Prior to use the ozone generator, it was connected to the air inlet of an Enaly Air Dryer with one end of 10cm hose. Then the other end of the 10 cm hose was connected to the air inlet of the unit. For water treatments, a 100 cm hose was connected on one end to the ozone outlet and the other end to a diffuser stone. The power switch followed by the ozone output adjuster was first turned on. The ozone was adjusted to 40% of the maximum output with a connection to an air dryer. The diffuser stone was placed in the water bottle and also immersed at least 10 cm below the liquid surface to prevent backflow. The water sample was then processed with ozone for a contact time of 2 min. This provided an ozone dose of 3.3 mg/L, which was in a range previously used to treat pharmaceuticals in water samples.[8]

C. For the household microwave oven, the water sample in its amber glass bottle was placed inside and heated for 5.5 min. This reached approximately 98 °C (the temperature was initially measured with blank tap water samples). Some vapor bubbles may be seen due to its point of boiling. Once the water sample has cooled down, it was capped. The heated/treated water was then refrigerated in a cold room (4°C) prior to the extraction.

7. The other half of the spiked water samples (500 mL) remained untreated.

8. All bottles of water were refrigerated (4°C) prior to the solid phase extraction.

4.3.3 Solid phase extraction

The SPE method was used to clean-up and pre-concentrate water samples as described in a previous paper, [1] with one exception. The volume of each water sample was extracted and concentrated from 500 mL to 500 μL for a constant enrichment factor of 1000.

4.3.4 LC-MS/MS analysis

The water samples extracts were analyzed using a LC-MS/MS method described in a previous paper.[1]

4.3.5 Data analysis

Percent removal/degradation by GAC/ion resin, ozone, and microwave water processing were calculated based on the levels (amount) of target compounds in treated and untreated water samples. A percent removal is equal to the amount of target compounds removed in a treated water sample dividing by the amount of target compounds in an initial (untreated) water sample times a hundred percent (%). Mean (arithmetic) percent removal and relative standard deviation (%RSD) for each compound were calculated for treated water using triplicate samples at both concentrations (200 ng/L and 2 μg/L). In addition, a harmonic mean of overall percent removal was calculated for each water-process as well as at each concentration (200 ng/L vs. 2 μg/L). The harmonic mean was chosen because of the type of data created by these experiments. It gives greater weight to the low removal efficiencies of many of these water treatment-

processes for these target compounds. The harmonic mean is the reciprocal of the

arithmetic (average) mean of the reciprocals as follows:

$$H = \frac{n}{\frac{1}{x_1} + \frac{1}{x_2} + \cdots + \frac{1}{x_n}} = \frac{n}{\sum_{i=1}^{n} \frac{1}{x_i}}, \qquad x_i > 0 \text{ for all } i.$$

Where, H represents a harmonic mean, x is the percent removal of target compounds (i),

and n is the number of target compounds (i).

The efficiency of each removal/degradation method was characterized based on

three of the main physicochemical parameters: molecular weight, octanol-water partition

coefficient (log Kow) and acid dissociation constant (pKa) of all target compounds

collected. The values for these parameters were based on a literature review and are

shown in **Table 4.1**. Literature values were used for all compounds studied. The

literature value was either measured experimentally or calculated using available

software *(e.g. ACD/LogP)*. The pKa and log Kow values of these target compounds were

compiled based on experimental data as well as calculated values when a lack of

experimental data existed. All pKa values were determined at a standard condition

(25ºC). The steroid hormones progesterone and testosterone were not included due to a

limitation of current pKa values.[15] In addition, the degradation product of cimetidine was

included for the quantification, however, its physicochemical properties (MW, log Kow

and pKa) were not available.

4.3.6 Statistical analysis

Analysis of variance (ANOVA) was performed to check for significant difference

in mean removal efficiencies of water-processes tested (GAC/ion resin, ozonation and

microwave). The *ANOVA: Single-Factor* in Excel™ software was used to run this

statistical test. The null hypothesis was that all of these removal efficiency means of water processes were equal and alpha (α) =0.05 was used for its statistical criteria.

A paired t-test was performed to test for a significant difference between concentrations of 200 ng/L and 2 µg/L for the removal by water-processes tested (Null hypothesis (H_0): $C_{200ng/L} = C_{2\mu g/L}$). This statistical analysis was used to check for a possible saturation of removal sites by looking for a percent removal difference between 200 ng/L and 2 µg/l. For a specific compound of interest, ibuprofen was tested for a saturation of removal (adsorption) sites due to its behavior as a potential outlier in the GAC/ion resin system. The statistical analysis was performed under *t-Test: Paired Two Sample for Means* in Excel™ software. For the input range for variable 1, 3 values (from triplicate samples) of percent removal in group "2µg/L" were selected for ibuprofen. For the input range for variable 2, 3 values (from triplicate samples) of percent removal in group "200ng/L" were selected for ibuprofen. The hypothesized mean difference was zero. Alpha (α) was 0.05.

Each water treatment-process was also tested for an overall difference between 200 ng/L and 200 µg/L for the percent removal of all target compounds. For the input range for variable 1, 20 values of percent removal in group "2µg/L" were selected, For the input range for variable 2, 20 values of percent removal in group "200ng/L" were selected. The hypothesized mean difference was zero. Alpha (α) was 0.05. To optimize this analysis, the difference between the paired values at two concentrations ($C_{200ng/L}$ and $C_{2\mu g/L}$) was calculated and a new column of the difference created was called "DIFF". Then an average mean was calculated. Finally, descriptive statistics on the difference values were calculated using *Descriptive Statistics* in Excel™ software. For each

analysis, outputs of t-Stat, two-tail p-value, and confidence interval (95%) were mainly

used to determine the statistical difference in their removal at the two concentrations for

all target compounds.

4.4 Results and Discussion

4.4.1 Percent removal calculation

The mean percent removal and percent relative standard deviation (%RSD) for

each target compound were calculated. %RSD were not calculated for the target

compounds with their percent removal close to zero (<5%). **Table 4.2** lists by increasing

order of MW (from bottom to top) the overall results of percent removal/decomposition

of 20 target compounds by each water-process tested. Because of its rapid degradation in

water, the degradation product of cimetidine was used as a surrogate for calculating

removal efficiency. Harmonic and arithmetic mean percent removal of all target

compounds were calculated for each water-process tested. Using the harmonic mean, the

GAC/ion resins had removal means of 37% and 39%; the ozonation process had means of

24% and 15%; microwave had means of 11% and 9% at 0.2 µg/L and 2 µg/L,

respectively. Using the arithmetic means, the GAC/ion resins had removal means of 65%

and 66%; the ozonation process had means of 54% and 48%; microwave had means of

36% and 30%. Compared to arithmetic mean values, these harmonic values were

lowered by as much as 70% of its arithmetic mean value. The arithmetic mean shows

dominance of the higher removal rates of some compounds to drive the overall evaluation

of the methods removal efficiency.

4.4.2 Efficiency of granular activated carbon and ion exchange resins

Previously, filtration with granular activated carbon (GAC) showed effective removal of pharmaceuticals.[8] In this work, a commercial Brita™ filter using GAC and cation exchange resins was examined for its efficiency at removing target compounds in water samples **Figure 4.1** is a bar graph of removal efficiencies of all target compounds (in alphabetical order) using GAC/ion resins of a commercial Brita™ filter. The removal efficiency of the degradation product of cimetidine was used due to its rapid degradation. The degradation product of cimetidine was selected for the quantification, *not* for its characterization by physicochemical properties. The main mechanism for a compound's removal in GAC and cation exchange resins was believed to be adsorption. Two adsorption mechanisms were expected to occur with this system based on a previous study[14]; one was governed by surface area of the adsorbent as a surrogate for size and distribution of GAC/ion resin pores. Another was governed by the surface charge of adsorbent from ion exchange resins. In this study, these two adsorption mechanisms were examined for the removal of these 20 target compounds.   Three main physiochemical properties; molecular weight (MW), octanol-water partition coefficient (log Kow) and acid dissociation constant (pKa) were used to characterize the efficiency of removal.

A plot was created for MW vs. percent removal of 19 target compounds by the GAC/ion resin system as shown in **Figure 4.2**. Target compounds with smaller MWs from 129 g to 415 g had higher removal efficiencies while chlortetracycline (515.35 g) and PFOS (500.13 g) with greater MWs had lower removal efficiencies in the GAC/ion resin system. A relationship was determined that showed larger molecular weight

compounds (>500 g) were less likely to be adsorbed on GAC/ion resin adsorbent than lower molecular weight compounds. This relationship may be explained by the adsorption properties, in which pore sizes and distribution of GAC and ion resins determined the amount of internal space available for adsorption of target compounds, since adsorption is often determined by both the size and distribution of GAC pores (micro-pores, meso-pores and macro-pores).[14] It is possible that small sized pores do not effectively trap large adsorbate molecules and large sized pores does not effectively trap small adsorbates, whether they are charged, polar molecules or uncharged, non-polar compounds. As a result, larger MW compounds were not effectively removed by the smaller pore sizes of GAC/ion resins. The best fit of the relationship between MWs (x) and percent removal (y) of target compounds was shown in **Figure 4.3**. Estradiol as a potential outlier was not included in this relationship. The removal behavior of estradiol is thought to be made unstable by local conditions because of its highly hydrophobic nature; the presence of other organic compounds at the adsorbent surface may or may not encourage hydrophobic partitioning.[16] This can affect the prediction of an accurate percent removal by the GAC/ion resin adsorbent. Estradiol as one of the well-known endocrine disrupting compounds and because of its ubiquitous nature was studied extensively for its removal rates using drinking water treatment technologies. A membrane-type of treatment-process was previously recommended for estradiol because of its retention characteristics for the steroid hormones seen elsewhere.[16, 17, 18] In **Figure 4.3**, the second order relationship was created with $y = -x^2 + 0.62x - 3.20$ suggesting that removal rates increased and then decreased once the MW reached a threshold value possibly due to the size and distribution of the pores in GAC/ion resins. The threshold

value was calculated for a MW of 284 based on the second order relationship created.

Unfortunately, manufacture's data were not available on the size and distribution of

GAC/ion resins in Brita™ filtration to reference against the calculated threshold value.

The goodness of fit, R^2 =0.71 was achieved for the relationship and higher R^2 may be

obtained with a further selection of target compounds based on their physicochemical

properties.

The removal efficiency of a GAC/ion resin system were viewed for their

relationship to octanol-water partition coefficients (log Kow) of target compounds A

direct association between log Kow and percent removal of 19 target compounds was

observed as shown in **Figure 4.4**. A positive correlation of removal efficiencies in

GAC/ion resins of target compounds with log Kow ranging from -2.6 to 4.7 was shown.

Both perfluorinated compounds, PFOA and PFOS had lower removal efficiencies of 51%

and 35%, respectively, which may be due to their low hydrophobicity of 1.02 and 1.92,

respectively. Less correlation was observed for chlortetracycline, estradiol and ibuprofen.

The antibiotic chlortetracycline was removed least effectively at <5% in the GAC/ion

resin system. This low removal efficiency may be due to a high MW (515.35) and low

hydrophobicity (log Kow = -0.36) of chlortetracycline, which were less likely to be

adsorbed onto GAC/ion resins. The low removal efficiency of estradiol (49%) was

previously described. For ibuprofen, the exact physicochemical property influencing its

low removal rate (59%) was not apparent. However, a slightly higher statistically

significant removal rate of ibuprofen (73 $\pm$ 3%) was observed at a lower concentration

(200 ng/L). A saturation of adsorption sites at a higher concentration (2 µg/L) of

ibuprofen may have occurred. Among the compounds with removal rates above 60%, a

casual relationship between percent removal and log Kow was observed. In **Figure 4.5**, a curvilinear regression ($R^2 = 0.70$) was created to show increasing removal rates of target compounds in log Kow ranged from -2.6 to 4.7. An exception was noted for albuterol (93%), because its estimated log Kow varied from source to source as appeared to be dependant on pH. The multiple hydroxyl functions may cause it's behavior to differentiate significantly for other compounds that have less and would be less affected by pH. Inclusion of albuterol changes the R^2 from 0.7 to 0.5. The relationship between log Kow and percent removal suggested that adsorption of target compounds were affected by hydrophobic and non-polar properties of GAC/ion resins in Brita™ filtration.

The efficiency of removal by the GAC/ion resin system was also viewed with respect to pKa values of the target compounds. A plot of percent removal vs. pKa of target compounds showed that target compounds with percent removal ranging from 5% to 94% were positively associated with their pKa values ranging from 3.2 to 13.2 (**Figure 4.6**). The relationship generally agreed with the hypothesis that the higher the pKa of the target compound the higher percent removal in the GAC/ion resin system. In other words, weakly acidic compounds with greater pKa were retained with higher efficiency on GAC/ion resin adsorbent than more acidic compounds with lower pKa. This relationship can be explained by cation-exchange resins that were more favorable to weakly acidic (basic) compounds with higher pKa values because they are proton acceptors (e.g., NH_3). However, more than one removal process may have occurred in this wide range of pKa ($3 < pKa < 13$) of target compounds. While the majority of target compounds of weak acids ($3 < pKa < 10$) can be seen as a potential curvilinear regression ($R^2 = 0.86$) shown in **Figure 4.7**, a second regression was preferred with target compounds of higher pKa

values (> above 10).   Noticeably, removal rates of basic compounds (pKa >10) were observed parallel to ones of acidic compounds. These basic compounds (i.e., estradiol, metformin and caffeine) as proton acceptors were more likely to be governed by the negatively charged surface area of cation exchange resins. Because of a broad range of acidic to basic target compounds with different physicochemical properties, these target compounds could have adsorbed on GAC/ion resins at different rates. Overall, the positive association of .removal efficiencies vs. pKa of target compounds demonstrated that a surface charge of adsorbent in Brita™ filter may have affected the removal efficiencies of target compounds.

Finally, overall results of removal efficiencies characterized by physicochemical properties of MW, log Kow and pKa showed an agreement with Lorphensri *et al.* [19] in which the adsorption of ionizable pharmaceuticals is strongly dependent on the system pH, the pharmaceutical properties of pKa, hydrophobicity, and the nature of the surface charge. The general concept of an adsorption mechanism with hydrophobic and non-polar properties was applicable to remove the majority of target compounds including pharmaceuticals although partial removal of target compounds was achieved. The adsorption relationship of log Kow vs. percent removal of target compounds showed hydrophobic and non-polar properties in the GAC/ion resin system. In addition, the effects of sizes and distribution of GAC/ion pores was observed on the relationship of MW vs. percent removal of target compounds. The removal efficiency of target compounds based on the negatively charged surface area of cation-exchange resins was appeared likely in the relationship of percent removal vs. pKa.

4.4.3 Efficiency of ozonation

An ozonation process previously demonstrated effective removal of polar compounds including pharmaceuticals.[8] The effective removal (>90%) of the antiphlogistic diclofenac and the antiepileptic carbamazepine were reported at 0.5mg/L ozone dose. The lipid regulator bezafibrate was less effectively removed, 50% at 1.5 mg/L ozone while clofibric acid was not removed even at 3 mg/L ozone in the same study.[8] Rate constants of ozone reactions for organic and inorganic compounds in water were previously studied and used to reference some of the target compounds for this work.[20, 21] For the study reported here, a household ozonator was examined for its efficiency of removing 20 target compounds including pharmaceuticals in drinking water. **Figure 4.8** shows a bar graph of removal efficiencies of all target compounds (in alphabetical order) by ozonation. The removal efficiency of the degradation product of cimetidine was used due to its rapid degradation. The degradation product of cimetidine was selected for quantification, *not* for its characterization by physicochemical properties.

The percent removal of target compounds by ozonation compared against their MWs, log Kow and pKa. No significant relationship was found based on MW of target compounds suggesting the physical nature of these target compounds with their MW did not affect their reactivity with ozone (**Figure 4.9**). Similarly, no obvious relationship between percent removal and log Kow of target compounds was observed. A higher percent removal (≥ 88 %) was observed for target compounds with log Kow in the range of 0.49 and 2.8 with one exception of warfarin (19%) (**Figure 4.10**). The higher removal efficiency in this hydrophilic range of log Kow showed that some polar pharmaceuticals were more effectively removed by ozonation.[8] PFOA ($\leq$5%) and PFOS ($\leq$31%) were not

effectively removed possibly due to their hydrophobic properties or/and low pKa values.

A direct relationship was observed between pKa and percent removal of target

compounds by ozonation. This positive association demonstrated that target compounds

with a higher pKa were removed more efficiently by ozonation.   Notable exceptions, the

lipid regulating agent, gemfibrozil and the central nerve system agent, methylphenidate

had slightly higher and lower removal efficiencies, respectively than other compounds in

a similar pKa range. A higher reactivity of gemfibrozil (100%) may probably be due to

its functional groups (i.e. substituted benzenes, -OH) that are more reactive with ozone.

Methylphenidate (43%) however was slightly less reactive with ozone, possibly be due to

its protonated acetate ester (pKa = -4.61)[22] attached. These results agreed with a previous

study, which suggested all reactions of ozone are highly selective and electrophilic.[20]

Finally, a linear regression ($R^2 = 0.86$) was created for the ozone relationship of percent

removal vs. pKa (<10) of target compounds as shown in **Figure 4.11**. A slight decreased

percent removal was however observed on target compounds with pKa ranges above 10

and suggested a selected pKa range of target compounds for the optimal removal by the

ozone treatment-process. Overall, these results suggested that weakly acidic (basic)

compounds of higher pKa values were more reactive to ozone and therefore likely to be

eliminated by chemical ozonation.

4.4.4 Efficiency of microwave heating system

A microwave enhanced - advanced oxidation process (AOP) was recently

demonstrated to favor promoting the degradation efficiency of pharmaceuticals in

wastewater.[13] The microwave system has been a well known water-process to heat/boil

drinking water in common households and therefore selected for its potential influence on

target compounds (e.g., pharmaceuticals) in drinking water. The household microwave

oven was tested for its potential in removing these emerging unregulated contaminants in

drinking water by dielectric heating and thermal degradation mechanisms. The behavior

of target compounds including the microwavable degradation/decomposition of target

precursors was reviewed based on the MW, log Kow and pKa of target compounds.

Figure 4.12 showed a bar graph of removal efficiencies of all target compounds (in

alphabetical order) by microwave heating. The removal efficiency of the degradation

product of cimetidine was used due to its rapid degradation. The degradation product of

cimetidine was selected the quantification, *not* for its characterization by

physicochemical properties.

No significant relationship was found for removal efficiency with these

physicochemical parameters of MW, log Kow, and pKa, as shown in **Figures 4.13, 4.14**

and **4.15,** respectively. Only a few of the target pharmaceuticals were effectively

degraded by the microwave system: albuterol (98 ±1%), warfarin (86±1%),

methylphenidate (96±0%), and fluoxetine (96±2%). Polar target compounds having

electrical dipole moments are more likely to be decomposed by dielectric heating because

of dipole rotation in microwave systems. Target compounds with lower log Kow (i.e.,

albuterol, warfarin, and methylphenidate) may have decomposed more favorably than

those with higher log Kow (i.e., fluoxetine) using the microwave oven. The

antidepressant fluoxetine (log Kow = 4.65) was decomposed exceptionally well by

dielectric heating (or thermal degradation). This may be due to the physical structure of

fluoxetine, which is expected to have a high permittivity (ε) to dielectric heating.

However, the majority of the other target compounds were poorly eliminated by both thermal degradation (> 98 ℃) and dielectric heating mechanisms.

4.4.5 Target compounds of least removal

Among 20 unregulated compounds in drinking water samples, the degradation product of cimetidine and PFOS were removed with the least efficiency in all of the water-processes tested. Although PFOS is hydrophobic by fluorocarbons, its sulfonic acids/sulfonate group with an enhanced polarity made it difficult to adsorb onto GAC/ion resins.   In addition, PFOS as a perfluorinated surfactant is very persistent in the environment. It may also be more resistant to thermal degradation and transparent to microwave energy. Using chemical ozonation, PFOS was slightly removed (31%) because fluorine and a sulfonic acid of PFOS can still be reactive to ozone.   The removal of the degradation product of cimetidine was disappointing in these water-processes. Previous studies showed that transformation (degradation) products may be generated that are less biodegradable and/or more toxic than the precursor compounds.[5] In agreement with these studies, it is possible that some degradation products of pharmaceuticals (e.g., cimetidine) may be produced, then remain throughout drinking water treatment with their concentrations increasing when compared to an initial concentration of the precursors. In addition, the antibiotic chlortetracycline was not removed ($\leq$5%) by either adsorption or ozonation mechanisms although it was somewhat removed (52%) by microwave heating. However, the removal efficiency of the antibiotic tetracycline was previously reported to be as high as 80-85% using activated sludge in wastewater treatment plants (WWTP).[24] The antibiotic tetracycline may be removed by

activated sludge system during WWTP prior to becoming a source for drinking water and therefore would be less likely to occur in drinking water samples.[1]

4.4.6 Statistical Analysis

The ANOVA was performed to check for statistical difference in mean removal efficiency for the water-processes tested. The F statistic = 6.71 was larger than the critical F = 3.17, causing rejection the null hypothesis at critical value (α) of 0.05 (see **Table 4.3**). Therefore the mean removal efficiency of GAC/ion resins (66%) was significantly higher than that of either Ozonation (50%), Microwave (27%).

The paired t-test was performed to determine if there is a significant difference between concentrations of 200 ng/L and 2 µg/L for the removal of these compounds by all water-processes tested (H_0: $C_{200ng/L} = C_{2µg/L}$). For the GAC/ion resin system, the removal efficiencies were governed by size and distribution of adsorption pores and therefore, were highly susceptible to saturation of adsorption sites. The statistical analysis was performed if the saturation of adsorption sites has occurred for ibuprofen, which was a potential outlier in the relationship of log Kow and percent removal. **Table 4.4 a** and **b** showed the result of a paired t-test for ibuprofen at two concentrations, 200 ng/L and 2 µg/L. The mean difference (M=-14.16, SD=2.12, N=3) was significantly different than zero, t (2) = -4.73, two-tail p=0.04, providing evidence that the removal was concentration dependent. A 95% confidence interval (C.I.) about mean difference was (-20.89, -7.42). A saturation of adsorption sites may have occurred at the higher concentration (2 µg/L) of ibuprofen. However, the difference was not statistically significant at the 1% level.

An overall difference of percent removal in all target compounds between the two concentrations was not statistically significant in all water-treatment processes. **Table 4.5a** and **b** showed the result of a paired t-test for the GAC/ion resin system. The mean difference (M=1.55, SD=3.81, N=20) was not significantly greater than zero, t (19) = 0.39, two-tail p=0.70, providing evidence that the removal was not concentration dependent. A 95% C.I. about mean difference was (-6.402, 9.51). **Table 4.6a** and **b** showed the result of a paired t-test for the ozonation system. The mean difference (M=-6.27, SD=2.61, N=20) was not significantly different than zero, t (19) = -2.28, two-tail p=0.03, providing evidence that the removal was not concentration dependent. However the p-value (0.03) was lower than the $\alpha = 0.05$ and greater than the $\alpha=0.01$ therefore, 1% of significance level was implied to reduce the chance of rejecting a true H_0 (Type I error). A 99% C.I. about mean difference was (-13.71, 1.16). **Table 4.6a** and **b** showed the result of a paired t-test for the microwave heating system. The mean difference (M=-5.61, SD=2.86, N=20) was not significantly different than zero, t (19) = -1.86, two-tail p=0.08, providing evidence that the removal was not concentration dependent. A 95% C.I. about mean difference was (-11.58, 0.36).

4.5 Conclusions

Based on utilization of current household water treatment processes, a Brita™ filter (GAC/ion resins), an ozonator and a microwave oven were tested for their efficiencies at removing 20 emerging unregulated compounds in drinking water samples. The (arithmetic) mean percent removal using these three household water process was decreased in the order GAC/ion resins (66% and 65%), ozonation (48% and 54%), and microwave heating (30% and 36%). This suggested adsorption (GAC/ion resins) was the

most effective household water process over ozonation and dielectric heating (microwave oven) for removing the target compounds. The mean removal efficiency of these water-processes was tested and showed significant difference using ANOVA. The adsorption properties of GAC/ion resins (Brita™ filter) were governed by these removal factors in a relation to physicochemical properties of target compounds. The relationship of percent removal vs. MW showed a potential factor from size and distribution of pores in GAC/ion resins. The relationship of percent removal vs. log Kow showed hydrophobic and non-polar properties in GAC/ion resins. Lastly, the relationship of pKa vs. percent removal showed the removal by negatively charged surface area of GAC/ion resins. Overall, removal efficiencies of target compounds varied from nearly 0 to 100% among all water processes tested. For all three water-processes, removal efficiencies were not generally affected by concentrations of 2 µg/L vs. 200 ng/L based on paired t-tests. However, the potential saturation of adsorption sites was observed for ibuprofen. Overall, the unregulated compounds tested were partially removed from drinking water by a GAC/ion resin (Brita™), an ozonator or microwave heating.

Among the 20 unregulated compounds in drinking water samples, the degradation product of cimetidine and PFOS were poorly removed ($\leq$27% and $\leq$36%, respectively) in all water-processes tested. This demonstrated the need to consider degradation products of pharmaceuticals and PFCs, which may also be present as unregulated drinking water contaminants. In addition, the antibiotic chlortetracycline was not effectively removed ($\leq$5%) by either adsorption or ozonation mechanism and only partially removed (52%) by microwave heating.

4.6 References

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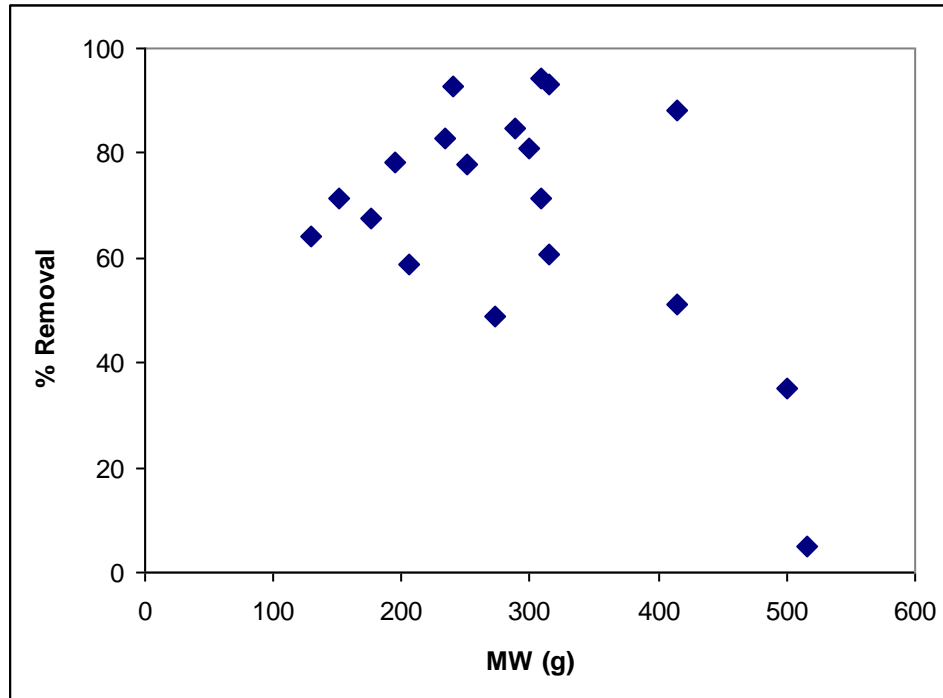
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Figure 4.1 Removal efficiencies of Brita™ for 20 target compounds (in alphabetical order)
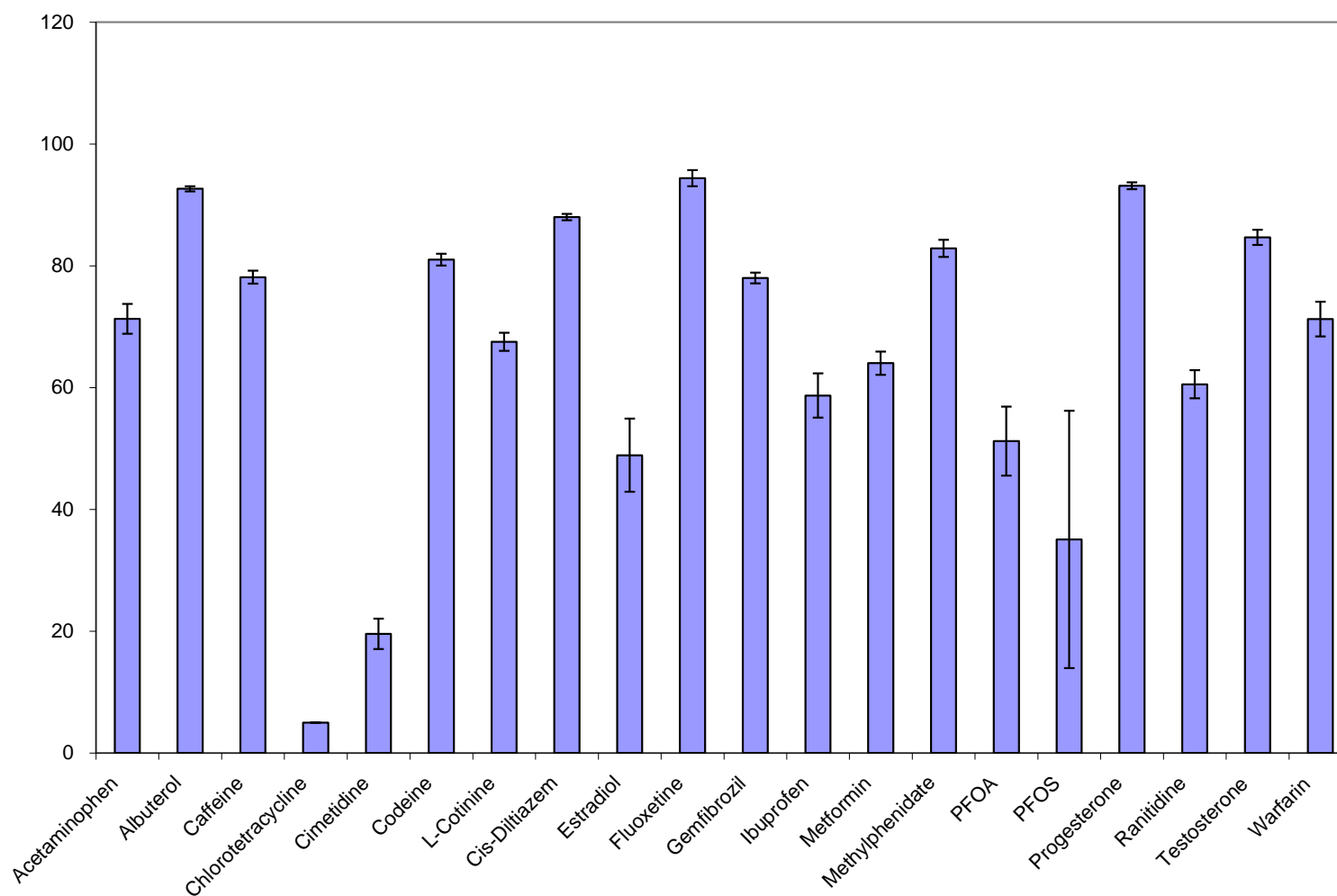
Figure 4.2 Percent removal by GAC/ion resins vs. MW

Figure 4.3 A relationship of percent removal by GAC/ion resins vs. excluding estradiol

The chart shows % Removal on the y-axis (0 to 100) versus MW (g) on the x-axis (0 to 600), with data points and a fitted curve:

$$y = -0.00x^2 + 0.62x - 3.20$$
$$R^2 = 0.71$$

Figure 4.4 Percent removal by GAC/ion resins vs. Log Kow
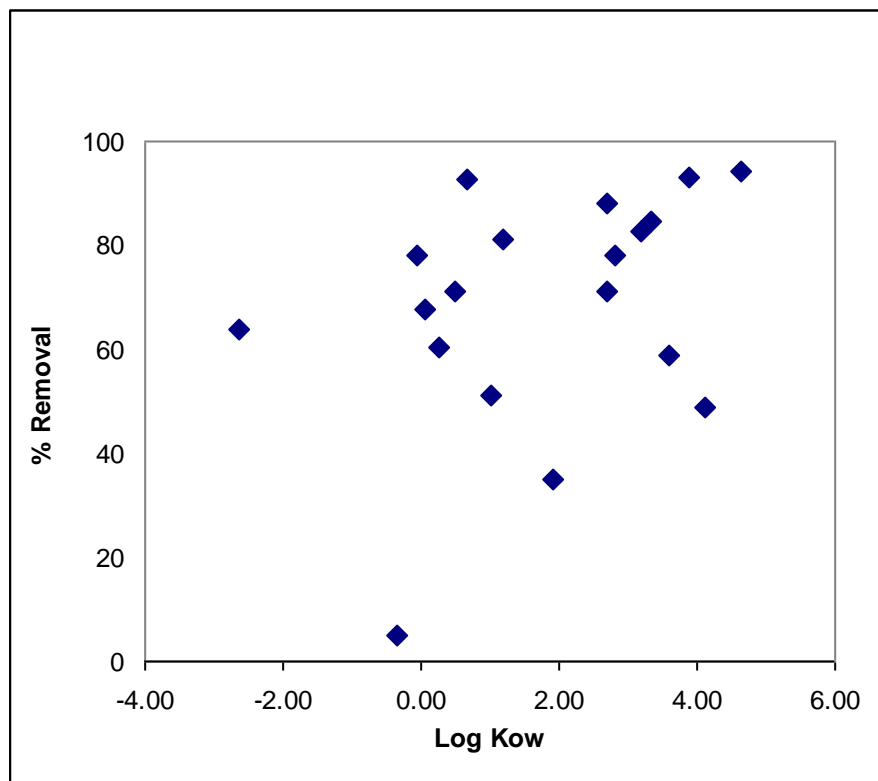
Figure 4.5 A relationship of percent removal by GAC/ion reins vs. log Kow at higher removal rates
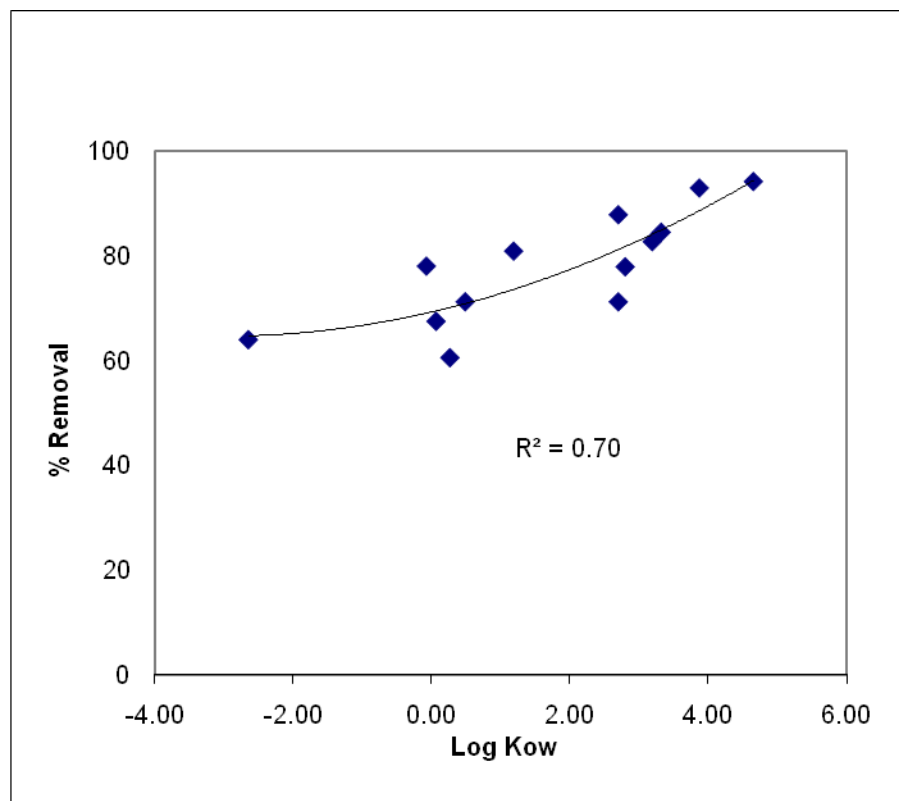
Figure 4.6 Percent removal by GAC/ion resins vs. pKa excluding testosterone and progesterone

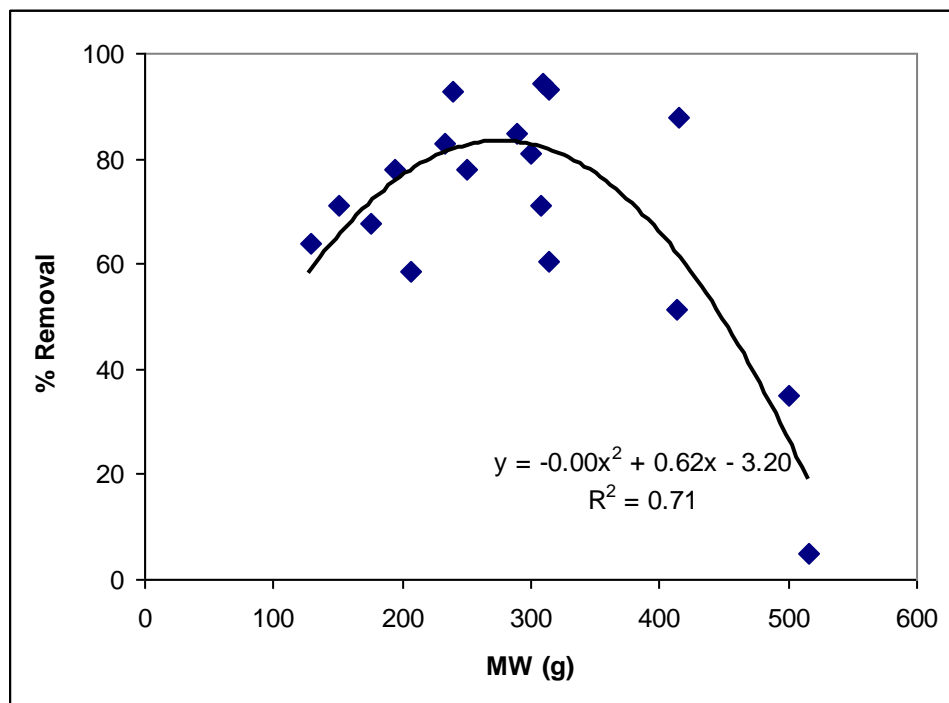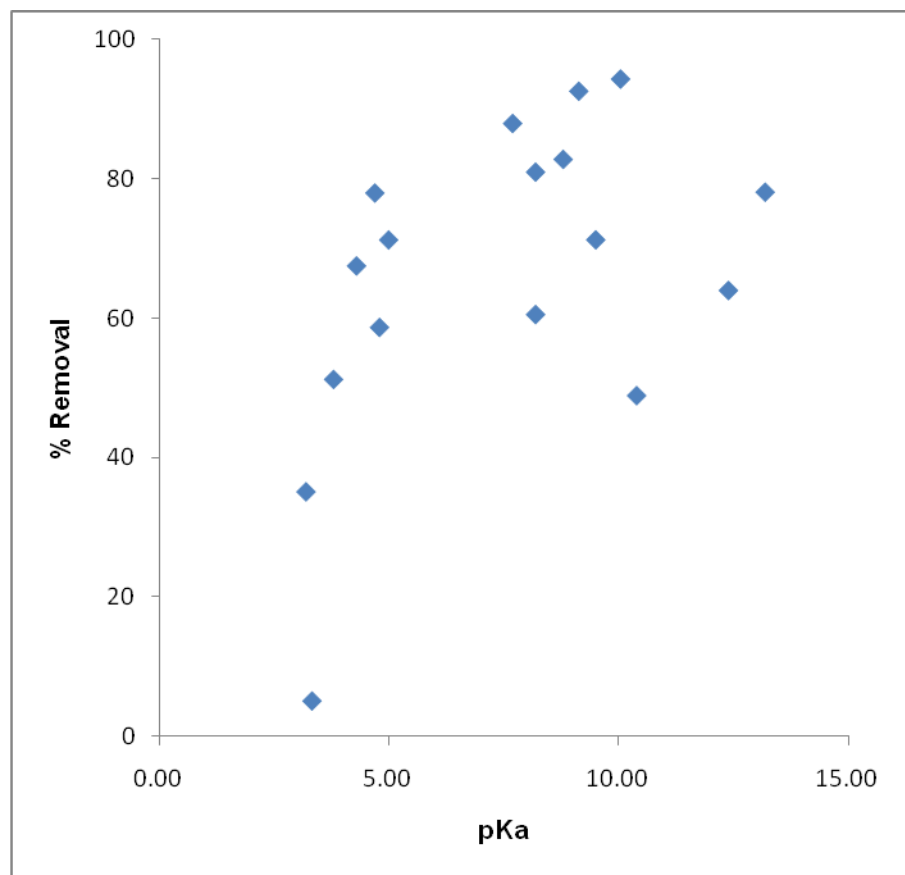**Figure 4.7 Percent removal for GAC/ion resins vs. pKa (≤10)**
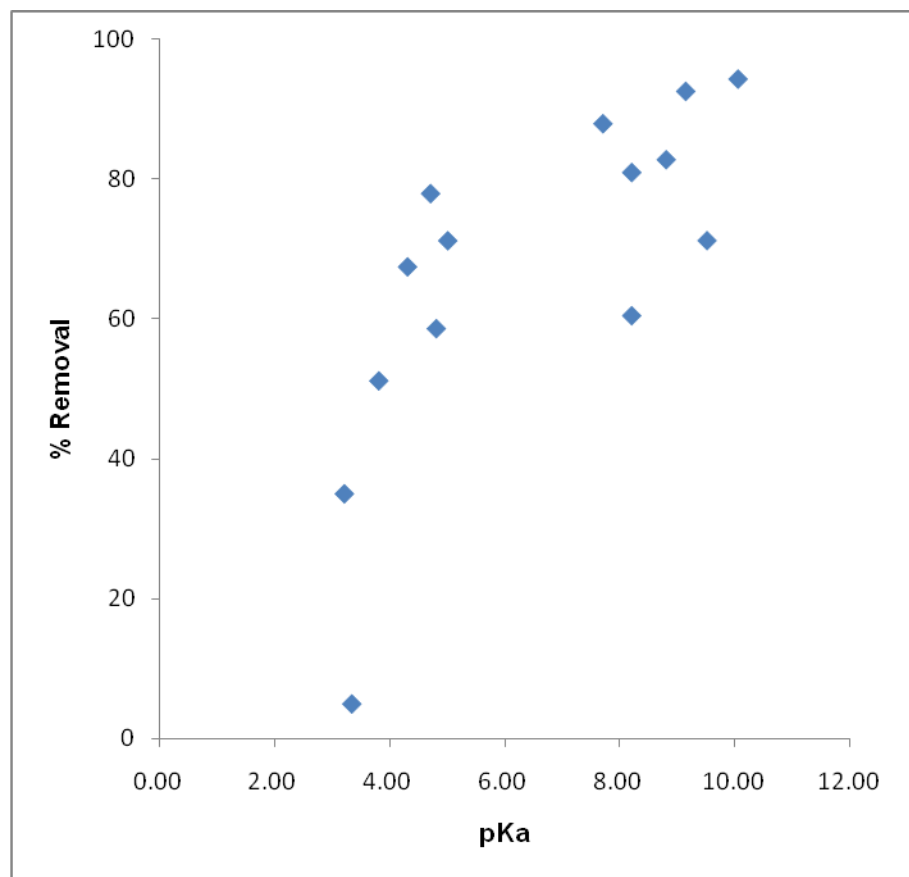
Figure 4.8 Removal efficiencies of ozonation for 20 target compounds (in alphabetical order)
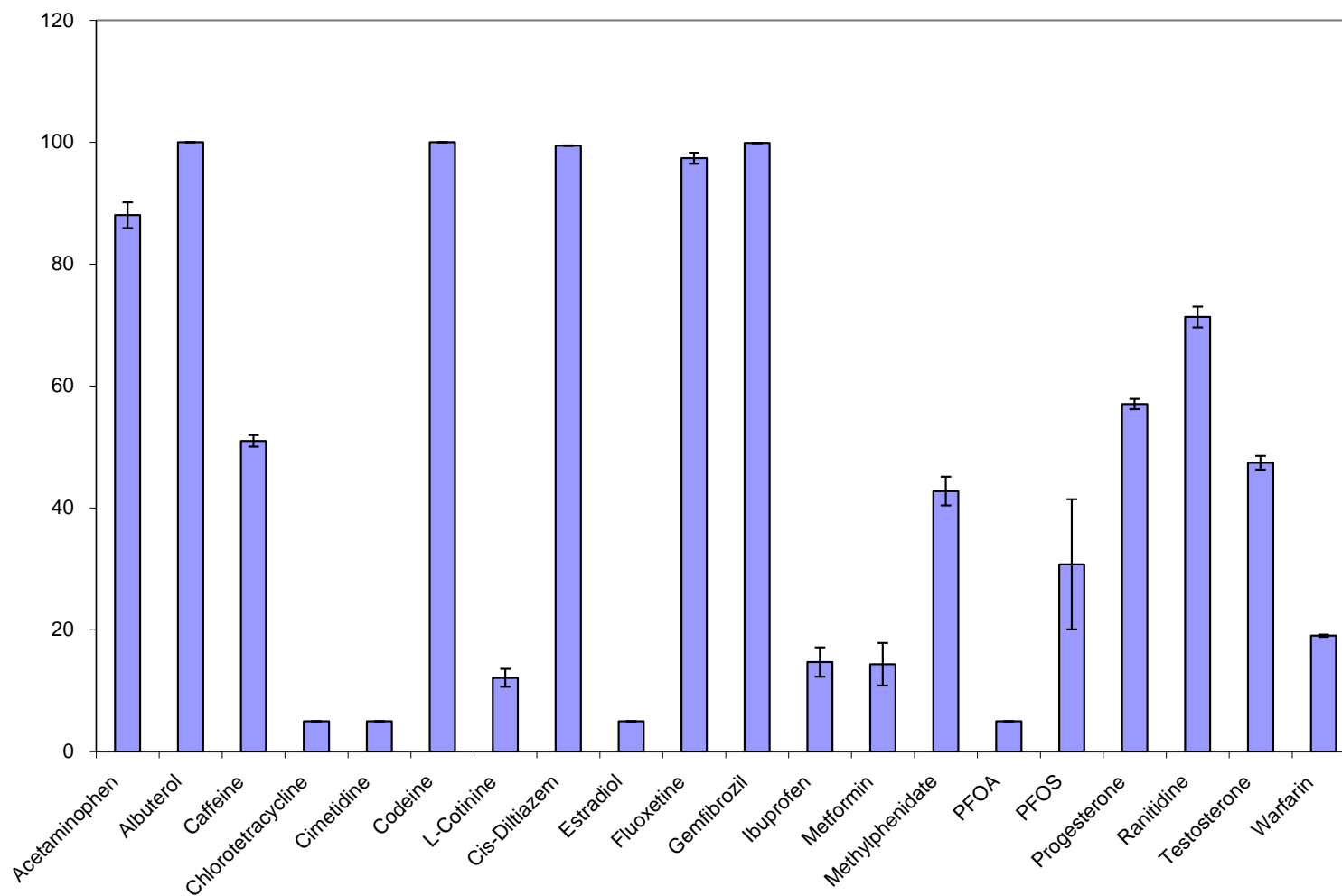
Figure 4.9 Percent removal by ozonation vs. MW
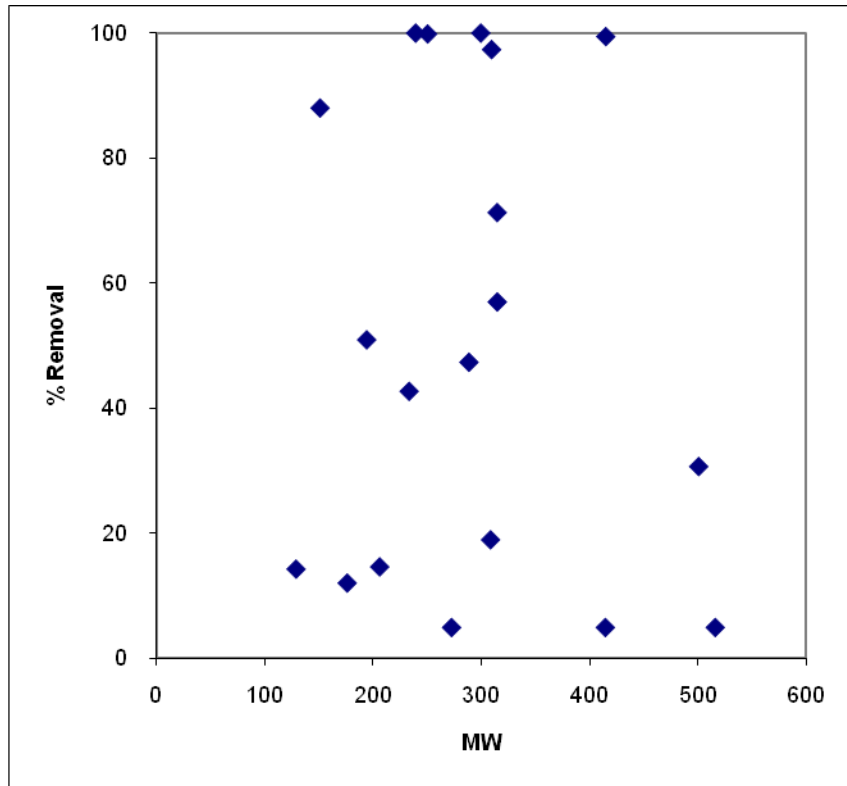
Figure 4.10 Percent removal by ozonation vs. log Kow
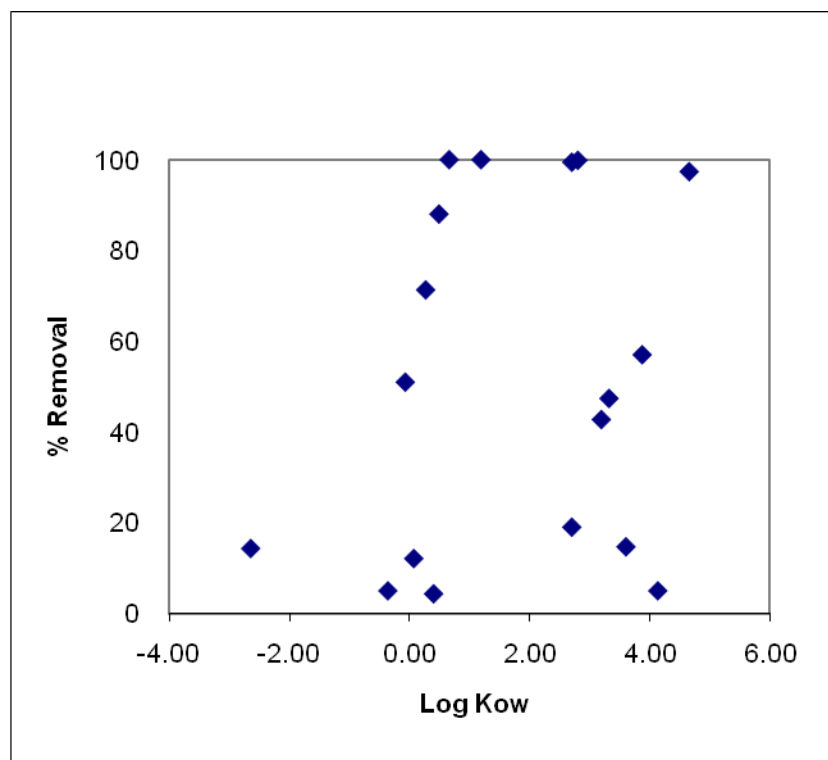
Figure 4.11 A relationship of percent removal by ozonation vs. pKa (≤10) excluding potential outliners
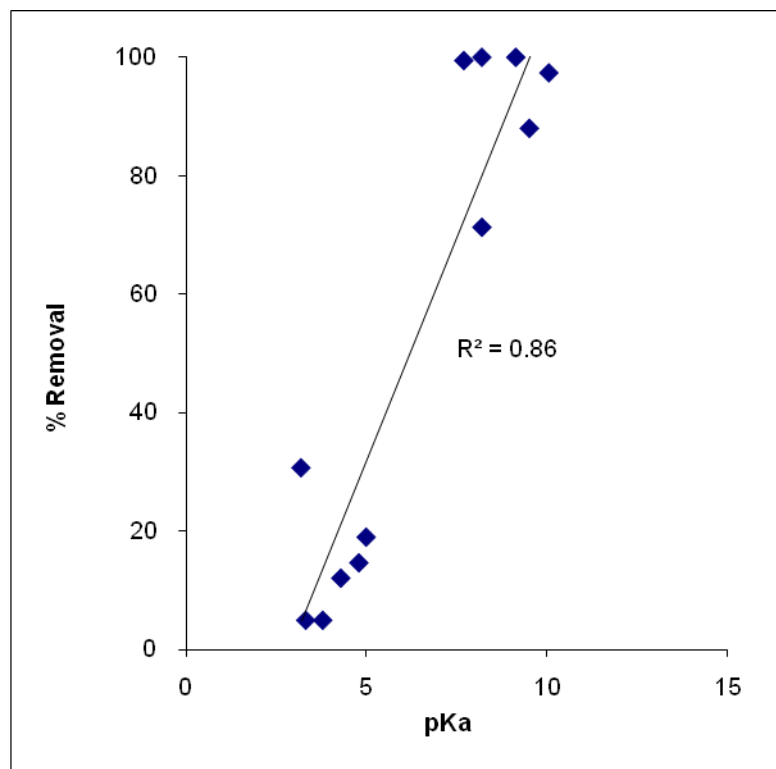
Figure 4.12 Removal efficiencies of microwave for 20 target compounds (in alphabetical order)

Figure 4.13 Percent removal by microwave vs. MW

Figure 4.14 Percent removal by microwave vs. log Kow

Figure 4.15 Percent removal by microwave vs. pKa
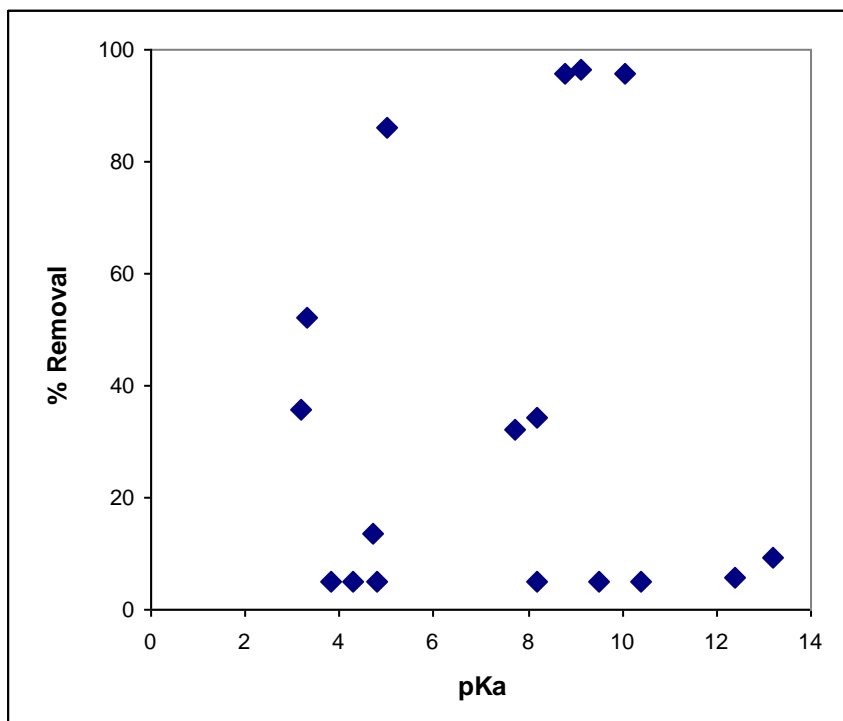
Table 4.1 Physiochemical properties of 20 target compounds: MW, log Kow and pKa

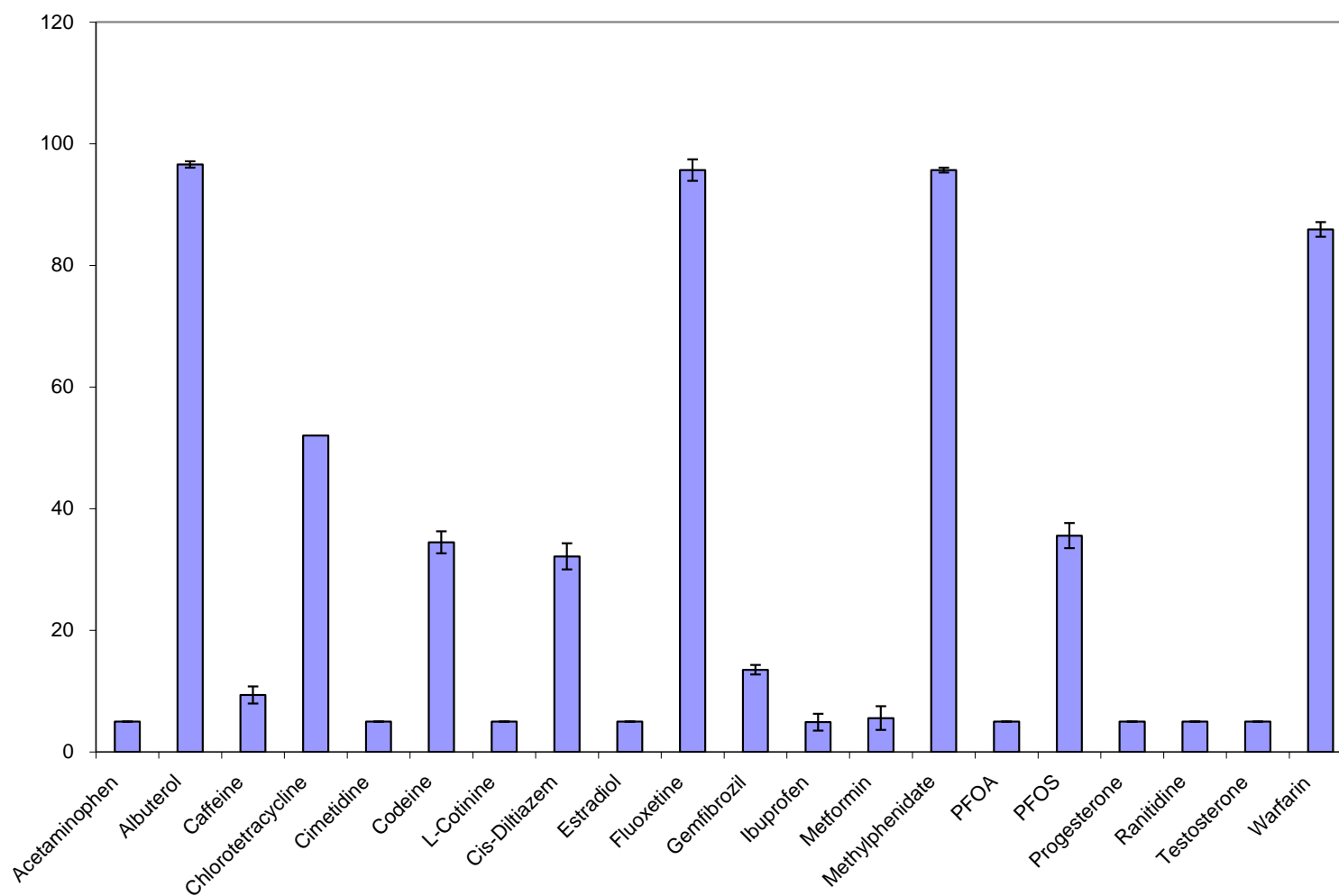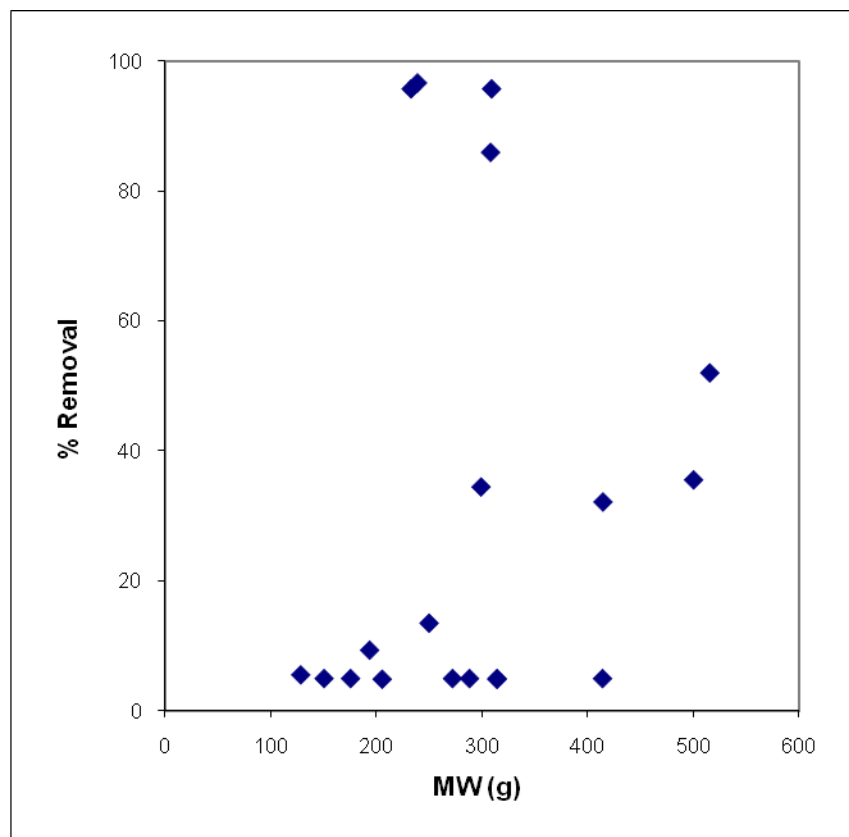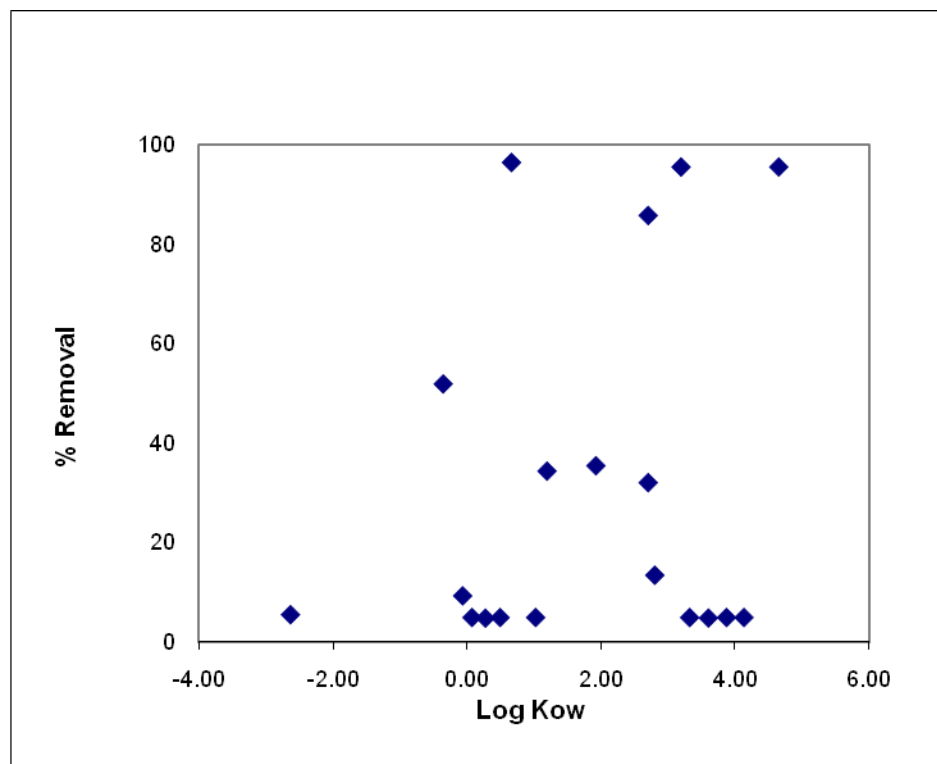| Target compounds	MW(g)	log Kow	References for log Kow	pKa (25°C)	References for pKa
Metformin	129.17	-2.64	liu&Coleman, 2009	2.8	NC van de Merbel, 1998
Acetaminophen	151.17	0.49	Foye's Prin Medic Chem	9.51	Seegers *et al.*, 2006; Bailey & Briggs, 2004
L-Cotinine	176.22	0.07	Arnaud *et al.*, 2004	4.3	Ghosheh *et al.*, 2001
Caffeine	194.19	-0.07	Dias *et al.*, 2007; Khan et al., 2005	13.2	Seeger et al., 2006
Ibuprofen	206.10	3.60	Beetge *et al.*, 2000	4.8	Janjikhel & Adeyeye, 1999
Methylphenidate	233.31	3.19	Qiu *et al.*, 2009 ed.	8.8	Markowitz *et al.*, 2001
Albuterol	239.31	0.66	Foye's Prin Medic Chem	9.14	MSDS (Environmental test; GSK 08/21/01)
Gemfibrozil	250.34	2.8	Luner *et al.*, 1994	4.7	Amjadi *et al.*, 2008
Cimetidine	252.34	0.4	Collett *et al.*, 1999	6.93	Avdeef & Berger, 2001
Estradiol	272.39	4.13	Biber *et al.*, 2005	10.4	Lorphensri *et al.*, 2006
Testosterone	288.42	3.32	Khan *et al.*, 2005	n/a	Heikkinen *et al.,* 2008
Codeine	299.37	1.19	Avdeef *et al.* (1996)	8.2	Wang *et al.*, 2008; Croes *et al.*, 1995
Warfarin	308.33	2.70	Okamoto *et al.*, 2006	5	Zhou *et al.*, 2003
Fluoxetine	309.33	4.65	Fernandes *et al.*, 2006	10.05	Kwon & Armbrust, 2008
Ranitidine	314.41	0.27	Collett *et al.*, 1999	8.2	Khan *et al.*, 2007
Progesterone	314.47	3.87	Bedmar *et al.*, 1996	n/a	Glass *et al.*, 1982
PFOA	414.07	1.02	Higgins et al 2007;Dias *et al.*,2004	3.8	DC Burns *et al.*, 2008
Cis-Diltiazem	414.52	2.70	*Kolovanov and Petrauskas (ACD/LogP)*	7.7	Sammes & Taylor 1990
PFOS	500.13	1.92	Rayne *et al.*, 2009	3.2	Rayne, 2009
Chlortetracycline	515.35	-0.36	Ganellin (Dictionary of Pharm Agents)	3.33	Biswas *et al.*, 2007

Table 4.2 Percent removal and percent relative standard deviation of 20 target compounds in tested water-processes (in alphabetical order)

Treatment process	GAC/ion resin				Ozonation				Microwave			
Concentration	2 ug/L		0.2 ug/L		2 ug/L		0.2 ug/L		2 ug/L		0.2 ug/L	
Target analytes	%removal	%RSD	%removal	%RSD	%removal	%RSD	%removal	%RSD	%removal	%RSD	%removal	%RSD
Acetaminophen	71	3	73	8	88	2	79	5	<5		57	8
Albuterol	93	0	94	0	100	0	100	0	97	1	96	0
Caffeine	78	1	79	2	51	2	68	7	9	15	<5	
Chlortetracycline	<5		55	44	<5		32	47	52	n/a	70	2
Cimetidine	20	13	14	24	<5		27	11	<5		<5	
Codeine	81	1	80	4	100	0	100	0	34	5	55	2
L-Cotinine	68	2	44	19	12	12	44	13	<5		8	26
Cis-Diltiazem	88	1	90	2	99	0	99	0	32	7	33	20
Estradiol	49	12	47	16	<5		<5		<5		<5	
Fluoxetine	94	1	90	2	97	1	97	1	96	2	86	10
Gemfibrozil	78	1	85	5	100	0	99	0	14	6	20	31
Ibuprofen	59	6	73	3	15	16	11	24	5	28	15	20
Metformin	64	3	73	2	14	24	29	10	6	35	13	11
Methylphenidate	83	2	88	1	43	5	62	8	96	0	97	2
PFOA	51	11	48	4	<5		<5		<5		<5	
PFOS	35	60	<5		31	35	30	25	36	6	26	5
Progesterone	93	1	74	1	57	1	55	6	<5		10	31
Ranitidine	61	4	35	13	71	2	61	7	<5		<5	
Testosterone	85	1	62	3	47	2	55	9	<5		<5	
Warfarin	71	4	84	1	19	1	33	26	86	1	97	0
Harmonic mean	39		37		15		24		9		11	
Arithmetic mean	66		65		48		54		30		36	

Table 4.3 Analysis of Variance (ANOVA) for comparing tested water processes

SUMMARY

Groups	Count	Sum	Average	Variance
GAC/ion resin	19	1254.535	66.02816	630.3897
Ozonation	19	946.0137	49.7902	1478.399
Microwave	19	515.3965	27.12613	1131.524

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	14507.76	2	7253.881	6.715909	0.002485	3.168246
Within Groups	58325.62	54	1080.104			
Total	72833.38	56				

Table 4.4 Paired t-test for ibuprofen

a) t-Test: Paired Two Sample for Means

	Variable 1	Variable 2
Mean	58.69	72.85
Variance	13.16	4.24
Observations	3.00	3.00
Pearson Correlation	-0.63	
Hypothesized Mean Difference	0.00	
df	2.00	
t Stat	-4.73	
P(T<=t) one-tail	0.02	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.04	
t Critical two-tail	4.30	

b) Descriptive Statistics

DIFF	
Mean	-14.16
Standard Error	2.12
Median	-14.01
Mode	#N/A
Standard Deviation	4.23
Sample Variance	17.91
Kurtosis	1.50
Skewness	-0.21
Range	10.35
Minimum	-19.48
Maximum	-9.13
Sum	-56.63
Count	4.00
Confidence Level (95.0%)	6.73

Table 4.5 Paired t-test for GAC/ion resin system

a) t-Test: Paired Two Sample for Means

	Variable 1	Variable 2
Mean	66.29	64.74
Variance	598.58	652.08
Observations	20.00	20.00
Pearson Correlation	0.74	
Hypothesized Mean Difference	0.00	
df	19.00	
t Stat	0.39	
P(T<=t) one-tail	0.35	
t Critical one-tail	1.73	
P(T<=t) two-tail	0.70	
t Critical two-tail	2.09	

b) Descriptive Statistics

DIFF	
Mean	1.55
Standard Error	3.81
Median	0.68
Mode	#N/A
Standard Deviation	17.48
Sample Variance	305.51
Kurtosis	2.93
Skewness	-0.87
Range	80.32
Minimum	-50.26
Maximum	30.06
Sum	32.63
Count	21.00
Confidence Level (95.0%)	7.96

Table 4.6 Paired t-test for ozonation system

a) t-Test: Paired Two Sample for Means

	Variable 1	Variable 2
Mean	48.22	54.49
Variance	1450.96	1084.54
Observations	20.00	20.00
Pearson Correlation	0.95	
Hypothesized Mean Difference	0.00	
df	19.00	
t Stat	-2.28	
P(T<=t) one-tail	0.02	
t Critical one-tail	2.54	
P(T<=t) two-tail	0.03	
t Critical two-tail	2.86	

b) Descriptive Statistics

DIFF	
Mean	-6.27
Standard Error	2.61
Median	0.00
Mode	0.00
Standard Deviation	11.97
Sample Variance	143.38
Kurtosis	-0.42
Skewness	-0.76
Range	42.46
Minimum	-32.17
Maximum	10.29
Sum	-131.68
Count	21.00
Confidence Level (99.0%)	7.43

Table 4.7 Paired t-test for microwave system

a) t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	30.06	35.67
Variance	1245.09	1268.98
Observations	20.00	20.00
Pearson Correlation	0.93	
Hypothesized Mean Difference	0.00	
df	19.00	
t Stat	-1.86	
P(T<=t) one-tail	0.04	
t Critical one-tail	1.73	
P(T<=t) two-tail	0.08	
t Critical two-tail	2.09	

b) Descriptive Statistics

DIFF	
Mean	-5.61
Standard Error	2.86
Median	-1.63
Mode	0.00
Standard Deviation	13.13
Sample Variance	172.29
Kurtosis	7.66
Skewness	-2.37
Range	62.31
Minimum	-52.16
Maximum	10.16
Sum	-117.81
Count	21.00
Confidence Level (95.0%)	5.97

Implications and Conclusions

This work was focused on a growing environmental and public concern, U.S. drinking water contamination. Organic wastewater contaminants (OWCs), pharmaceuticals, antibiotics, steroid hormones and perfluorinated compounds (PFCs) were identified as emerging unregulated organic contaminants in drinking water sources. Some of these contaminants were determined to be "likely carcinogens" to humans and/or endocrine disruptors. More significantly, toxicological effects were previously studied on individual compounds, but not yet understood for mixed and/or altered forms of pharmaceuticals and other OWCs in drinking water. These OWCs were however, frequently found in U. S. drinking water supplies as reported by the U.S. Geological Survey (USGS).

In previous studies, volatile and semi-volatile organic contaminants had been measured in raw and finished water using a gas chromatography coupled to mass spectrometry (GC-MS). The GC-MS method was able to detect pesticides including organochlorine pesticides, organophosphorus pesticides, and nitrogen containing pesticides as well as polynuclear aromatic hydrocarbons and polychlorinated biphenyls. The method was not, however, able to analyze many other organic non-volatile contaminants found in drinking water. The development of a liquid chromatography - mass spectrometry method was performed to analyze a broad range of organic contaminants including non-volatiles in drinking water.

An analytical method was developed for quantitative isolation and measurement of unregulated organic contaminants in aqueous matrices. Twenty target unregulated compounds from various classes of pharmaceuticals, antibiotics, steroid hormones and

PFCs were selected for this work based on their frequency of detection in a previous USGS study. The method was optimized for solid phase extraction (SPE) and liquid chromatography – tandem mass spectrometry (LC-MS/MS), creating enhanced sensitivity amenable to drinking water samples. The optimized extraction protocols demonstrated high recoveries (~100%) for PFCs as well as relatively high recoveries (>70%) for 20 target compounds determined simultaneously, with one exception; cimetidine was recovered at $50 \pm 11\%$ in water samples (1L) from this study.

Two LC-ion trap MS systems were compared for rapid, reliable, and sensitive detection using PFCs. An ultra performance LC-linear ion trap MS/MS achieved the lowest detection limits measured at 0.03pg and 0.24pg for PFOA and PFOS respectively, which were approximately two orders of magnitude more sensitive than a similar LC-MS/MS. The enhanced sensitivity of the method allowed for the direct analysis of PFOA/S in drinking water. This method therefore eliminates a potential background contamination issue created by SPE steps and substantially reduces its time and cost for routine drinking water analysis.

Simultaneous detection of twenty target compounds including pharmaceuticals was demonstrated firstly using a rapid polarity switching LC-MS/MS method. Sensitive method detection limits were achieved in the range of 0.18 ng/L to 3.6×10^2 ng/L for all target compounds. This optimized method was applied to quantify 1 ng/L to 6 µg/L levels of unregulated compounds in field samples of tap, well (raw and treated) and storm water in New Jersey. Eight target compounds were measured below 1µg/L and two target compounds (i.e., metformin and estradiol) were measured slightly above 1µg/L in field water samples collected throughout New Jersey.

Finally, commercial household water treatment processes, Brita™ using granular activated carbon (GAC) and ion resin filtration, ozonation, and microwave heating were examined for the efficacy at removing these 20 target compounds from drinking water samples. The Brita™ filtration showed greater removal of the target compounds than the other two systems however, it only partially removed them from drinking water samples with a mean removal of 65-66%.

In the GAC/ion resin treatment-process, the adsorption was believed to be the dominant process, which was governed by molecular weights and log Kow of these target compounds. In the ozone treatment-process, an ozone reactivity of target compounds was mainly governed by pKa, which targeted compounds with higher pKa (but not exceeding pKa =10) that were more reactive and more effectively removed by ozonation. In the microwave (dielectric) heating process, no apparent relationship with any of the 3 physicochemical properties was found; however, compounds with high polarity and permittivity were observed to be targeted for greater removal. For all three water-processes, efficacies with one exception were not significantly affected by concentration (200 ng/L vs. 2 µg/L) based on paired t-test.

Overall, these studies demonstrated the need for monitoring of drinking water for unregulated OWCs, based both on frequency of detection and an inability of household treatment processes to effectively remove them. All twelve field water samples of raw or finished water had detectable quantities of a number of target compounds ranged from 6 to 11 with a mean frequency of detection of 80%.

Future work should target removal by combined drinking water treatment processes with higher efficacies at removing these unregulated OWCs. More

sophisticated physicochemical characteristics/relationship between target compounds of one or more classes and their removal efficiencies may be used to create a more effective removal process. The degradation products of histamine H_2-receptor antagonists (i.e., cimetidine and ranitidine) had higher concentrations than the precursors demonstrating degraded and/or altered forms of many other pharmaceuticals may be included as a part of the unregulated OWCs targeted for removal from drinking water. The adaptation and optimization of the analytical method should therefore be continued for measurement of new and/or other emerging organic contaminants in drinking water sources.

Curriculum Vita

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Education

September 2001 – May 2005   Rutgers University, New Brunswick, NJ

Bachelor of Arts, Statistics

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Publications

Min K. Yoon, R. Lee Lippincott, Ill Yang, Eileen Murphy and Brian Buckley, "An Analytical Method Using Sequential Positive and Negative Ionization Modes for the Measurement of Twenty Unregulated Compounds In Drinking Water Samples by LC-MS/MS". Submitted to *Journal of Mass Spectrometry,* **2010** Manuscript ID: JMS-10-0056.

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