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Assessment of the Occurrence and Potential Effects of Pharmaceuticals and Personal Care Products in South Florida Waters and Sediments

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ASSESSMENT OF THE OCCURRENCE AND POTENTIAL EFFECTS OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN SOUTH FLORIDA WATERS AND SEDIMENTS

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by

Chengtao Wang

2012

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Chengtao Wang, and entitled Assessment of the Occurrence and Potential Effects of Pharmaceuticals and Personal Care Products in South Florida Waters and Sediments, having been approved in respect to style and intellectual content, is referred to you for judgment.

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DEDICATION

It is not easy to start pursuing a Ph.D and it becomes even harder towards the end. It is a kind of job that makes me start to question myself; it is a period of time in my life that I am desperate to call for help. But I am indeed blessed.

I dedicate this dissertation to my parents, without their understanding and support and most of all love, it is impossible for me to walk so far on my way of pursuing my dream. They not only gave me life but also taught me how to become a better person.

I dedicate this dissertation to my husband, without him, I will never know the colorful and beautiful part of life. He lets me feel peaceful and powerful when I face difficulties.

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ABSTRACT OF THE DISSERTATION

ASSESSMENT OF THE OCCURRENCE AND POTENTIAL EFFECTS OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN SOUTH FLORIDA WATERS AND SEDIMENTS

by

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Florida International University, 2012

Miami, Florida

Professor Piero R. Gardinali, Major Professor

A LLE-GC-MS method was developed to detect PPCPs in surface water samples from Big Cypress National Park, Everglades National Park and Biscayne National Park in South Florida. The most frequently found PPCPs were caffeine, DEET and triclosan with detected maximum concentration of 169 ng/L, 27.9 ng/L and 10.9 ng/L, respectively. The detection frequencies of hormones were less than PPCPs. Detected maximal concentrations of estrone, 17β-estradiol, coprostan-3-ol, coprostane and coprostan-3-one were 5.98 ng/L, 3.34 ng/L, 16.5 ng/L, 13.5 ng/L and 6.79 ng/L, respectively.

An ASE-SPE-GC-MS method was developed and applied to the analysis of the sediment and soil area where reclaimed water was used for irrigation. Most analytes were below detection limits, even though some of analytes were detected in the reclaimed water at relatively high concentrations corroborating the fact that PPCPs do not significantly partition to mineral phases.

An online SPE-HPLC-APPI-MS/MS method and an online SPE-HPLC-HESI-MS/MS method were developed to analyze reclaimed water and drinking water samples. In the reclaimed water study, reclaimed water samples were collected from the sprinkler for a year-long period at Florida International University Biscayne Bay Campus, where reclaimed water was reused for irrigation. Analysis results showed that several analytes were continuously detected in all reclaimed water samples. Coprostanol, bisphenol A and DEET's maximum concentration exceeded 10 µg/L (ppb). The four most frequently detected compounds were diphenhydramine (100%), DEET (98%), atenolol (98%) and carbamazepine (96%). In the study of drinking water, 54 tap water samples were collected from the Miami-Dade area. The maximum concentrations of salicylic acid, ibuprofen and DEET were 521 ng/L, 301 ng/L and 290 ng/L, respectively. The three most frequently detected compounds were DEET (93%), carbamazepine (43%) and salicylic acid (37%), respectively. Because the source of drinking water in Miami-Dade County is the relatively pristine Biscayne aquifer, these findings suggest the presence of wastewater intrusions into the delivery system or the onset of direct influence of surface waters into the shallow aquifer.

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LIST OF ABBREVIATIONS

ABBREVIATION	FULLNAME
APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photoionization
ASE	accelerated solvent extraction
BCNP	Big Cypress National Preserve
BNP	Biscayne National Park
BSTFA	N,O-bis- (trimethylsilyl)trifluoroacetamide
CE	Capillary electrophoresis
CI	Chemical ionization
DA	dopant-assisted
DEET	N,N-Diethyl-3-methylbenzamide
E1	Estrone
E2	17β-estraiol
EDCs	endocrine disrupting compounds
EE2	ethynylestradiol
EI	electron impact
ENP	Everglades National Park
EPA	Environmental Protection Agency
EQuan	Environmental Quantitation
ESI	electrospray ionization

GC	gas chromatography
HLB	Hydrophilic-Lipophilic Balanced
HPLC	high performance liquid chromatography
IT	iontrap
LLE	Liquid-liquid extraction
LIT	linear ion trap
LOEL	lowest observable effect level
MDLs	method detection limits
MEA	microwave-assisted extraction
MGD	million gallons per day
MS	mass spectrometry
MSTFA	N-methyl-trimethylsilyltrifluoroacetamide
MTBSTFA	N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide
NI	negative ionization
OTC	over-the-counter
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated biphenyls
PI	positive ionization
PLE	pressurized liquid extraction
PPCPs	Pharmaceuticals and Personal Care Products
QqQ	Triple quadrupole
RRF	relative response factor
S/N	signal to noise

SPME	solid phase microextraction
SPE	solid phase extraction
SIM	selective ion monitoring
TMCS	trimethylchlorosilane
TMSI	trimiethylsilylimidazole
TOF	time of flight
UAE	ultrasonic assisted extraction
WWTPs	wastewater treatment plants

CHAPTER 1

INTRODUCTION

1.1 What are PPCPs?

Pharmaceuticals and Personal Care Products (PPCPs) are defined as any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock. Pharmaceuticals and Personal Care Products comprise a diverse collection of thousands of chemical substances, including prescription and nonprescription drugs, veterinary drugs, fragrances, and cosmetics (URL1). Pharmaceuticals and Personal Care Products are a wide variety of important “unrecognized” or “emerging” pollutants in everyday urban activities. The United States Environmental Protection Agency (EPA) defines emerging pollutants as new chemicals without regulatory status and the influence of emerging pollutants on environmental and human health are poorly understood to say the least.

1.2 Why do we need to monitor them?

Many pharmaceuticals are not completely eliminated by the human body and often are excreted only slightly transformed or even unchanged (Reddersen et al., 2002). The disposal of unused medication via a household sink or toilet brings pharmaceuticals directly to the wastewater treatment plants (WWTPs) in relatively high concentrations. Several investigations have shown that current wastewater treatment processes (physical and biological) could not remove PPCPs completely from effluents of WWTPs (Ingrand et al., 2003; Esperanza et

al., 2004; Sui et al., 2010; Lacey et al., 2011; Ryu et al., 2011). The typical rate of removal of analgesics, anti-inflammatories and beta-blockers are 30% to 40%. The average rate of removal is about 50% for antibiotics and 71% for compounds like bisphenol A (Deblonde et al., 2011). More recent research indicates that advanced treatment steps (e.g., ultrafiltation, flocculation, ozonation, advanced oxidation or osmosis) are usually required to increase removal of micropolluants (Fatta-Kassinos et al., 2010). However, these treatment steps are seldom used in the WWTPs because of their high costs. Therefore, the effluents of wastewater treatment plants bring pharmaceuticals to the surface waters when the effluents are discharged into rivers, lakes or oceans. If the effluent is reused for irrigation or landscape, PPCPs may be transferred to soil and enter to surface water through runoff (Heberer et al., 2002). When active recharge is used, PPCPs may also leach into an aquifer and be transported into ground waters, which are potential drinking water sources. That is likely the main reason of why PPCPs are reported in the ground water and drinking water samples. The presence of PPCPs in the ground water may also be caused by the influence of landfill leachates. Meanwhile, large use of veterinary drugs may also cause the occurrence of PPCPs residues in the environment. Figure 1 shows the possible sources and pathways of PPCPs in the environment.

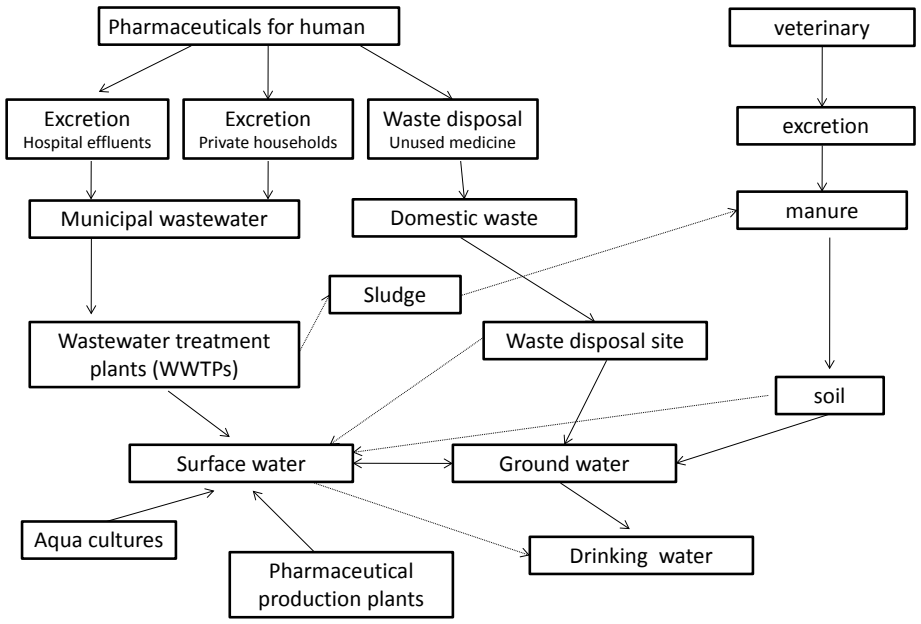


Figure 1. Possible sources and pathways for the occurrence of PPCPs residues in the environment

One issue about PPCPs present in the environment is that the endocrine disrupting effect of some PPCPs may occur even at very low concentration (Caliman and Gavrilescu, 2009). Until now, very little is known about the long-term effect of PPCPs on aquatic organisms. For most human pharmaceuticals, it is unlikely that they will have acute effects on aquatic organisms except for the cases of a direct spill/disposal. However, understanding the chronic effects and toxicity of pharmaceuticals mixtures are more important because many aquatic species are continuously exposed to PPCPs over their entire life cycle. Despite this, there is very little data about chronic effects of pharmaceuticals on the aquatic organisms except for ethynylestradiol (EE2). Ethynylestradiol shows estrogenic effects in many fish at extremely low concentrations. For fathead

minnows, egg fertilization of female was significantly decreased at extremely low concentration of 0.32 ng/L ethynylestradiol. For males, demasculinization (decreased male secondary sex characteristic index) happened when males exposed to EE2 at a concentration of 0.96 ng/L (Parrott and Blunt, 2005). In addition, life exposure of zebrafish to EE2 at a concentration of 0.05 ng/L caused the secondary sexual characteristics of males to become significantly feminized (Larsen et al., 2008). Very few chronic effect data of analgesics, non-steroid anti-inflammatories and beta-blockers are available. Diclofenac was found to have chronic histopathological effects in rainbow trout at a concentration of 5 µg/L (Schwaiger et al., 2004). Propranolol has chronic toxicity for fish not only on the cardiovascular system but also on reproduction. Reproduction of *C.dubia* and *H. azteca* was affected by propranolol at 250 µg/L and 100 µg/L after 14 days of exposure, respectively (Huggett et al., 2002). Typically toxicity of single compound may show no or only little effects at certain concentration, but mixtures of many pharmaceuticals may have effects at the same concentrations due to synergistic effects. Study on mixtures of NSAID (diclofenac, ibuprofen, naproxen, acetylsalicylic acid) on *Daphnia* and algae demonstrated the mixture followed the concept of concentration addition (Michael, 2003). Even though the reported lowest observed effect concentrations (LOEC) of PPCPs are usually higher than measured concentrations in the effluent of WWTPs or surface water, monitoring PPCPs in the environment is still an urgent need. The general lack of chronic toxicity data on pharmaceuticals requires not only more investigation about potential ecotoxicological effects such as endocrine disruption,

immunological status, or gene activation and silencing during long-term exposure but also the development of robust, low-level, selective, high throughput analytical techniques.

Besides aquatic organisms like fish, plants may also be affected, to a lesser extent, by PPCPs when treated wastewater is reused for irrigation. It is not clear the negative effects on plants are from direct damage of PPCPs to plant or from the indirect damage of PPCPs to the soil microorganisms (Sabourin et al., 2009; Matamoros and Salvado, 2012).

Another important issue is the presence of chronic levels of antibiotics in the environment. Presence of antibiotics is often suggested as a potential link to the development of antibiotic-resistant bacteria. The resistant bacteria can be transferred to human via water or food if plants are irrigated by reclaimed water, surface water or sludge with antibiotic-resistant bacteria which have escaped from treatment (Fatta-Kassinos et al., 2010).

1.3 What are the typical procedures to detect them?

The two key challenges for the detection of emerging microconstituents is interference from complex matrices and the relatively low concentration of analytes in the environment. However, with the development of sophisticated and sensitive analytical protocol, such as more efficient extraction techniques and

more sensitive detectors, trace level PPCPs can be detected in the environment. Typical analysis procedure for aqueous and solid samples is shown in figure 2.

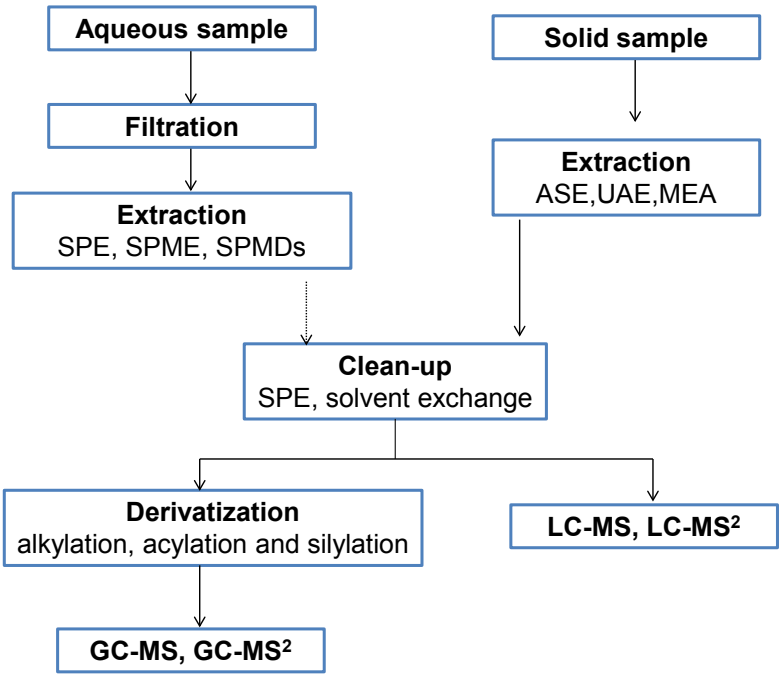


Figure 2. Typical analysis procedure for aqueous and solid samples

1.3.1 Sample preparation for aqueous samples

Currently, available extraction techniques for aqueous samples (e.g., tap water, surface water and wastewater) include Liquid-liquid extraction (LLE) (Zafra et al., 2003), solid phase extraction (SPE) (Zaharie, 2006; Gómez et al., 2007; Gros et al., 2009) and solid phase microextraction (SPME) (Pablo Lamas et al., 2004) etc. Solid phase extraction is the most widely used extraction method. Solid phase extraction cartridge sorbents include non-polar phase, ion-exchange phase and polymeric phase. Among them, the Oasis HLB (Waters Corp, Hydrophilic-Lipophilic Balanced phase) cartridge is able to extract both polar and nonpolar

analytes under the same conditions and improve simultaneous detection of analytes with markedly different chemical properties. In order to increase the capacity of multiple-residues extraction, several researches started to use tandem or serial mixed-mode cartridges to increase analyte recoveries (Gros et al., 2009; Laven et al., 2009). The development of on-line SPE simplifies the extraction procedure and reduces the sample preparation time. Therefore, more and more studies begin to focus on on-line SPE method development (Segura et al., 2007; Garcia-Ac et al., 2009; Lopez-Serna et al., 2010). Because of the repeated use of the SPE mini-cartridges, development of on-line methods require many additional optimization of parameters, such as sample size, the sample loading flow, and wash step, but the additional work is clearly offset by the gain in processing speed as a result of the system automation.

Solid phase microextraction is another extraction technique that starts to attract interest for the analysis of many organic compounds in aqueous samples. Solid phase microextraction extracts target compounds from sample to an absorptive layer of sorbent coated on a fiber. The quantity of target compounds extracted is proportional to the concentration of target compounds in the sample. After extraction, the fiber can be transferred to the injection port of GC, where desorption happens and analysis starts. The advantage of SPME is solvent free and very simple sample preparation (Carballa et al., 2004; Pablo Lamas et al., 2004; Fatta-Kassinos et al., 2010). However, the use of SPME is still limited by the fiber activation and cleanup steps and is much more difficult to automate.

1.3.2 Sample preparation for solid samples

For solid matrices (e.g., soils, sediments, sludge and fish tissues), accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE), ultrasonic assisted extraction (UAE) and microwave-assisted extraction (MEA) have been used to enhance extraction efficiency (Löffler and Ternes, 2003; Burkhardt et al., 2005; Xu et al., 2008; Jelic et al., 2009; Vazquez-Roig et al., 2010). Usually SPE is used as a clean-up step after the extraction mentioned above to reduce the interference from environmental samples due to the complexity of matrices.

1.4 Instrumental detection

Detection and quantification of PPCPs in the environment were a big challenge several years ago because of the complexity of matrices and PPCPs' low concentration of occurrence. Currently, gas chromatography (GC), coupled with mass spectrometry (MS) and high performance liquid chromatography (HPLC), coupled with MS, provide the opportunity to detect PPCPs down to extremely low concentrations in the ng/L (parts per trillion) range. The choice of GC or HPLC depends on the chemical properties of the target compounds. Generally, GC is appropriate for identification and quantification of volatile or volatizable compounds, while HPLC is used to determine more polar and less volatile compounds. For the investigation of PPCPs in the environment, GC-MS, GC-MS^2, LC-MS, LC-MS^2 have become indispensable tools. Capillary electrophoresis (CE) also is used to analyze pharmaceuticals and personal care products. However, CE without preconcentration can only reach concentrations

in the µg/L range limiting its application. Therefore, GC or LC coupled with MS is still the primary tools for analysis of PPCPs in the environment.

1.4.1 GC-MS and GC-MS^2

The use of GC-MS to determine PPCPs in the environment started in the nineteen seventies (Garrison et al., 1976). Nowadays, GC-MS and GC-MS^2 are still the most widely used techniques because of their availability in environmental laboratories. The major advantage of GC coupled with MS is that the ionization modes like electron impact (EI) or chemical ionization (CI) are generally less affected by the sample matrix than electrospray ionization (ESI), the main ionization mode used for liquid chromatography mass spectrometry.

A derivatization step is usually needed for highly-polar, thermally-fragile compounds to make them suitable for GC analysis. Usually, GC-MS analysis after derivatization is an effective alternative to liquid chromatography mass spectrometry. Derivatization is usually done by substitution on the polar functional group and the most common reactions are alkylation, acylation and silylation (Shareef et al., 2006; Schummer et al., 2009). The largest limitation to this approach is the derivatization step itself. The efficiency of derivatization is influenced by the derivatizing agent and solvent, reaction temperature and reaction time. These parameters need to be optimized to increase the signal to noise (S/N) for analytes. The most commonly used derivatization technique is silylation, and common reagents include trimethylchlorosilane (TMCS),

trimiethylsilylimidazole (TMSI), N-methyl-trimethylsilyltrifluoroacetamide (MSTFA), N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), where the last two are the most frequently reported in the analysis of PPCPs (Boyd et al., 2003; Rice and Mitra, 2007; Xu et al., 2008; Durán-Alvarez et al., 2009).

The column used to separate PPCPs in GC analysis includes DB5, DB5-MS, HP5-MS and their equivalents. The dimension of the column is usually 30 m × 0.25 mm × 0.25 μm and longer columns or thicker film phases can be used to improve the separation of PPCPs. Normal injection volume is 1-2 μL. While large volume injection has been reported to decrease the method detection limits (MDLs) of GC analysis the introduction of co-extractants and residues from the derivatizing agents do compromise the column integrity so its use is not widespread. Typical temperature program is from 50 °C to 300 °C with a run time of 30 - 45 mins (Boyd et al., 2003; Zafra et al., 2003; Weigel et al., 2004; Gibson et al., 2007; Gómez et al., 2007).

Most of the publications focused on EI with -70 eV standard ionization energy. Electrons are continuously emitted from a heated filament (200-280 °C) and collide with analytes that elute from the end of the GC column. Qualification analysis of PPCPs and their metabolites can be achieved by full-scan mass spectra with the help of computer libraries, which have thousands of standard mass spectra. Quantification analysis is usually achieved by compounds

molecular ions and fragment ions in selective ion monitoring (SIM). Selective ion monitoring improves sensitivity of target compounds by only acquiring data of target compounds with no attempt to acquire data of non-target compounds (Boyd et al., 2003; Zafra et al., 2003; Weigel et al., 2004; Lishman et al., 2006; Gibson et al., 2007).

Gas chromatography tandem mass spectrometry is able to achieve excellent selectivity and sensitivity by suppression of matrix backgrounds. The MS^2 experiment can be implemented by ion-trap and triple-quadrupole mass analyzers. The precursor ions are selected and the fragmentations are optimized to obtain the best S/N ratio. Therefore, MS^2 has been used for the detection of trace level analytes present in complex matrices like wastewater, sediment and sludge (Verenitch et al., 2006; Gómez et al., 2007).

1.4.2 LC-MS and LC-MS^2

Although MS has the ability to simultaneously identify target compounds, LC separation is still needed, especially for isomeric chemicals. Reversed-phase analytical columns are most commonly used to separate pharmaceuticals and personal care products. The typical particle size of analytical columns is between 1.9 μm and 3 μm. The organic mobile phase includes methanol, acetonitrile or a combination of these two solvents, while the aqueous phase is water with the addition of formic acid, acetic acid, ammonium hydroxide, ammonium formate or ammonium acetate to adjust pH.

1.4.3 Ionization techniques

High performance liquid chromatography mass spectrometry has been shown as a valuable alternative for detection of PPCPs and EDCs to overcome the drawbacks of GC-MS (Gardinali and Zhao, 2002; Gentili et al., 2002; Ingrand et al., 2003; Cahill et al., 2004; Castiglioni et al., 2005; Schlüsener and Bester, 2005; Martnez Bueno et al., 2007; Gros et al., 2009; Laven et al., 2009; Huerta-Fontela et al., 2010; Jian-lin et al., 2010). Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are the three most common ionization techniques coupled with liquid chromatography (Marchi et al., 2009). Electrospray ionization dominates the field of environmental analysis at trace level because of its simplicity and versatility.

Electrospray ionization and atmospheric pressure chemical ionization have both been widely used for analysis of polar molecules in the aqueous environmental samples in many studies (Cahill et al., 2004; Castiglioni et al., 2005; Gros et al., 2009). Several studies that described multi-target detection of up to 74 compounds by ESI have been recently published in the literature (Gros et al., 2009; Lopez-Serna et al., 2010). However, ESI and APCI also have many critical limitations. For example, some steroids, and generally nonpolar compounds, such as PAHs, are poorly ionized or cannot be ionized by ESI or APCI (Hanold et al., 2004). Therefore, it is not surprising that most of the studies using ESI are focused on the most polar, easily ionizable pharmaceuticals. Only a handful of studies have tried to detect steroid hormones that are difficult to ionize by ESI or

APCI with marginal results (Jeannot et al., 2002; Ingrand et al., 2003). Not surprisingly, there is abundant literature for compounds ameanable to ESI but reports are scarce for those that present an ionization challenge. The critical issue is that the most EDC active compounds are not well ionized by electrospray ionization.

Atmospheric pressure photoionization is based on the interaction of a photon beam created by a discharge lamp with the vapors of a nebulized liquid solution (Marchi et al., 2009). Atmospheric pressure photoionization is a technique that has the capability to ionize compounds with a wide range of polarities while being remarkably tolerant of matrix components of HPLC additives. The rapidly growing number of publications in this area clearly demonstrates the advantages of atmospheric pressure photoionization (Raffaelli and Saba, 2003; Bos et al., 2006; Marchi et al., 2009). At the beginning, APPI was introduced as a complement of ESI and APCI. So far, APPI has been proved to be a valuable tool for analytes which are poorly ionized or not ionized by ESI and APCI. In particular APPI was shown to be able to detect steroid hormones down to several ng/L and had been proven to have much higher sensitivity than ESI. (Yamamoto et al., 2006; Viglino et al., 2008). Atmospheric pressure photoionization is the ionization of choice for PAHs and showed results comparable to gas chromatography mass spectrometry (Itoh et al., 2006; Cai et al., 2009). Indeed, APPI not only gives superior performance on nonpolar compounds but also works great for analytes which are out of the reach of ESI and APCI. Cai et al. demonstrated that APPI

could be considered as a universal ionization method since APPI was able to ionize more compounds, with greater structural diversity, than ESI and APCI (Cai et al., 2005). Because of APPI's capacity to ionize compounds with various polarities, it has been increasingly applied in the environmental and pharmaceutical areas.

1.4.4 Detection techniques

In single quadrupole MS, SIM is the mode used for qualitative and quantitative analysis of target compounds (Cahill et al., 2004). Compared with MS, MS^2 can reduce more interference from matrix. The unique ability of ion trap-MS (IT-MS) for MS^n makes it an ideal tool in identification of analytes of interest (Ingrand et al., 2003) but because of its space-charge limitations is not generally used for quantitation. Triple quadrupole (QqQ) is the most frequently used MS^2 detector in the muti-residue analysis of pharmaceuticals and EDCs in the environmental samples (Trenholm et al., 2008). For quantitative analysis, QqQ is excellent, but qualitative information, which is needed for structure identification, is lost. The drawback of QqQ can be overcome by using triple quadrupole time of flight (QqTOF) or quadrupole linear iontrap (QqLIT). Triple quadrupole time of flight is appropriate for identification of unknown compounds or metabolites due to its ability of providing exact mass. Quadrupole linear iontrap is excellent for both due to the unique capabilities of linear ion traps. LIT can run in two different modes, acting as the classical triple quadruple scan or sensitive ion trap scan. However, QqTOF and QqLIT are not widely available in environmental analysis

so far due to their extremely high cost. Only a few papers reported on their application for trace lever determination of emerging contaminants (Gros et al., 2009; Laven et al., 2009; Huerta-Fontela et al., 2010).

1.5 What is the concentration and fate of PPCPs in the environment?

With the development of sensitive detection techniques, a wealth of information about the occurrence of PPCPs in the complex environment samples have been produced in the last decade (Verenitch et al., 2006; Gómez et al., 2007; Durán-Alvarez et al., 2009; Xu et al., 2009). Pharmaceutical and personal care products have been documented in almost every water resource around the world. Pharmaceutical and personal care products are generally divided into several groups based on their mode of action including anti-inflammatory/analgesics, lipid regulators, H_2-receptor antagonists, betablockers, personal care products and hormones.

1.5.1 Anti-inflammatory/ analgesics

Pharmaceuticals in this section are primarily used as painkillers. The most prominent drugs of this group are aspirin, ibuprofen and naproxen. Large amounts of painkillers are sold without prescription as “over-the-counter” (OTC) drugs. Because of the diversity of manufacturers it is hard to estimate the amount of OTC drugs sold worldwide. Acetaminophen (paracetamol) is one of the most frequently used OTC painkillers all over the world. About 140 tons of acetaminophen was dispensed in Wales (Kasprzyk-Hordern et al., 2008) and

more than 500 tons were sold in Germany (Thomas, 2002). In the investigation of 139 surface streams in the U.S., acetaminophen was detected in 23.8% of samples at a maximum concentration of 10,000 ng/L (Kolpin et al., 2002). Acetaminophen is easily degraded and removed by WWTPs but it was still frequently detected in the environmental samples because its large usage. For example groundwater samples collected from 18 states from the USA, showed maximum concentration of acetaminophen of 380 ng/L (Barnes et al., 2008). Acetaminophen was even detected in drinking water in France at concentrations as high as of 210 ng/L (Mompelat et al., 2009).

Ibuprofen is also a popular painkiller. Ibuprofen was detected in 50% of samples from UK estuaries at the maximal concentration of 928 ng/L and the median concentration of 48 ng/L. It was detected in 139 streams in the U.S. at the maximum concentration of 1000 ng/L with 9.5% detection frequency. The high concentration of ibuprofen in surface water is not surprising because it has been reported at a high concentration in effluents of wastewater treatment plants. The high concentration of ibuprofen WWTP effluents is because of a combination of the high usage and low degree of human metabolism. In the WWTPs, ibuprofen was detected at concentrations from 14.3 to 22,700 ng/L in the influent and from 30 to 12,600 ng/L in the effluent of WWTPs, respectively (Deblonde et al., 2011). Ibuprofen has also been detected in drinking water with maximal concentrations of 3.0 ng/L, 0.6 ng/L, 8.5 ng/L and 1350 ng/L in Germany, France, Finland and the USA, respectively (Mompelat et al., 2009).

Other than these compounds mentioned above, naproxen, diclofenac, indomethacine, ketoprofen are also in high demand and were also widely reported in the wastewaters and surface waters (Mompelat et al., 2009; Deblonde et al., 2011).

1.5.2 Lipid regulators

Clofibric acid, the active metabolite of the blood regulator clofibrate, has been widely reported in the effluents of WWTPs and has a removal rate of 40% (Metcalfe et al., 2003; Deblonde et al., 2011). Clofibric acid has been reported in surface waters at concentrations of 100 ng/L in UK estuaries (Thomas and Hilton, 2004) and 0.4-18 ng/L in the river samples from Ebro River Basin (Gros et al., 2009). In tap water, clofibric acid was reported up to 270 ng/L in distribution systems in Germany (Mompelat et al., 2009).

The metabolites of fenofibrate—gemfibrozil, bezafibrate and fenofibric acid—have been routinely detected up to μg/L in WWTPs effluents and surface waters (Thomas, 2002; Metcalfe et al., 2003; Kim et al., 2007; Deblonde et al., 2011). In drinking water, gemfibrozil was detected up to 70 ng/L in Canada (Mompelat et al., 2009).

1.5.3 Antidepressants and anticonvulsants

Antidepressants are a psychiatric medication used to alleviate mood disorders. Fluoxetine is a widely used antidepressant. Although the relative removal of

fluoxetine is higher than other pharmaceuticals (~98%) (Deblonde et al., 2011), fluoxetine was still reported in many surface water and groundwater samples. In national reconnaissance of the USA, the maximum concentration of fluoxetine in streams, groundwater and sources of drinking water were 12 ng/L, 56 ng/L and ND, respectively (Kolpin et al., 2002; Barnes et al., 2008; Focazio et al., 2008).

Anticonvulsants are used in the treatment of epileptic seizures. Carbamazepine is one the most frequently detected anticonvulsants in wastewater, surface waters and drinking water. Because of its environmental stability carbamazepine is one of the most commonly detected PPCPs in surface waters worldwide. In WWTPs, the mean concentrations of carbamazepine in the influent and effluent were 732 ng/L and 774 ng/L, respectively, which indicated that carbamazepine had an extremely low removal rate in WWTPs (Deblonde et al., 2011). The concentrations of carbamazepine had been detected up to 1075 ng/L in surface waters in Germany (Heberer et al., 2002). Carbamazepine has even been detected in ground waters and drinking waters because of its persistence. The maximal concentrations of carbamazepine detected in drinking water were 24 ng/L, 258 ng/L, 43.2 ng/L and 60 ng/L in Canada, France, Germany and the USA, respectively (Mompelat et al., 2009).

1.5.4 H2-receptor antagonists

The function of H2 receptor antagonists is to block the action of histamine on parietal cells in the stomach. Cimetidine, ranitidine, famotidine and nizatidine are

the available OCT H2 receptor antagonists in the USA. Ranitidine was detected in streams in the USA at a maximum concentration of 10 ng/L with the detection frequency of 1.2% (Kolpin et al., 2002). In surface water in South Wales, concentrations of ranitidine were reported up to 8 ng/L (Kasprzyk-Hordern et al., 2008). Until now, ranitidine was not detected in groundwater or sources of drinking water in national reconnaissance in the United States (Barnes et al., 2008; Focazio et al., 2008).

1.5.5 Betablockers

Betablockers are used to manage cardiac dysrhythmia, cardioprotection after heart attack and hypertension. Betablockers are not very widely studied in wastewater. Concentration of metoprolol and propranolol in the influent of WWTPs ranged from 20 to 4900 ng/L and 36 to 510 ng/L, respectively. In the effluent of WWTPs the concentration of metoprolol and propranolol ranged from 19 to 1700 ng/L and 30 to 180 ng/L, respectively. The removal rate was about 60% (Deblonde et al., 2011). Propranolol was detected up to 56 ng/L in UK estuaries in 40% of the samples (Thomas and Hilton, 2004). Kasprzyk-Hordern's study suggests that betablockers, like atenolol, propranolol and metoprolol, are very persistent in the aqueous environment because they were present in 100% of the samples collected downstream from a wastewater discharge point and showing very small decreases in concentration with distance from the wastewater discharge point (Kasprzyk-Hordern et al., 2008).

1.5.6 Industrial chemicals and personal care products

Bisphenol A is used to make polycarbonate polymers and epoxy resins, which are used to make plastic along with other materials. Over six million tons of bisphenol A are produced worldwide each year (Welshons et al., 2006). The concentration of bisphenol A ranged from 88 to 11800 ng/L in the influent of wastewater treatment plants. With the removal rate of 71%, the concentration of bisphenol A was between 6 and 4090 ng/L in the effluent (Deblonde et al., 2011). Because of the incomplete removal of bisphenol A in the WWTPs, it also been detected in surface waters, ground waters and drinking waters. Bisphenol A was detected in 41.2% of samples collected from 139 streams in the USA and the maximum concentration was 12000 ng/L (Kolpin et al., 2002). In the groundwater in the USA, bisphenol A was detected up to 2550 ng/L with 29.8% detection frequency (Barnes et al., 2008). In the Australia, bisphenol A was detected up to 600 ng/L in surface water and 930 ng/L in groundwater. Water samples were collected from 27 surface water sites and 59 groundwater sites all across Australia (Hohenblum et al., 2004).

Personal care products are used for beautification and in personal hygiene. Triclosan is an antimicrobial disinfectant. Triclosan was detected up to 2300 ng/L with a very high detection frequency (57.6%) in streams in the United States (Kolpin et al., 2002). The removal rate of triclosan was 76.8% (Deblonde et al., 2011) and the incomplete removal of triclosan from effluents brought triclosan to the surface waters or even the drinking water. Triclosan's maximum

concentrations were 95 ng/L in South Wales' river water (Kasprzyk-Hordern et al., 2008) and 56.7 ng/L in Romania's river water (Zaharie, 2006). Triclosan was detected in drinking water sample in the USA with the maximal concentration of 734 ng/L (Mompelat et al., 2009).

1.5.7 Hormones

A hormone is a chemical released by a cell or a gland in one part of the body, which can affect cells of other parts of the body by triggering chemically induced messages. Hormones, which can behave as endocrine disruptors in the environment, may induce unexpected effect in no-mammalian organism in the aqueous environment, such as algae, invertebrate and fish. Disturbance of reproductive system and hormone system, immune depression, neurobehavioral changes may have effects on the population level (Fent et al., 2006). Estrone (E1), 17β-estradiol (E2) and ethynylestradiol (EE2) are the three hormones that have been studied most. The natural and environmental concentrations of hormones are lower than many other pharmaceuticals, but we still need to pay attention to them because they have endocrine disrupting effect at the ng/L level. A study of WWTPs located at Galicia, Spain showed that the concentration of E2 in the influent was 3 ng/L and in the effluent of plant the concentration was below detection limit (1 ng/L). The overall removal rate of E2 in WWTPs was around 65%, but E2 is usually converted to E1 during secondary treatment (conventional activated sludge) (Carballa et al., 2004), which explains why sometimes E1 was detected at a higher concentration in the effluent of WWTPs than E2 (Kim et al.,

2007; Ryu et al., 2011). Estrone was detected in the surface water and ground water up to 4.6 ng/L and 1.6 ng/L, respectively, in Australia (Hohenblum et al., 2004). In the study of U.S. drinking water, the maximum concentrations of E1, E2 and E3 were 0.9 ng/L, 17 ng/L and 1.4 ng/L in the source water, respectively. But none of them were above the detection limit (0.2 ng/L, 0.5 ng/L and 1.0 ng/L, respectively) in the finished water (Benotti et al., 2009).

1.6 Control of PPCPs pollution

More efforts need to be made to decrease the pollution in the environment with PPCPs. Several methods, including segregation of source, improvement of the disposal system for expired medicines, application of the pharmaceutical return program and the development of “green” pharmaceuticals, can be used to prevent the release of PPCPs into the environment. Source segregation can be an effective way to prevent pharmaceuticals from entering the environment. Wastewater from hospitals can be separated from domestic wastewater and advanced treatment can be applied to wastewater form hospitals to remove pharmaceutical and personal care products. Presently, most of the expired medicines are disposed of via sinks or toilets, ending up in WWTPs or household waste ending up in landfill sites. The US federal prescription drug-disposal guidelines (2007) allow flushing certain drugs if it is safe, but suggests returning unused, unneeded, or expired medicines to pharmaceutical take-back locations for safe disposal (URL2). Development of “green” pharmaceuticals requires more efforts and time but would be an effective way to increase the rapid removal of

PPCPs from the environment. Keeping in mind that these compounds are produced with an intended biological effect, in the absence of good data describing their ecological effects caution should be exerted to prevent their chronic release into environmental systems. Because of the lack of an environmental regulatory framework continued efforts to document their occurrence is still the best approach to keep the discussion going. The work presented here is a step in that direction and provides a set of tools and findings to advance the knowledge on the environmental occurrence of these chemicals in sensitive areas in South Florida. Water resources in Florida are under persistent stress due to the continued expansion of the urban population, the constant changes of land use and the competition for good quality water between the human population and ecosystem sustainability.

1.7 Objectives

The overall objective of this study is to monitor the PPCPs and hormones in reclaimed waters, surface waters, drinking waters, sediments and soils in South Florida in order to provide a general overview of the quality of these resources.

In order to accomplish this task, specific aims include to:

• Develop a GC-MS method for the detection of PPCPs and hormones in surface waters, sediments and soils.

- Because GC-MS systems are still the most widely used instrument in environmental labs. A sensitive GC-MS method is still valuable

for the analysis of PPCPs in the environment samples. (Chapters 2 and 3)

• Develop a fully automated, high throughput online SPE-LC-MS/MS method for the detection of PPCPs and hormones in different water matrices to avoid sample preparation.

- The online SPE method simplified sample preparation procedure and increased the productivity of analysis. Tandem MS enhanced both the selectivity and sensitivity. The online SPE-LC-MS/MS method largely increased the efficiency of analysis compared to GC-MS method. (Chapters 4 and 5)

• Compare different ionization sources including HESI, APCI and APPI on the ionization efficiency for PPCPs and hormones and produce a single, yet comprehensive method for the analysis of multiple compounds at environmentally relevant concentrations. (Chapters 4 and 5)

• Assess the occurrence of the target compounds in surface, reclaimed and drinking waters in South Florida and provide information regarding the present state of the quality of water resources with respect to PPCPs.

- A better understanding of distributions of PPCPs in South Florida can be achieved by analyzing the results from surface water samples. By analyzing reclaimed water, more information will be

provided about the potential effects of water reuse. Results from drinking water samples will offer information about current water quality in South Florida and the implications for system integrity. (Chapters 6 and 7)

CHAPTER 2

Detection of PPCPs and hormones in aqueous samples using GC-MS

2.1 Introduction

The wide spread occurrence of pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) have shifted the attention of environmental and toxicological research beyond traditional environmental pollutants, such as polychlorinated biphenyls (PCBs), dioxins, and pesticides.

Pharmaceuticals and personal care products are continuously released into the environment in vast quantities from many sources but in general municipal wastewater has been recognized as one of the main routes bringing human pharmaceuticals into the environment. Traditional wastewater treatment processes, such as aerated lagoons, conventional activated sludge and filtration, do not completely remove drugs and estrogens from their effluents (Lishman et al., 2006; Verenitch et al., 2006; Gibson et al., 2007; Gros et al., 2009). Many PPCPs and hormones have been detected in the effluent of wastewater treatment plants (WWTPs) (Boyd et al., 2003; Gibson et al., 2007) as well as in the receiving surface water (Kolpin et al., 2002). The residue of hormones can have adverse effects on organisms in the environment at very low concentrations (1-10 ng/L). These endocrine disrupting compounds (EDCs) can influence the endocrine system under long-term chronic exposure. Synthetic estrogens such as mestranol and ethynylestradiol (EE2) have the lowest observable effect level (LOEL) on the order of 1 ng/L (Christiansen, 2002). Concentrations above LOEL

have already been found in surface water (Kolpin et al., 2002) and current data suggests that hormones can travel considerable distance from the source of pollution (Barel-Cohen et al., 2006). Therefore, it is essential to monitor concentrations of PPCPs and EDCs in the aquatic environment at trace levels to provide adequate risk evaluation.

The most common difficulties for the detection of PPCPs are low occurrence concentration levels (μg/L or ng/L), which requires highly sensitive instruments and the interference coextraction of environment samples, which made it very difficult to identify and quantify target compounds. Therefore, proper clean-up procedures are required during sample preparation. In addition, there is a need for detection of diverse PPCPs in a single run. Therefore, this chapter reviews simultaneous detection of PPCPs in the environment.

The analysis of trace level contamination in environmental samples can be achieved by sophisticated analytical techniques such as liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS). Ionization sources such as electrospray ionization (ESI) and atmospheric chemical ionization (APCI) that are coupled to LC can ionize polar and non-volatile compounds (Kolpin et al., 2002; Cahill et al., 2004). The high selectivity of tandem mass allows simple sample preparation of complex matrix samples such as wastewater (Ingrand et al., 2003; Vanderford et al., 2003; Weigel et al., 2004; Castiglioni et al., 2005; Verenitch et al., 2006; Gómez et al., 2007; Gros et al., 2009). However, those sophisticated

analytical techniques are still not as common as GC-MS because of their high cost. In addition, certain steroids such as coprostan-3-ol and coprostanone, are very hard to be ionized in ESI or APCI. Gas chromatography mass spectrometry is still a very useful common technique to simultaneously detect trace level hormones and PPCPs in the environment, despite the fact that GC-MS may require an extra derivatization step during sample preparation (Kolpin et al., 2002; Boyd et al., 2003; Weigel et al., 2004; Lishman et al., 2006; Gibson et al., 2007).

In my study, I developed a reliable method to simultaneously detect 20 PPCPs in surface water using liquid-liquid extraction followed by GC-MS analysis. I detected 20 PPCPs of different properties in a single run. The method was used to detect compounds in water samples from Big Cypress National Preserve, Everglades National Park and Biscayne National Park in south Florida in order to understand the current status with respect to PPCPs occurrence and potential sources. The research investigates the influence of human activities on the surface waters from national parks and provides information on overall water quality in South Florida.

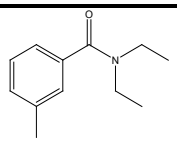
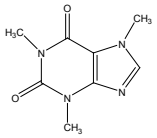
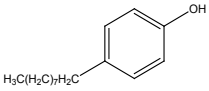
2.2 Experimental

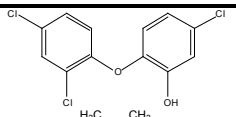
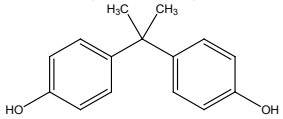
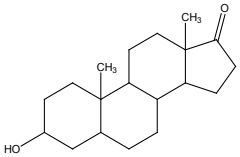
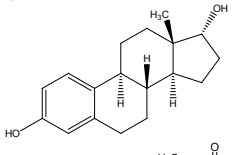
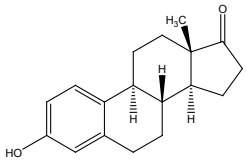
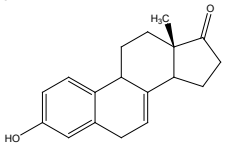
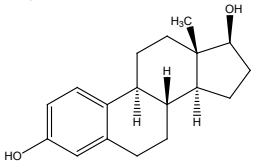
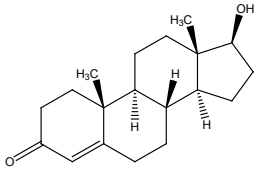
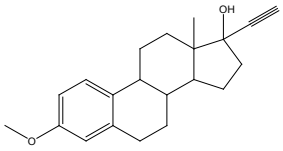
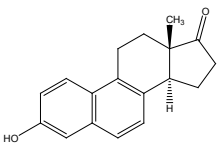
2.2.1 Chemicals

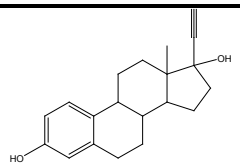
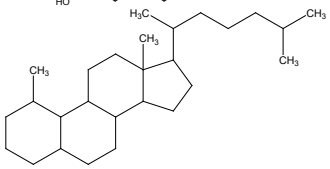
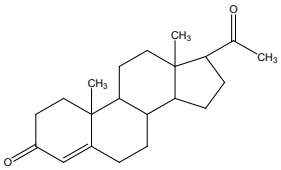
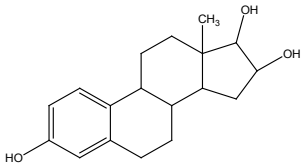
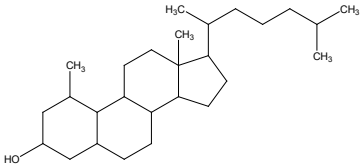
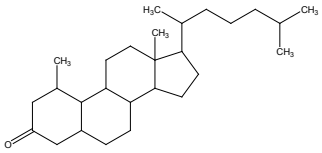
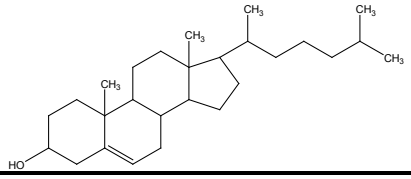
Caffeine was purchased from Fisher Scientific (Suwannee, GA, USA). N,N-Diethyl-3-methylbenzamide (DEET), 4-nonylphenol, triclosan, bisphenol A, androsterone, 17α-estradiol, estrone, equilin, 17β-estradiol, testosterone, 17α-

ethynylestradiol, coprostane, progesterone, estriol, coprostan-3-ol, coprostan-3-one and cholesterol were purchased as solids from Sigma and Aldrich (St. Louis, MO, USA). Mestranol and equilenin were purchased as a certified standard solution from Dr. Ehrenstorfer (Augsburg, Germany). Caffeine-$^{13}C_3$ used as surrogate was purchased from Cambridge Isotope Laboratories, Inc (Andover, MA, USA). Deuterated 17β-estradiol (17β-estradiol-d5), bisphenol A (bisphenol A-d16), estrone (estrone-d4) and progesterone (progesterone-d9) were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). All the reference standards were >95% purity. Detailed information of selected PPCPs is shown in table 1. Intermediate solutions were prepared at concentrations of 200 ppm in methanol and stored in the dark below 4°C. All laboratory materials were either made of glass or Teflon to avoid contamination. Glassware used in extraction was cleaned with soap and rinsed with DI water and combustion took place at 450 °C for at least six hours. Teflon materials were rinsed with methanol, acetone, methylene chloride and hexane before use.

Table 1. Target PPCPs, structure, CAS and intended usage

Name	Structure	CAS	Use
DEET		134-62-3	Insect repellant
Caffeine		58-08-2	Stimulant
Nonylphenol		104-40-5	Product formed during process phenols

Name	Structure	CAS	Use
Triclosan		3380-34-5	antibiotic
Bisphenol A		80-05-7	Polymer additive
Androsterone		53-41-8	androgen
17α-estradiol		57-91-0	estrogen
Estrone		53-16-7	estrogen
Equilin		474-86-2	Estrogen replacement
17β-estradiol		50-28-2	estrogen
Testosterone		58-22-0	androgen
Mestranol		72-33-3	Ovulation inhibitor
Equilenin		517-09-9	Estrogen replacement

Name	Structure	CAS	Use
17α-ethynyl Estradiol		57-63-6	Synthetic estrogen
Coprostane		481-20-9	Fecal steroid
Progesterone		57-83-0	estrogen
Estriol		50-27-1	Reproductive hormone
Coprostan-3-ol		360-68-9	Fecal steroid
Coprostan-3-one		601-53-6	Fecal steroid
Cholesterol		57-88-5	Plant/animal steroid

2.2.2 Sample collection and sample treatment

Samples were collected from three different protected areas in South Florida, Big Cypress National Preserve (BCNP), Everglades National Park (ENP) and Biscayne National Park (BNP). The sampling sites are shown in figure 3. Soap, beverages, sun screen, repellent, caffeinated drinks and pharmaceuticals were

not allowed to be used during sampling to minimize potential contamination of samples. Water samples were collected in previously combusted glass bottles to avoid contamination. Samples were filtered through 0.45 μm before being transferred into separatory funnels. The walls of the glass bottles were rinsed with methylene chloride to guarantee that all analytes were transferred into the separatory funnels.

2.2.3 Extraction method

Each 1 L water sample was extracted by liquid-liquid extraction (LLE) using a separatory funnel equipped with Teflon cap and stopper. Samples were fortified with surrogate standards (caffeine-$^{13}C_3$, bisphenol A-d16, estrone-d4, 17β-estradiol-d5 and progesterone-d9) and extracted three times using 50 mL methylene chloride. All the organic layers were dried over anhydrous sodium sulfate and collected in a 250 mL flat-bottom round flask. Extracts were evaporated to about 10 mL in a water bath at 65 °C, transferred to concentration tubes, concentrated to 1 mL under nitrogen gas, transferred into 1.5 mL amber vial and brought to dryness using nitrogen gas.

2.2.4 Silylation method

N,O-bis(trimethysilyl)trifluoroacetamide (BSTFA) (100 μL) was added to each amber vial and samples were heated at 60 °C for 45 min in the GC oven. Internal standard (chrysene-d12) working solution (100 μL) was added to the sample extracts before injection to gas chromatography mass spectrometry.

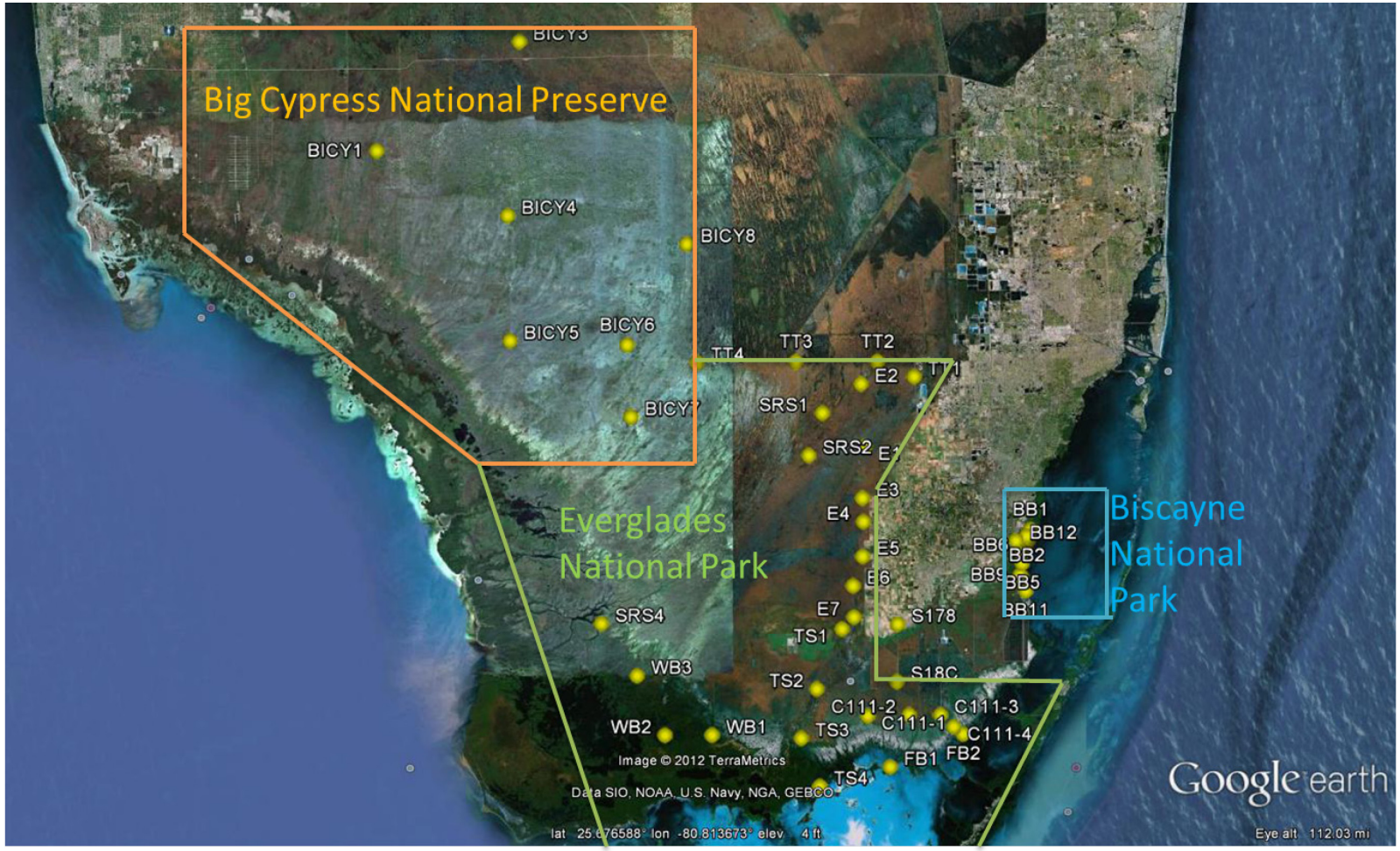


Figure 3. Location of sampling sites

2.2.5 GC-MS analysis

All the chromatographic measurements were performed using a Thermo GC/MS system comprised of a Finnigan Trace GC Ultra fitted with an autosampler AS 3000 and a Finnigan Trace DSQ operated in an EI at 70 eV. Derivatized sample extracts (2 μL) were injected into a DB-5MS column (30 m with 0.5 μm film thickness and 0.25 mm I.D.) under the splitless mode. The helium flow rate was held constant at 1.2 mL/min and the GC oven was programmed from 85 °C (1 min hold) at a rate of 15 °C /min to 270 °C (1 min hold), then at a rate of 5 °C /min to 300 °C (10 mins hold). The transfer line was 280 °C. The MS operated in EI mode using selected ion monitoring (SIM) to enhance sensitivity.

2.2.6 Quantification

Quantification of target compounds was accomplished by isotope dilution. The target compound was identified by retention time and ions (usually one quantitation ion and one or two confirmation ions). The quantitation ion and confirmation ion of target compounds are shown in table 2. The ion ratio was monitored to distinguish the matrix interference from the target compounds. In each sequence of samples, relative response factor (RRF) was calculated by calibration solutions. Seven calibration solutions with concentration of target compounds representing 5 ng/L to 1000 ng/L were run before samples. A continuous calibration check was also performed after each set of samples to assure the instrument's stability through the run.

Table 2. Molecular weight, retention time, quantitation ion and confirmation ion of PPCPs

Name	type	Molecular weight	RT (min)	Quantitation ion	Confirmation ion	Confirmation ion
DEET	Analyte	191.27	14.33	190	191	119
Caffeine-$^{13}C_3$	Surrogate	197.21	17.51	197	196	198
Caffeine	Analyte	194.19	17.51	194	195	109
Nonylphenol	Analyte	220.3	18.08	292	179	180
Triclosan	Analyte	289.54	20.72	360	347	345
Bisphenol A-d_{16}	Surrogate	244.38	21.55	368	386	
Bisphenol A	Analyte	228.29	21.67	357	358	372
Chrysene-d_{12}	Internal standard	240.37	26.26	240	239	236
Androsterone	Analyte	290.00	26.50	272	271	347
17α-estradiol	Analyte	272.38	28.21	416	285	
Estrone-d_4	Surrogate	274.39	28.35	346	347	261
Estrone	Analyte	270.37	28.41	342	218	257
Equilin	Analyte	268.35	28.55	340	341	242
17β-estradiol-d_5	Surrogate	277.42	28.78	421	422	287
17β-estradiol	Analyte	272.38	28.83	416	285	
Testosterone	Analyte	288.42	29.23	270	360	226
Mestranol	Analyte	310.43	29.73	227	242	367
Equilenin	Analyte	266.33	29.83	338	339	295
17α-ethynyl estradiol	Analyte	296.4	30.48	425	426	285
Coprostane	Analyte	372.67	31.06	217	218	357
Progesterone-d_9	Surrogate	323.52	31.50	323	324	279
Progesterone	Analyte	314.46	31.70	314	272	229
Estriol	Analyte	288.38	31.75	311	345	504
Coprostan-3-ol	Analyte	388.67	35.32	370	355	371
Coprostan-3-one	Analyte	386.65	37.33	231	232	386
Cholesterol	Analyte	386.65	37.55	329	368	458

2.3 Results and discussion

2.3.1 Method development and validation

2.3.1.1 pH range experiment

The pH is an important factor that affects the recovery of analytes when analytes with different functional groups are presented in the sample. Compounds in neutral form are more willing to partition to the organic phase, while in their ionized form they prefer to stay in the aqueous phase. The pH was adjusted to 3.0, 6.0 and 10 to assess recoveries across the pH range. Recoveries of target analytes are shown in Figure 4. Recovery did not differ significantly at different pH values expect for androsterone, equilin, testosterone, and coprostan-3-ol, whose recoveries were lower at pH=10. Therefore, pH was not adjusted for the real samples. Additionally, there were many advantages of doing extraction at natural pH: ① It simplified sample handling: no extra steps were required to remove acid or base; ② The pH is amenable to the derivatization step; ③ It eliminated the risk of acidic hydrolysis of susceptible analytes during sample preparation.

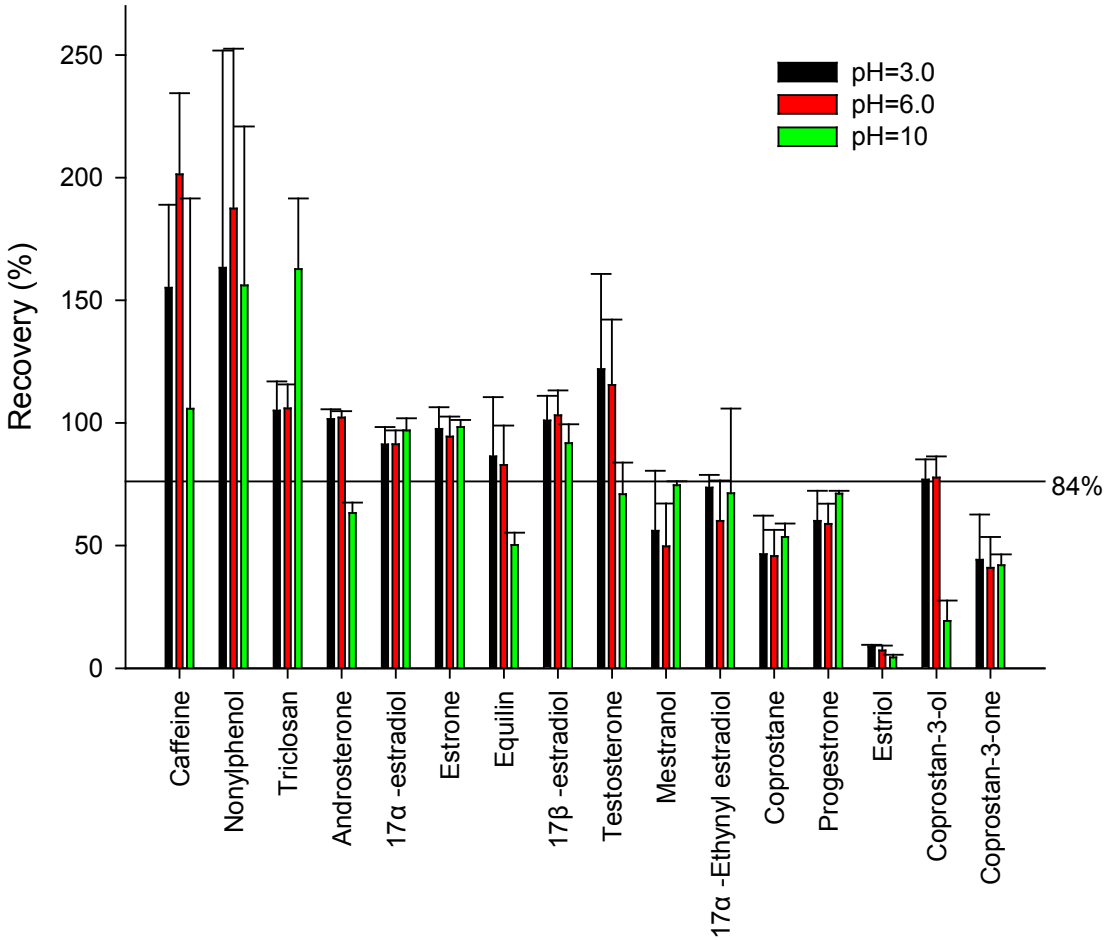


Figure 4. Recovery of PPCPs when extraction at different pH

2.3.1.2 Method validation

Batch quality assurance and quality control included a method blank, a fortified blank, one matrix spike sample and one duplicate sample per set of 20 samples or less analysis. Recoveries of the matrix spike were consistent between analytes. The duplicate sample agreed within 30% of target compounds except for cholesterol. Method detection limits (MDLs) are shown in table 3. Analytes

were spiked in DI water at environmental concentrations ranging from 2.00 ng/L to 20.0 ng/L) and MDLs were between 0.14 ng/L and 2.61 ng/L, respectively. The average recovery of analtyes was 79%.

Table 3. Performance data for PPCPs (linearity, method limit of detection, spike level and recovery)

Analyte	Surrogate	RRF	R^2	Spike level (ng/L)	MDL (ng/L)	Recovery (%)
DEET	Caffeine-$^{13}C_3$	0.7251	0.9744	2.00	0.24	87
Caffeine	Caffeine-$^{13}C_3$	1.0351	0.9990	2.00	2.61	96
Nonylphenol	Bisphenol A-d16	0.2575	0.9980	2.00	0.47	67
Triclosan	Bisphenol A-d16	0.0918	0.9973	2.00	1.20	145
Bisphenol A	Bisphenol A-d16	1.3781	0.9986	2.00	1.46	89
Androsterone	Estrone-d4	0.5796	0.9973	2.00	0.21	107
17α-estradiol	17β-Estradiol-d5	1.0726	0.9999	2.00	0.14	90
Estrone	Estrone-d4	1.5556	0.9964	2.00	0.22	81
Equilin	Estrone-d4	0.6110	0.9975	2.00	1.09	60
17β-estradiol	17β-Estradiol-d5	1.1809	0.9999	2.00	0.18	93
Testosterone	Estrone-d4	0.0526	0.9989	4.00	0.37	64
Mestranol	Estrone-d4	0.2421	0.9914	4.00	2.00	107
Equilenin	Estrone-d4	1.6533	0.9963	2.00	0.33	56
17α-ethynylestradiol	17β-Estradiol-d5	0.2845	0.9964	4.00	0.65	94
Coprostane	Progesterone-d9	1.5742	0.9994	8.00	0.48	85
Progesterone	Progesterone-d9	2.4044	0.9953	8.00	0.41	83
Estriol	17β-Estradiol-d5	0.3663	0.9957	2.00	0.29	10[b]
Coprostan-3-ol	Estrone-d4	1.0304	0.9944	4.00	0.51	45[b]
Coprostan-3-one	Progesterone-d9	0.6814	0.9974	8.00	1.45	60
Cholesterol	Estrone-d4	0.5336	0.9962	a	150	63

RRF- relative response factor

R^2-coefficient of determination of a linear regression

MDL- method detection limit

a MDL was set at 150 n/L because cholesterol is a common contamination on the glassware

b recovery of estriol is low because of no proper surrogate for it.

2.3.2 Analysis of real samples

The method was successfully applied to 80 samples from surface water collected from three protected areas in South Florida. The detailed description of the sampling sites and the concentrations of PPCPs are summarized in table 4. Detection frequencies and concentrations of PPCPs are shown in figure 5.

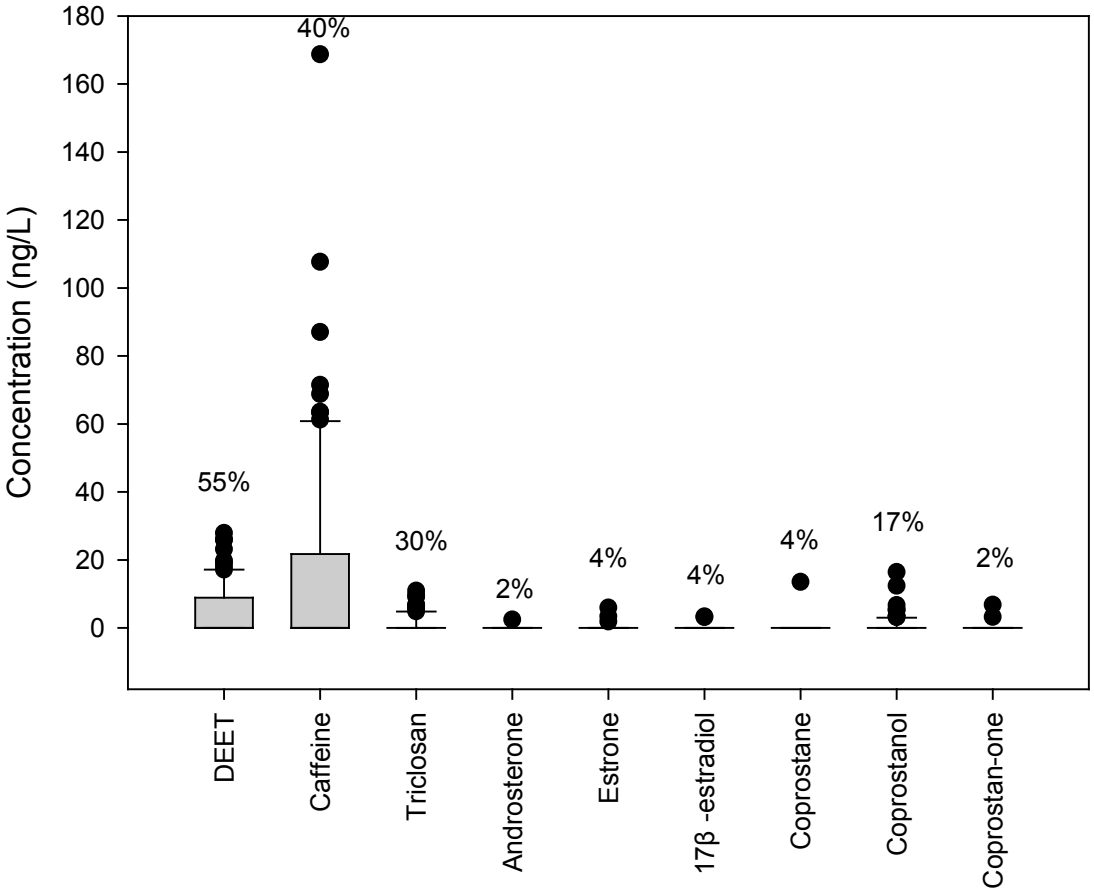


Figure 5. Concentrations and frequencies of detection of selected microconstituents in all sampling sites

DEET was detected in 55% of sampling sites with concentrations ranging from ND to 27.9 ng/L. DEET is the most common active ingredient in insect repellents. On the basis of the 1990-1999 estimates, approximately four to seven million pounds of DEET are used every year in the U.S. (Cahill et al., 2004). Therefore, it is not surprising that DEET is the most frequently detected pollutant in ground water in the United States (Barnes et al., 2008). Concentrations as high as 1100 ng/L have been reported for U.S. streams (Kolpin et al., 2002). Nevertheless, concentrations of DEET are usually related to recreational use and not an indicator of water quality issues related to wastewater intrusion.

Caffeine was detected in 40% of the sampling sites with concentrations ranging from ND to 169 ng/L. Caffeine was one of the most frequently detected compounds in this study and results are consistent with previous studies in the area (Gardinali and Zhao, 2002; Singh et al., 2010) and other similar areas such as the Herault watershed, where caffeine was not completely degraded either in the wastewater treatment plant or in river water (Rabiet et al., 2006). Caffeine has been associated with coral bleaching at concentrations between 30×10^6 ng/L and 75×10^6 ng/L (Pollack et al., 2009). Although the concentrations are orders of magnitude larger than the concentration of caffeine detected in the sampling sites, potential impacts of chronic exposure effect at low concentrations and possible additive effect with other chemical pollutants should still be of concern. Caffeine was neither detected in the sampling sites in Big Cypress National Preserve nor in the sampling sites in the Everglades National Park like

SRS3, SRS4, WB1, WB2 and WB3. Caffeine was detected in the sampling sites along the canals and in the Biscayne Bay National Park, which are affected by human activities. Therefore, caffeine can be used as a valuable indicator of human activities.

Triclosan was detected in 30% of the sampling sites, ranging from ND to 10.9 ng/L. Triclosan is an antibacterial agent added to detergents and soap formulas. The annual production of triclosan exceeded a million pounds in the late 1990s (Vanderford et al., 2003). Triclosan is hard to be removed during primary clarification, aeration basin and secondary clarification stages of wastewater treatment. Concentrations of triclosan from a Louisiana treatment plant effluent were between 10 to 21 ng/L, which were similar to the concentrations we detected (Boyd et al., 2003). Additionally, triclosan was detected at less than 50 ng/L in the surface water in South Wales, UK (Thomas and Hilton, 2004) and found at 4.2 ng/L in surface water from Hamburg, Germany (Weigel et al., 2004).

Androsterone was detected at only one site at a concentration of 2.44 ng/L (C111-2). Few studies included androsterone in their target compounds. Nevertheless, androsterone is an important weak androgenic steroid hormone, which comes from the metabolism of testosterone. Androsterone was detected in 14.3 % of U.S. streams at a maximal concentration 214 ng/L and a median concentration of 17 ng/L (Kolpin et al., 2002). The source for androsterone in the area is unknown.

Estrone (E1) was detected in only 4% of the sampling sites at a maximal concentration of 5.98 ng/L (BB09). These results are much lower than the concentrations detected by Kolpin et al. in U.S. streams (112 ng/L) (Kolpin et al., 2002). Such high concentrations have not seen in any other studies. The study of Edward et al. detected estrone in an agricultural region at a maximal concentration of 0.9 ng/L in river waters and 17 ng/L in irrigation canals (Kolodziej et al., 2004). Estrone detected in BB09 indicated that leaks from the landfill or WWTP area nearby may be affecting the quality of surface water. Caffeine, DEET, bisphenol A and coprostan-3-ol were also detected in BB09.

Estradiol was detected at two sampling sites at a maximal concentration of 3.34 ng/L. By contrast, 17β-estradiol was detected in U.S. streams at a maximal concentration of 93 ng/L at 10.0% frequency of detection (Kolpin et al., 2002). However, such high concentrations were not seen in other studies. Estradiol was detected in spring water in Mexico City at a concentration of 0.17 ng/L (Gibson et al., 2007). No 17β-estradiol was detected in surface water in the Mississippi River (Boyd et al., 2003), effluents from various WWTPs in Italy (Castiglioni et al., 2005), or effluents from 12 municipal wastewater treatment plants along the Thames River in Canada (Lishman et al., 2006).

Coprostan-3-ol, copostane and coprostan-3-one are metabolites of cholesterol generated by fecal bacteria (Jeannot et al., 2002). Cholesterol was detected at sampling sites at a maximal concentration of 2736 ng/L. Cholesterol can be

produced from both nonanthropogenic and anthropogenic sources (Kolpin et al., 2002). Therefore, chlolesterol was detected at all sampling sites. Similar concentrations also were found in the Danube River (Sebok et al., 2009). Coprostan-3-ol was detected in 17% of the sampling sites at a maximal concentration of 16.5 ng/L (C111-4). Previously, bacterial indicators were used to determine the quality of water and the stress from anthropogenic activities. Nucleic acid sequences were detected in 93.3% of coral surface microlayer samples from Florida Bay. It indicated the accumulation of entire microorganisms in the reef environment may be a risk to public and environmental health (Lipp et al., 2002). However, bacteria indicators have their limitations, such as being time-consuming and lacking specificity. Therefore, chemical indicators of human feces were an alternative to identifying human sewage contaminations in water bodies. The fecal steroid coprostan-3-ol was first suggested to be an indicator of fecal pollution (Glassmeyer et al., 2005). Coprostane was detected only at one sampling site (TS1) at a maximal concentration of 13.5 ng/L. Coprostane was detected in river samples at 20 ng/L (Jeannot et al., 2002). Coprostan-3-one was detected at two sampling sites at a maximal concentration of 6.79 ng/L.

Average concentrations of analytes detected in each sampling site were shown in figure 6 using different colors. Average concentrations between ND and 10 ng/L were displayed in blue color. Average concentrations between 10 and 25 ng/L were displayed in green color. Average concentrations between 25 and 50 ng/L were displayed in yellow color. Average concentrations between 50 and 100

ng/L were displayed in red color. Result in figure 6 showed that average concentrations in BCNP were lower than the other two protected areas. The reason is there are almost no residents in BNP area and human activities in BNP are much less than the other two protected areas.

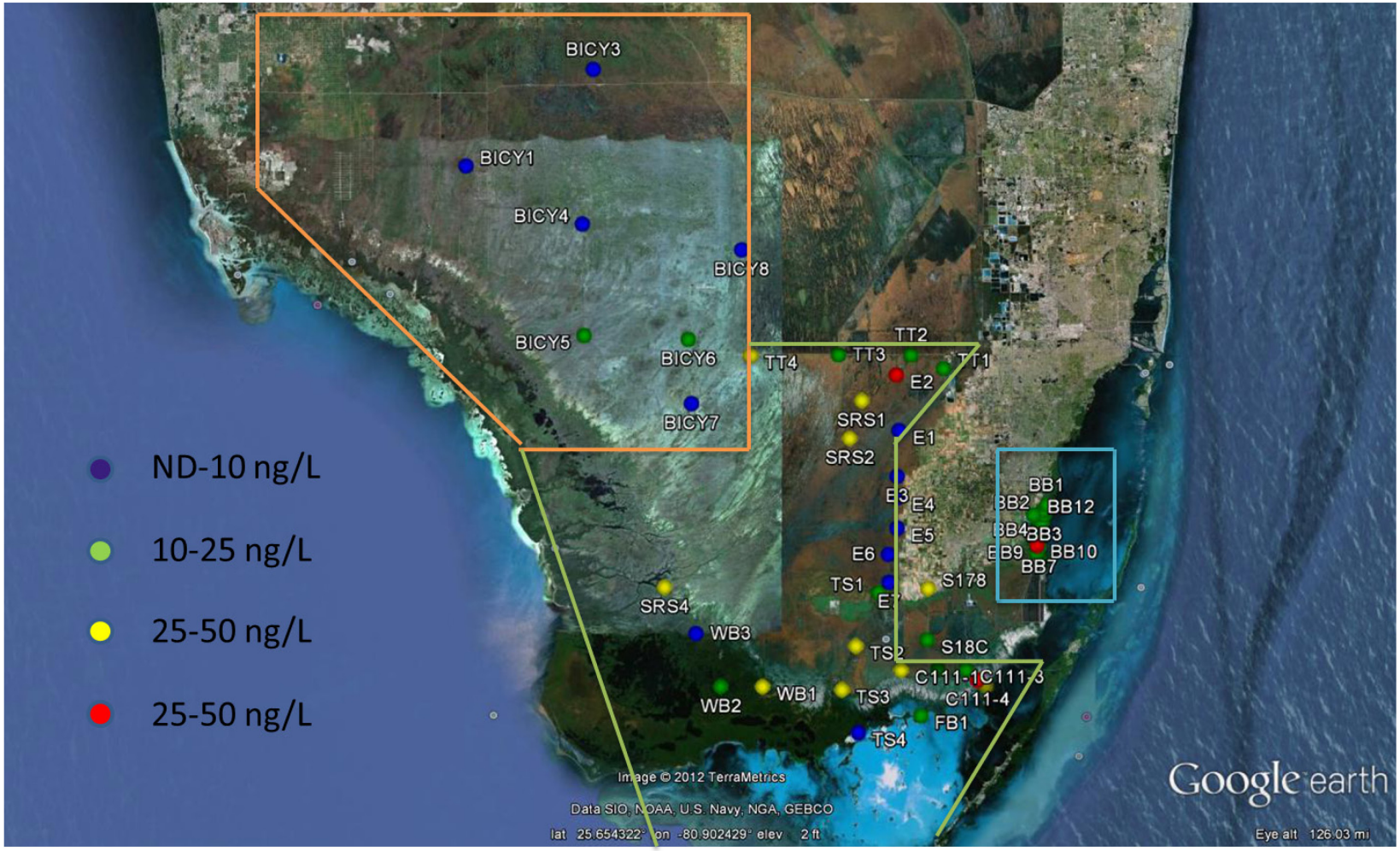


Figure 6. Map showing the distribution of average concentrations. ND-10 ng/L, blue dot; 10-25 ng/L, green dot; 25-50 ng/L, yellow dot; 50-100 ng/L, red dot.

In summary, the three most frequently detected compounds were DEET, caffeine and triclosan, if we do not consider cholesterol. The greater frequency of detection of these three compounds may be derived from their greater annual use. Mixture of PPCPs was prevalent during this study because forty three percent of sampling sites had more than three target compounds. Research has shown that certain chemical combinations can have additive toxic effects to organisms (Pomati et al., 2008). Therefore, the toxicity research should not only focus on individual compound effect but also the mixture of these compounds.

Table 4. Description of sampling sites and concentrations of PPCPs

STATION ID	STATION DESCRIPTOR	DEET	Caffeine	Triclosan	Androsterone	Estrone	17β-estradiol	Coprostane	Coprostan-3-ol	Coprostan-3-one	Cholesterol
BB01	Biscayne Bay	ND-8.87	ND-54.1	ND	ND	ND	ND	ND	ND	ND	ND-276
BB02	Biscayne Bay	ND-10.2	ND	ND	ND	ND	ND	ND	ND	ND	ND-418
BB03	Biscayne Bay	ND-25.9	ND	ND	ND	ND	ND	ND	ND	ND	ND -242
BB04	Biscayne Bay	ND-18.3	ND	ND	ND	ND	ND	ND	ND	ND	215-274
BB05	Biscayne Bay	ND-8.76	ND	ND	ND	ND	ND	ND	ND	ND	ND -312
BB06	Biscayne Bay	ND-6.21	ND	ND	ND	ND	ND	ND	ND -1.32	ND	208-420
BB07	Biscayne Bay	3.63-19.3	ND-38.5	ND	ND	ND	ND	ND	ND	ND	342-445
BB09	Biscayne Bay	ND-6.29	ND-25.4	ND	ND	ND-5.98	ND	ND	ND -12.4	ND	289-2337
BB10	Biscayne Bay	ND-9.29	ND	ND	ND	ND	ND	ND	ND	ND	ND -269
BB11	Biscayne Bay	ND-13.2	ND-22.9	ND	ND	ND	ND	ND	ND	ND	ND -279
BB12	Biscayne Bay	ND	ND	ND	ND	ND	ND	ND	ND	ND	321-389
BICY1	Big Cypress	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BICY3	Big Cypress	17.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
BICY4	Big Cypress	ND	ND	ND	ND	ND	ND	ND	3.16	ND	ND
BICY5	Big Cypress	ND-16.8	ND	ND	ND	ND	ND	ND	ND	ND	ND -423

STATION ID	STATION DESCRIPTOR	DEET	Caffeine	Triclosan	Androsterone	Estrone	17β-estradiol	Coprostane	Coprostan-3-ol	Coprostan-3-one	Cholesterol
BICY6	Big Cypress	ND	ND	ND	ND	ND	ND	ND	ND	ND	203-319
BICY7	Big Cypress	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BICY8	Big Cypress	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C111-1	C111 Basin	ND	22.9	2.66	ND	ND	3.34	ND	ND	ND	545
C111-2	C111 Basin	ND	10.6	4.34	2.44	ND	ND	ND	ND	ND	353
C111-3	C111 Canal	ND	ND	9.10	ND	ND	ND	ND	ND	ND	ND -434
C111-4	Highway Creek	6.71-17.2	ND - 35.5	ND	ND	ND-3.42	ND	ND	1.59-16.5	ND	287-2736
E1	East Boundary	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E2	East Boundary	12.1	39.6	ND	ND	ND	ND	ND	ND	ND	1561
E3	East Boundary	ND	33.6	ND	ND	ND	ND	ND	ND	ND	ND
E4	East Boundary	ND	ND	10.9	ND	ND	ND	ND	ND	ND	ND
E5	East Boundary	ND	61.3	ND	ND	ND	ND	ND	ND	ND	ND
E6	East Boundary	ND	68.8	ND	ND	ND	ND	ND	ND	ND	ND
E7	East Boundary	ND	ND	9.66	ND	ND	ND	ND	ND	ND	ND
FB1	Florida Bay	ND	ND	ND	ND	ND	ND	ND	ND	ND	164
FB2	Florida Bay	11.5	ND	ND	ND	ND	ND	ND	ND	ND	553
S178	Structure 178	ND	ND - 18.4	ND - 1.33	ND	ND	ND	ND	ND- 5.46	ND-3.22	309-859
S18C	Structure 18C	ND	63.5-169	ND	ND	ND	ND	ND	ND	ND	158-274

STATION ID	STATION DESCRIPTOR	DEET	Caffeine	Triclosan	Androsterone	Estrone	17β-estradiol	Coprostane	Coprostan-3-ol	Coprostan-3-one	Cholesterol
SRS1	Shark River Slough	6.25	87.0	ND	ND	ND	ND	ND	ND	ND	642
SRS2	Shark River Slough	23.2	40.8	ND	ND	ND	ND	ND	ND	ND	719
SRS4	Shark River	ND	ND	ND-1.72	ND	ND	ND	ND	ND	ND	ND - 1149
TS1	Taylor Slough	3.02-7.24	56.7-108	ND-9.66	ND	ND	ND	13.5	ND -3.26	ND	ND -623
TS2	Taylor Slough	ND	12.1	4.86	ND	ND	3.16	ND	6.72	ND	702
TS3	Taylor Slough	ND	15.3	ND	ND	ND	ND	ND	ND	ND	456
TS4	Florida Bay	ND	ND	ND	ND	ND	ND	ND	ND	ND	183
TT1	Tamiami Trail	ND - 10.9	51.2-71.5	ND-6.20	ND	ND	ND	ND	ND	ND	284-367
TT2	Tamiami Trail	ND	54.7	ND	ND	ND	ND	ND	ND	ND	191
TT3	Tamiami Trail	11.9-19.8	ND	ND-5.54	ND	ND	ND	ND	ND	ND	234-568
TT4	Tamiami Trail	ND - 26.1	ND	ND-6.66	ND	ND	ND	ND	ND	ND	183-899
WB1	West Boundary	ND - 8.91	ND	ND	ND	ND	ND	ND	3.49-5.20	6.79	150-855
WB2	West Boundary	11.6-27.9	ND	ND-2.61	ND	ND	ND	ND	ND	ND	ND -274
WB3	West Boundary	ND - 4.37	ND	ND-6.84	ND	ND	ND	ND	ND	ND	ND -178

2.4 Conclusion

The present study gave an overview of the occurrence of PPCPs and hormones in South Florida surface waters. Although there were a few reports about the occurrence of microconstituents in several typical environments in South Florida (Gardinali and Zhao, 2002; Singh et al., 2010), it was the first time to report the occurrence of PPCPs and hormones in surface waters from Big Cypress National Preserve, Everglades National Park and Biscayne National Park.

Pharmaceuticals and personal care products were detected at higher concentrations and higher frequencies compared to hormones. Caffeine can be used as an indicator of human activities. Potential impact of chronic exposure effects of caffeine at detected concentrations is still unknown. In our study, the highest detected concentration of estrone (5.98 ng/L detected in BB09) was high enough to require further investigation because of the toxicological implications for fish and probably other aquatic organisms. Results suggested that leakage from the landfill area and WWTP nearby may affect the quality of surface water. Results also indicated that future toxicity research should not only focus on the effect of individual compounds, but also on the influence of mixtures of compounds, since 43% of sampling sites have more than 3 compounds detected.

CHAPTER 3

Detection of PPCPs and hormones in sediment samples using GC-MS

3.1 Introduction

Current WWTPs do not completely remove PPCPs from wastewater. Measuring the concentration of PPCPs in the influent and effluent of WWTPs is insufficient to evaluate the efficiency of removal, because many PPCPs may be incorporated into sludge, which is used for landfill or as the fertilizer in agriculture (Jelic et al., 2009). No matter how the sludge is disposed of, PPCPs may enter the environment with the sludge and leach into sediment or soil. Moreover, PPCPs could enter surface water by runoff or enter groundwater through leaching from leakage. Therefore, it is essential to develop new methods for detection of PPCPs in the mineral phases, such as sediment, soil and sludge. By analyzing sediment or soil samples from the environment, the fate of PPCPs in the environment can be better understood.

The difficulty of sediment or soil sample analysis is because of the low concentration of PPCPs and the complex effect of matrices. The low concentration of analytes requires extensive extraction techniques and large concentration rates of samples. Accelerated solvent extraction (ASE) has demonstrated more advantages than Soxhlet extraction on automation, reduced extraction time and lower solvent consumption. Reduction of matrix effects requires proper cleanup steps (Löffler and Ternes, 2003; Burkhardt et al., 2005; Peng et al., 2006). Currently, SPE is the most widely used as cleanup step for

environmental sediment and soil samples (Burkhardt et al., 2005; Peng et al., 2006; Rice and Mitra, 2007; Xu et al., 2008; Durán-Alvarez et al., 2009). For instrument analysis, MS^2 or high resolution MS are preferred to decrease the interference from matrices (Löffler and Ternes, 2003; Gómez et al., 2007; Jelic et al., 2009). However, single quadrupole is still able to do the quantitation if proper cleanup steps are applied (Burkhardt et al., 2005; Rice and Mitra, 2007; Xu et al., 2008; Durán-Alvarez et al., 2009).

In the present study, a method to simultaneously determine many environmentally relevant PPCPs and steroid hormones in sediments and soils using ASE-SPE-GC-MS was developed. Recoveries of most analytes were adequate even when the matrix became complex at very low spike levels. MDLs are low enough to detect the target compounds at environmentally relevant levels.

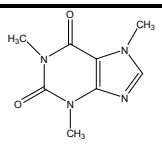
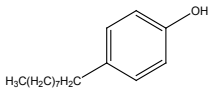
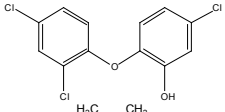
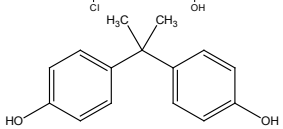
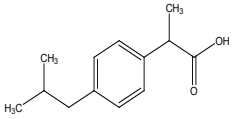
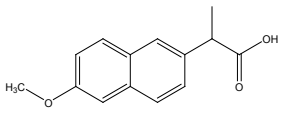
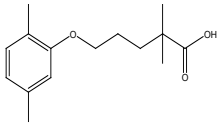
3.2 Experimental

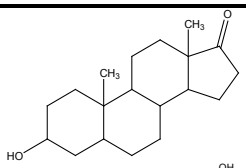
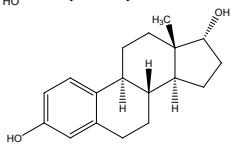
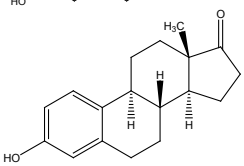
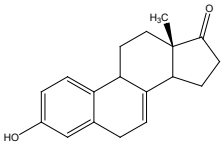
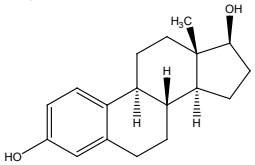
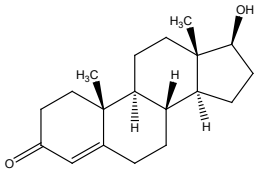
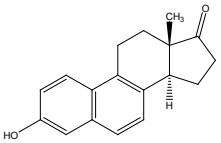
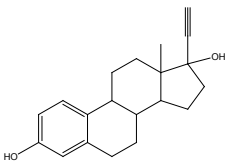
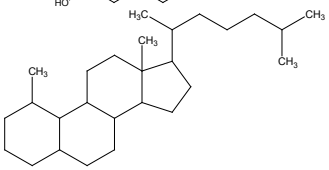
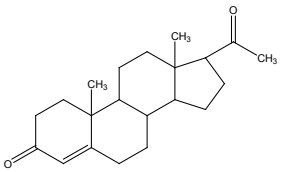
3.2.1 Chemicals

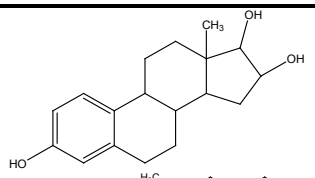
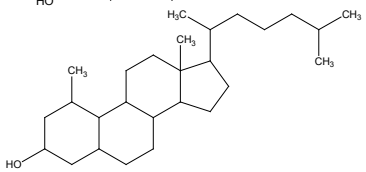
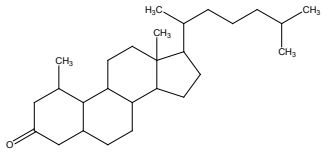
Caffeine was purchased from Fisher Scientific (Suwannee, GA, USA). Naproxen, ibuprofen, gemfibrozil, triclosan, bisphenol-A, 4-n-nonylphenol, androsterone, estrone, equilin, 17β-estradiol, 17α-estradiol, testosterone, 17α-ethynylestradiol, coprostane, progesterone, estriol, coprostane, coprostan-3-ol and coprostan-3-one were purchased as neat compounds from Sigma-Aldrich (St. Louis, MO, USA). Equilenin were purchased as certified standard solutions from Dr. Ehrenstorfer (Augsburg, Germany). Surrogate standard caffeine-trimethyl-$^{13}C_3$

was purchased from Cambridge Isotope Laboratories Inc (Andover, MA, USA); DEET-d7, triclosan-d3, 4-n-nonylphenol-d4, equilin-d4, 17β-estradiol-d5, estrone-d4, 17α-ethynylestradiol-d4, norgestrel-d6, progesterone-d9 and 5α-cholestan-3β-ol-d5 were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Bisphenol A-d16 was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the reference standards were >95% purity. Detailed information of analytes is shown in table 5.

Table 5. Name, structure, CAS and intended usage of analytes

Name	Structure	CAS	Use
Caffeine		58-08-2	Stimulant
Nonylphenol		104-40-5	Product formed during process phenols
Triclosan		3380-34-5	antibiotic
Bisphenol A		80-05-7	Polymer additive
Ibuprofen		15687-27-1	Analgesics and antiinflamatory
naproxen		22204-53-1	Analgesics and antiinflamatory
gemfibrozil		25812-30-0	Liqid regulator

Name	Structure	CAS	Use
Androsterone		53-41-8	androgen
17α-estradiol		57-91-0	estrogen
Estrone		53-16-7	estrogen
Equilin		474-86-2	Estrogen replacement
17β-estradiol		50-28-2	estrogen
Testosterone		58-22-0	androgen
Equilenin		517-09-9	Estrogen replacement
17α-ethynyl Estradiol		57-63-6	Synthetic estrogen
Coprostane		481-20-9	Fecal steroid
Progesterone		57-83-0	estrogen

Name	Structure	CAS	Use
Estriol		50-27-1	Reproductive hormone
Coprostan-3-ol		360-68-9	Fecal steroid
Coprostan-3-one		601-53-6	Fecal steroid

3.2.2 Sampling

Reclaimed water used for irrigation was collected directly from a sprinkler system using 500 mL PETE bottles and was stored in the freezer at <10 °C. The source of reclaimed water used in this study is the Miami-Dade Water and Sewer Department North District Wastewater Treatment Plant. The wastewater treatment plant was designed to have a flow of 120 million gallons per day (MGD) with average daily flow around 112.5 million gallons per day. Pure oxygen activated-sludge is used in the WWTP as the main secondary treatment process (Kasprzyk-Hordern et al., 2008). Extra filtration and disinfection are applied to effluents before release to make the reclaimed water ready for use in irrigation. Sediments and soil samples were collected in and near a fresh water pond in FIU Biscayan Bay Campus (Figure 7). Soil samples were collected under the sprinkler and on the side of the pond. Sediment samples were collected at the bottom of the pond.

Figure 7. Sampling sites of sediment and soil

3.2.3 Sample preparation

Samples were extracted using a Dionex ASE 200 accelerated solvent extraction system. Extraction cells were filled with 20 g of anhydrous sodium sulfate and 2 grams of the freeze-dried sediment sample. Blanks consisted of 20 g of sodium sulfate. The most efficient solvent tested for extraction was methanol and ASE conditions used were as follow: pre-heat (0 min), heating time (5 mins), 60% flush, 1 cycle. Temperature was held at 100 °C and pressure was 1500 psi. Solvent was evaporated to dryness and samples were reconstituted in 60 mL of DI water. NH_4OH (60 µL) was added to adjust pH to 10. Oasis MAX cartridges (225 mg) were conditioned with 5 mL of methanol followed by 5 mL of DI water (pH 10). Samples were loaded to the cartridges at a rate of 1-2 mL/min. The first fraction (5 mL of methanol: water 1:1) contained polar analytes. The second

fraction (5 mL of acetone and 5 mL of acetone with 4% formic acid) contained the steroid hormones. Hormone concentrations are usually lower than pharmaceuticals in environmental samples and the fractionation helped to decrease the background interferences in the gas chromatograph mass spectrometry. Fractions were evaporated to dryness using nitrogen gas. Pyridine (100 µL) and BSTFA (50 µL) were added to the residue. Compounds were derivatized at 60 °C for 45 minutes and analyzed on a Thermo Trace DSQ GC/MS. The procedure is shown in figure 8.

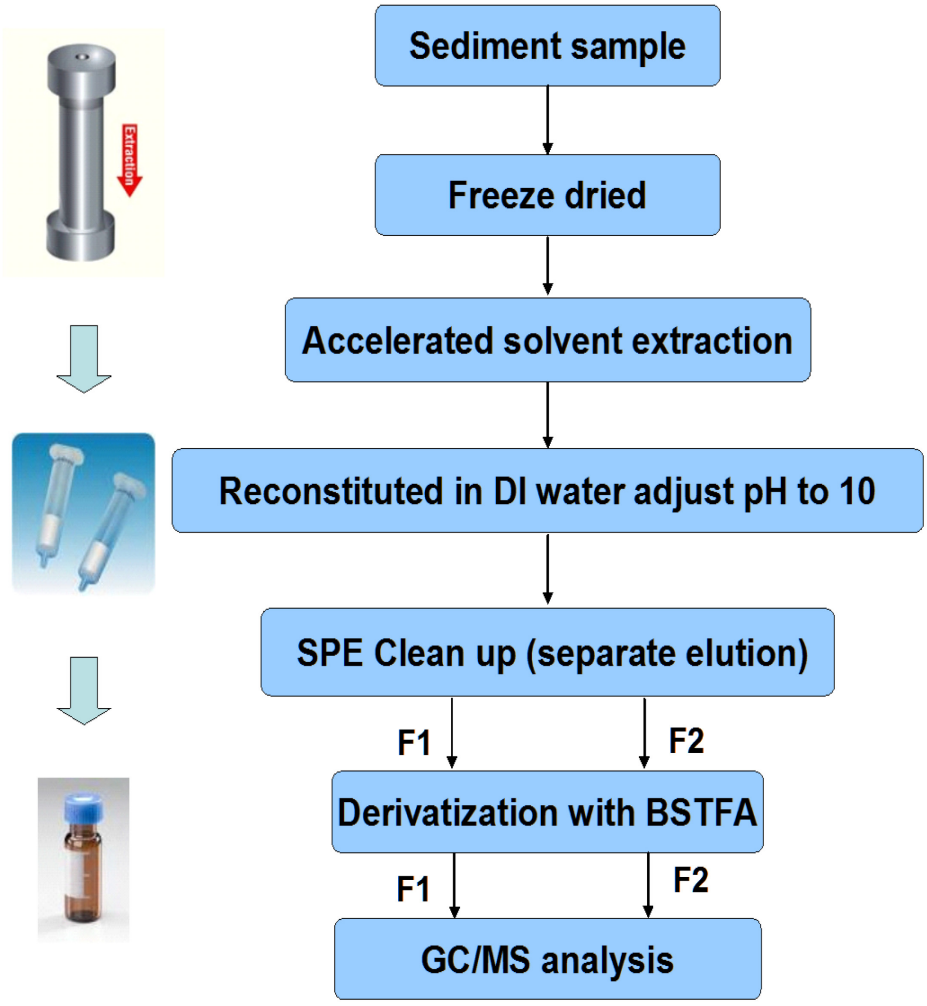


Figure 8. Sample preparation procedure for sediments and soils

3.2.4 GC-MS analysis

All the chromatographic measurements were performed using a Thermo GC/MS system comprised of a Finnigan Trace GC Ultra fitted with an autosampler AS 3000 and a Finnigan Trace DSQ operated in an EI at 70 eV. Derivatized sample extracts (2 μL) were injected into a DB-5MS column (30 m with 0.5 μm film thickness and 0.25 mm I.D.) under the splitless mode. The helium flow rate was held constant at 1.2 mL/min and the GC oven was programmed from 85 °C (1 min hold) at a rate of 15 °C /min to 270 °C (1 min hold), then at a rate of 5 °C /min to 300 °C (10 mins hold). The transfer line was 280 °C. The MS operated in EI mode using selected ion monitoring (SIM) to enhance sensitivity. Recoveries of PPCPs in different matrices and method detection limits (MDLs) were performed for method validation.

3.2.5 Extraction recovery and method detection limit

Analytes were spiked to sodium sulfate, pre-extracted sediment and sediment to check the recovery of method. Fortification concentrations are: caffeine, bisphenol A, ibuprofen, gemifibrozil, naproxen, nonylphenol, triclosan, estrone, equilin, equilenin androsterone, 17β-estradiol, 17α-estradiol, and estriol were spiked at 4.4 ng/g. Testosterone, coprostan-3-ol, 17α-ethynylestradiol were spiked at 8.9 ng/g. Progesterone, coprostan-3-one and coprostane were spiked at 17.8 ng/g.

Method detection limits (MDLs) were determined according to EPA guidelines (Ripp, 1996). Eight replicate pre-extracted sediment samples spiked with all compounds with concentration ranging from one to eight times of the tested detection limit (DL). Standard deviations (SD) were calculated from replicates and MDL = 2.998 SD.

3.3 Results and discussion

3.3.1 Method development and validation

3.3.1.1 Recovery of analytes from different matrices

Pharmaceuticals and personal care products and hormones were spiked into sodium sulfate, pre-extracted sediments and untreated sediments at very low fortification levels. Recoveries of PPCPs are shown in figure 9. Recoveries were not affected as the matrix complexity increased even for such low fortification levels. With the exception of equilin, naproxen and progesterone the method was very reliable for the rest of the compounds.

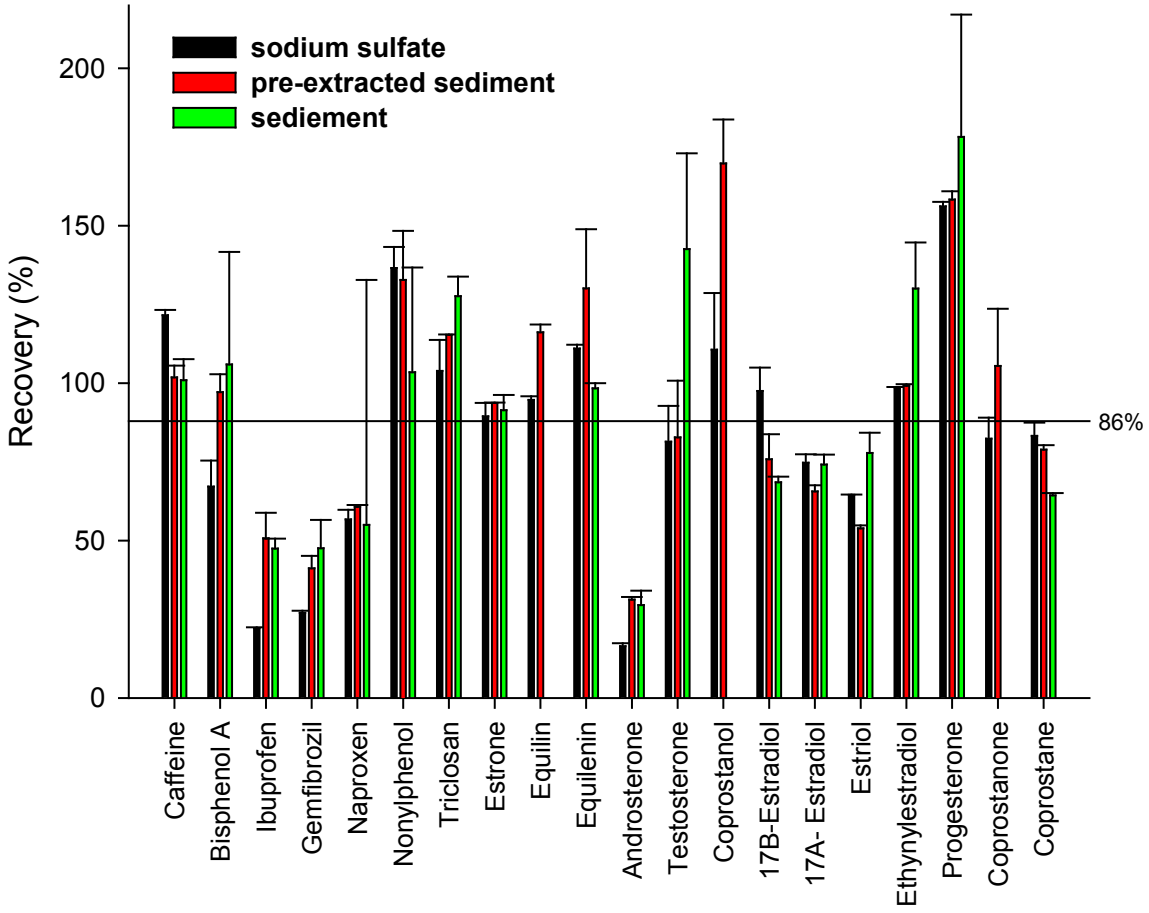


Figure 9. Recoveries of analytes in sodium sulfate, pre-extracted sediment and untreated sediment.

3.3.1.2 Method detection limit

Method detection limits of analytes in sediment and soil are shown in table 6. Method detection limits were low but varied among pharmaceuticals and hormones. Method detection limits for ibuprofen, gemfibrozil and naproxen were 1.86 ng/g, 1.96 ng/g and 0.85 ng/g, respectively. The detection limits for estrogen

hormones were between 1.27 ng/g and 14.5 ng/g. Faecal steroids' detection limits were between 3.58 ng/g and 15.5 ng/g.

Table 6. MDLs of PPCPs and hormones in sediment

analytes	MDL (ng/g)	analytes	MDL (ng/g)
caffeine	1.85	equilin	2.11
bisphenol A	2.51	equilenin	2.64
ibuprofen	1.86	androsterone	2.99
gemfibrozil	1.96	testosterone	8.61
naproxen	0.85	17α-ethynylestradiol	6.68
4-n-nonylphenol	3.34	estriol	1.27
tricolsan	1.35	coprostan-3-ol	5.50
estrone	2.30	progesterone	14.5
17β-estradiol	2.05	coprostan-3-one	15.5
17α-estradiol	1.66	coprostane	3.58

3.3.2 Analysis of real samples

The detected concentration of PPCPs and hormones are shown in table 7. Results of reclaimed water samples are analyzed by online SPE-LC-MS/MS method, which is shown in chapter 6.3. Most of analytes were below detection limits even though some of analytes were detected in the reclaimed water at relatively high concentrations. Results indicated that the higher the logkow value, the more possible that the compounds attach to the sediment or soil. Coprostanol was detected at sediment from the bottom of ponds but concentrations were below detection limit on soil samples from side of pond and under sprinkler. The most possible explanation is selective partition combined with degradation, probably aided by the microbial activity in the soil, the high temperatures of the region and long exposure to sunlight.

Table 7. Concentrations of PPCPs in reclaimed water, sediment and soil

	Logkow	Reclaimed water (ng/L)	Sampling site 1 Soil under the sprinkler (ng/g)	Sampling site 2 Soil on the side of the pond (ng/g)	Sampling site 3 Sediment at the bottom of pond (ng/g)
triclosan	4.76	ND-1035	3.78	4.22	7.66
caffeine	<0	ND-3249	ND	ND	ND
bisphenol A	3.3	ND-14306	6.14	ND	ND
gemfibrozil	4.75	ND-4177	ND	ND	ND
Coprostan-3-ol	8.2	ND-17197	ND	ND	235

3.4 Conclusion

A robust, reliable and sensitive analytical method was developed for the simultaneous determination of many environmentally relevant hormones, steroids and pharmaceuticals in sediments and soils. Good recoveries were achieved for different matrices except for equilin, coprostanol and coprostanone. Method detection limits were low enough to detect at environmentally relevant analysis. Compounds with low logkow value are willing to stay in the water and compounds with relatively high logkow value are more possible to attach to the sediment or soil. Even though some of analytes were detected in the reclaimed water at relatively high concentrations, most analytes were below detection limits in the sediment and soil samples. The microbial activity in the soil, the high temperatures of the region and long exposure to sun light may cause the degradation of analyte in the soil samples.

CHAPTER 4

Detection of PPCPs and hormones in aqueous samples using online SPE-LC-MS/MS assessing the utility of APPI for non ionizable compounds

4.1 Introduction

Because traditional wastewater treatment processes cannot completely remove drugs and estrogens from the final effluent of conventional wastewater treatment plants (WWTPs), pharmaceuticals and personal care products (PPCPs) could be continuously released into the environment in rather large quantities (Esperanza et al., 2004). It is particularly important when reuse water is used for irrigation. Many PPCPs, also named microconstituents or organic waste indicators, have been detected in both effluent of wastewater treatment plants and in the receiving surface waters (Jeannot et al., 2002; Boyd et al., 2003; Cahill et al., 2004; Weigel et al., 2004). Several PPCP residues even when present at trace levels may have adverse effects on organisms in particular those with endocrine disrupting capability (Ben-Jonathan and Steinmetz, 1998). Research has indicated that endocrine disrupting compounds (EDCs) can interact with the endocrine system of fish at very low concentrations by long-term exposure (Ben-Jonathan and Steinmetz, 1998; Pollack et al., 2009). Therefore, it is very important to accurately monitor concentrations of PPCPs and EDCs in the aquatic environment at environmental relevant concentrations (ng/L).

Gas chromatography mass spectrometry (GC-MS) has been traditionally used to detect many PPCPs and EDCs in aquatic matrices in a number of studies (Boyd

et al., 2003; Weigel et al., 2004; Lishman et al., 2006; Verenitch et al., 2006; Gibson et al., 2007; Gómez et al., 2007; Markman et al., 2007). Gas chromatography mass spectrometry is a very widely used and mature technique; however, it requires collection of large sample volumes, extensive use of solvent for extraction, several evaporation steps and in many cases, derivatization steps to make polar compounds amenable to gas chromatography. High performance liquid chromatography coupled to mass spectrometry has been shown as a valuable alternative for detection of PPCPs and EDCs to overcome the drawbacks of GC-MS (Gardinali and Zhao, 2002; Gentili et al., 2002; Ingrand et al., 2003; Cahill et al., 2004; Castiglioni et al., 2005; Schlüsener and Bester, 2005; Martnez Bueno et al., 2007; Gros et al., 2009; Laven et al., 2009; Huerta-Fontela et al., 2010; Jian-lin et al., 2010). Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are the three most common ion sources coupled with HPLC/UPLC. Electrospray ionization however because of its versatility dominates the field of environmental analysis at trace level (Hilton and Thomas, 2003; Martnez Bueno et al., 2007; Lopez-Serna et al., 2010).

Electrospray ionization and APCI have both been widely used for analysis of polar molecules in aqueous environmental samples in many studies (Cahill et al., 2004; Castiglioni et al., 2005; Gros et al., 2009; Lopez-Serna et al., 2010). However, ESI and APCI also have many limitations: 1. Because ionization efficiencies are charge affinity dependent, certain classes of compounds cannot

be ionized (some steroids, nonpolar compounds like PAHs, etc); 2. Adducts with common cations (Na^+ and K^+), routinely form during ESI, and these charge-bearing salt compounds could not only increase the chemical background but reduce the analyte signal (Hanold et al., 2004). 3. Target analyte molecules are often not ionized because of competition with other compounds with higher charge affinity. Therefore, most of the studies using ESI are focused on pharmaceuticals, which are readily ionizable by electrospray ionization. Fewer studies have tried to detect steroid hormones, which are difficult to ionize by ESI or APCI, with marginal results (Jeannot et al., 2002; Ingrand et al., 2003). Not surprisingly, several studies often rely on the use of chemical derivatization to increase sensitivity, even for LC/MS (Palmgren et al., 2005; Lien et al., 2009; Wang and Schnute, 2010).

Atmospheric pressure photoionization is on the basis of the interaction of a photon beam created by a high intensity discharge lamp with the vapors of a nebulized liquid solution entering the MS source (Marchi et al., 2009). Atmospheric pressure photoionization is an ionization technique that has the capability to ionize compounds with various polarities while being remarkably tolerant of matrix additives (Cai et al., 2005; Viglino et al., 2008). The rapidly growing number of publications in this area clearly demonstrates the advantages of atmospheric pressure photoionization (Raffaelli and Saba, 2003; Bos et al., 2006; Marchi et al., 2009). Atmospheric pressure photoionization was introduced as a complement of ESI and APCI. Currently, APPI has proven to be a valuable

tool for analytes, which are poorly or not ionized by ESI and APCI. Examples of the progress were shown in recent studies. For example, Viglino and coworkers developed a fully automated online method using LC-APPI-MS/MS to simultaneously detect selected natural and synthetic hormones at concentrations as low as 5 ng/L (Viglino et al., 2008). Yamanoto et al. compared detection of steroidal hormones using ESI and APPI and they found that APPI displayed higher sensitivity than ESI for most of the unconjugated steroids examined, with much greater sensitivity for testosterone and 4-androstene-3, 17-dione (Yamamoto et al., 2006). Itoh and coworker was able to detect 16 common polycyclic aromatic hydrocarbons (PAHs) with MDLs as low as 0.79-168 ng/L using LC/dopant-assisted (DA) APPI/MS (Itoh et al., 2006). Cai and his coworkers demonstrated a robust method to detect 16 priority PAHs in 3.5 min at low pictogram using chlorobenzene as the dopant. (Cai et al., 2009). Indeed, APPI not only gives superior performance on nonpolar compounds but also works well for many analytes which are properly ionized by ESI and APCI. Cai et al. suggested that APPI could be considered as a more universal ionization method since APPI was able to ionized more compounds, with greater structural diversity, than ESI and APCI (Cai et al., 2005). Because of the capacity of APPI to ionize compounds with various polarities, it has been successfully applied frequently to environmental and pharmaceutical samples (Cai et al., 2005; Yamamoto et al., 2006; Viglino et al., 2008; Garcia-Ac et al., 2011).

Most sample preparation protocols for the analysis of microconstituents in water samples uses off-line extraction as the main sample preparation. Although relatively straightforward, the disadvantages of off-line extraction are: lengthy extractions, and solvent evaporation and dervatization effects on reproducibility. Therefore, there is considerable interest in developing on-line sample extraction procedures that mainly overcome the need for the time consuming evaporation and reconstitution steps in the off-line procedure. Many recent articles have described on-line SPE procedure for detection of EDCs (Segura et al., 2007; Viglino et al., 2008; Garcia-Ac et al., 2009; Lien et al., 2009). However, further development in environmental monitoring should target simultaneous detection of multiple classes of compounds with diverse properties using a combination of on-line SPE and tandem MS to achieve high sample throughput.

In this work, a novel fully automated on-line preconcentration method coupled to HPLC-APPI-MS/MS was developed for a comprehensive list of analytes with environmental relevance for water tracking in semi pristine environment. The on-line procedure was optimized for cleanup efficiency and analyte retention. Different ionization methods, including HESI, APCI and APPI, were explored. Atmospheric pressure photoionization was evaluated with different dopants (acetone, anisole, chlorobenzene and toluene). Statistically determined MDLs for analytes in each method were generated to cross-evaluate the method's capability. Reclaimed water is often the released end product of a WWTP treatment. As a results of water scarcity, use of reclaimed water for agricultural

and landscape irrigation has been become increasing popular. However, the use of reclaimed water has received much less attention than WWTP effluent, and little is known about the risk of contamination of surface water and groundwater by this kind of source (Wang et al., 2005; Kinney et al., 2006). A comprehensive method should be capable of analyzing both source (reclaimed water) and the receiving water with equal robustness.

4.2 Experimental

4.2.1 Chemicals

Caffeine was purchased from Fisher Scientific (Suwannee, GA, USA). Acetaminophen, naproxen, carbamazepine, primidone, DEET (N,N-Diethyl-3-methylbenzamide), triclosan, bisphenol-A, gemfibrozil, androsterone, estrone, equilin, 17β-estradiol, testosterone, 17α-ethynylestradiol, coprostane, progesterone, estriol, coprostan-3-ol and coprostan-3-one were purchased as neat compounds from Sigma-Aldrich (St. Louis, MO, USA). 4-tert-octylphenol was from Restek (Bellefonte, PA, USA). Mestranol (ethynylestradiol 3-methyl ether) and equilenin were purchased as certified standard solutions from Dr. Ehrenstorfer (Augsburg, Germany); Surrogate standard caffeine-trimethyl-$^{13}C_3$ was purchased from Cambridge Isotope Laboratories Inc (Andover, MA, USA); DEET-d7, triclosan-d3, carbamazepine-d10, 4-n-nonylphenol-d4, equilin-d4, 17β-estradiol-d5, estrone-d4, 17α-ethynylestradiol-d4, norgestrel-d6, progesterone-d9 and 5α-cholestan-3β-ol-d5 were purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). Bisphenol A-d16 was purchased from Sigma-Aldrich (St.

Louis, MO, USA). All the reference standards were >95% purity. Detailed information for all analytes is shown in Table 8. Stock solutions were prepared at concentrations of 200 µg/mL in methanol and stored below 0 °C in the dark. All laboratory materials were either made of glass or Teflon to avoid contamination. All glassware used in extraction was cleaned with soap and rinsed with water before combustion. Glassware was combusted at 450 °C for at least six hours before using it. Teflon materials were washed with DI water, rinsed with methanol, acetone, methylene chloride and hexane before use.

4.2.2 Sampling

Reclaimed water used for irrigation was collected directly from a sprinkler system using 500 mL PETE bottles and was stored in the freezer at <10 °C. The source of reclaimed water used in this study is the Miami-Dade Water and Sewer Department North District Wastewater Treatment Plant. The wastewater treatment plant was designed to have a flow of 120 million gallons per day (MGD) with average daily flow around 112.5 million gallons per day. Pure oxygen activated-sludge is used in the WWTP as the main secondary treatment process (Kasprzyk-Hordern et al., 2008). Extra filtration and disinfection are applied to effluents before release to make the reclaimed water ready for use in irrigation.

4.2.3 Sample preparation

Water samples were allowed to reach room temperature, filtered through glass fiber filters with a pore size of 0.5 µm without loss of analytes and samples were

analyzed within 14 days in order to avoid potential degradation and transformation of analytes. No additional sample preparation was required beyond the addition of surrogates.

Table 8. Information of analytes

compound	CAS	Usage/source	compound	CAS	Usage/ source
acetaminophen	103-90-2	analgesic and anti-inflammatory	testosterone	58-22-0	natural hormone
caffeine	58-08-2	stimulant drug	progesterone	57-83-0	natural hormone
primidone	125-33-7	anticonvulsants	androsterone	53-41-8	natural hormone
estriol	50-27-1	Hormone	mestranol	72-33-3	synthetic hormone
carbamazepine	298-46-4	anticonvulsant	coprostan-3-one	601-53-6	fecal sterol
DEET	134-62-3	insect repellent	coprostan-3-ol	360-68-9	fecal sterol
equilenin	517-09-9	natural hormone	bisphenol A	80-05-7	intermediate in synthesis of plastics
naproxen	22204-53-1	analgesic and anti-inflammatory	ibuprofen	15687-27-1	analgesic and anti-inflammatory
17α-ethynylestradiol	57-63-6	synthetic hormone	triclosan	3380-34-5	antibacterial and antifungal agent
equilin	474-86-2	natural hormone	4-tert-octylphenol	27193-28-8	degradation of octylphenolethoxylates

compound	CAS	Usage/source	compound	CAS	Usage/ source
17β-estradiol	50-28-2	natural hormone	gemfibrozil	25812-30-0	Lipid regulator
estrone	53-16-7	natural hormone			

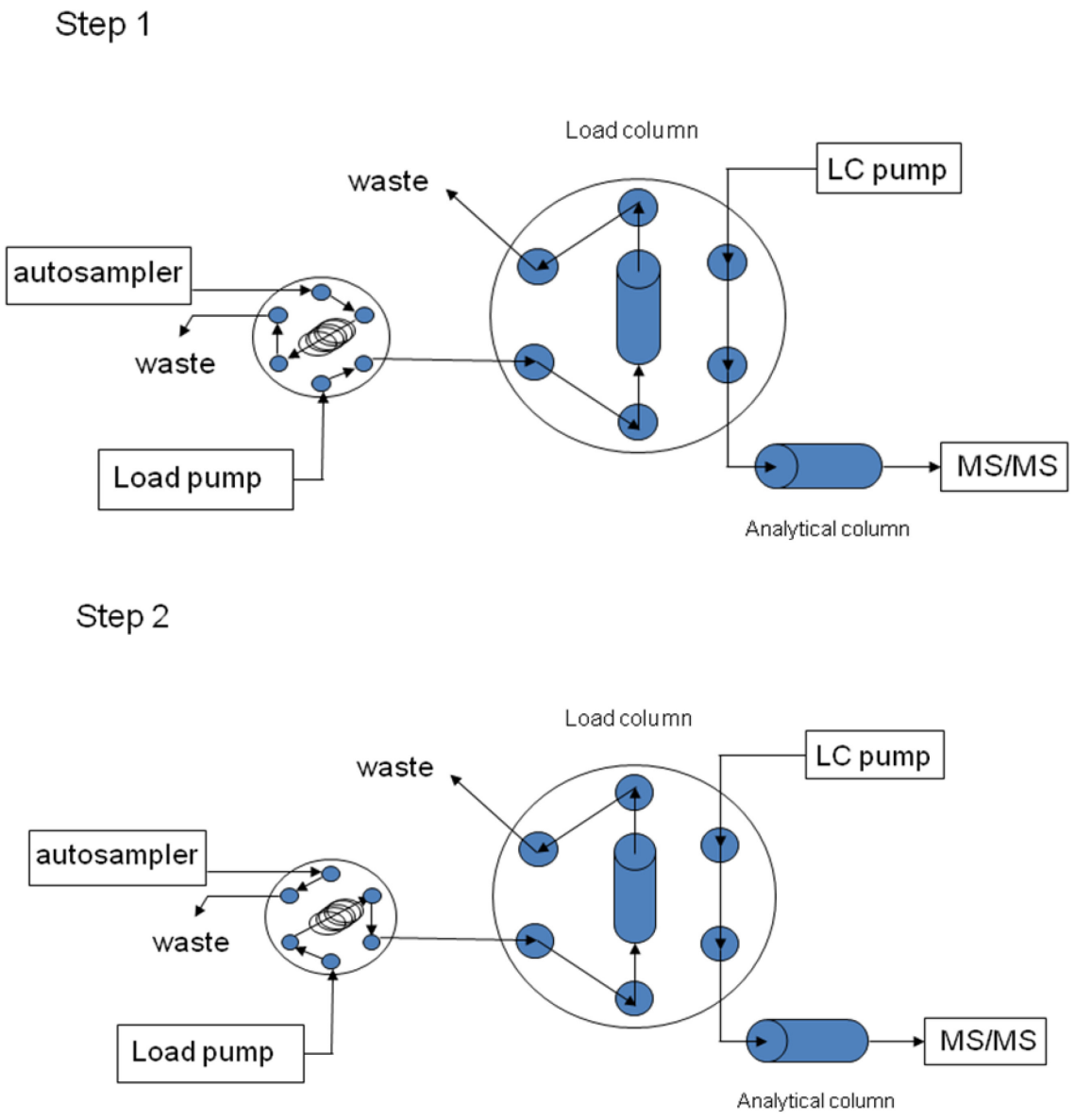
4.2.4 Instrumentation

An EQuan Environmental Quantitation system manufactured by Thermo Fisher Scientific was used to preconcentrate microconstituents from reclaimed water. The EQuan^{TM} system is based on a dual switching-column system, which consists of a sample delivery system, a switching-column array and an LC-MS/MS system. Its sample delivery system consists of an autosampler manufactured by CTC analytics AG (Zwingen, Switzerland) and an Accela 600 loading pump (Thermo Fisher Scientific, San Jose, CA, USA). Its column-switching array is composed of a Rheodyne 7750E-205 six-port switching valve system made by IDEX (Oak Arbor, WA, USA), a preconcentration column and an analytical column. A Thermo Hypersil aQ (20 mm × 2.1 mm, 12 µm particle size) was used as the loading column and a Thermo Hypersil Gold (50 mm × 2.1 mm, 1.9 µm particle size) was used as the analytical column. Mass spectrometry analysis was performed using a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

4.2.5 On-line SPE setup

Depending on the expected target analyte concentration, 5 mL or 20 mL samples were injected into the sample loop (step 1 in figure 10) and then loaded to the preconcentration column at a flow rate of 1 mL/min (step 2 in figure 10). This loading rate allowed good overall recoveries of analytes. The preconcentration column was washed by 1000 µL of water with varied amount of methanol and connected to the analytical column after the valve had switched to the inject

position. The loading column and analytical column underwent the same gradient in the positive ionization (PI) mode or negative ionization (NI) mode (step 3 in figure 10). The gradient details are shown in Table 9.



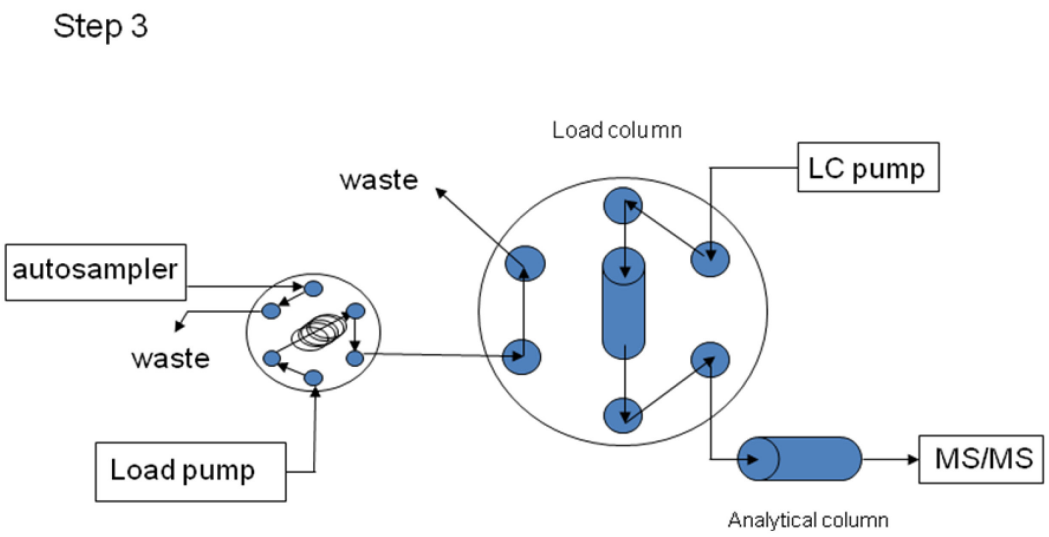


Figure 10. On-line SPE procedure

4.2.6 Mass spectrometry

Mass spectrometry analysis was performed using a TSQ Quantum Access QqQ Mass Spectrometer (Thermo Scientific, San Jose, CA, USA). Quantitation for all sources was performed using selected reaction monitoring. Instrument control and data acquisition were performed using Xcalibur software (rev. 2.1, Thermo Scientific, San Jose, CA, USA). Source parameters for analytes were optimized using HESI, APCI and APPI independently. A mobile phase of 0.1% fomic acid/MeOH (50:50, v/v) was used for the positive ionization mode and water/MeOH (50:50, v/v) was used for the negative ionization mode. Each analyte and surrogate was injected to the ion source at a concentration of 10 µg/mL. Compound-dependent parameters such as tube lens and collision energy were optimized to obtain maximum signals. The APPI source was further

optimized for the dopants described before. Results are shown in Table 10a, 10b and 10c.

Table 9. Gradient of load pump and analytical pump

Time (min)	Load Pump			Analytical Pump			Valve position
	Solvent A water (%)	Solvent B methanol (%)	Flow rate (µL min^{-1})	Solvent B methanol (%)	Solvent D 0.1% FA in water (%)	Flow rate (µL min^{-1})	
				PI mode			
0.0	75	25	1000	10	90	200	Load
6.0	75	25	1000	↓	↓	↓	Inject
6.1	0	100	250	↓	↓	↓	
7.0	↓	↓	↓	10	90	↓	
14.0	↓	↓	↓	100	0	↓	
26.0	↓	↓	↓	100	0	↓	
27.0	↓	↓	250	10	90	↓	Load
27.1	0	100	1000	↓	↓	↓	
28.0	0	100	↓	↓	↓	↓	
28.1	100	0	↓	↓	↓	↓	
33.0	100	0	↓	10	90	200	
				NI mode			
	Solvent A water (%)	Solvent B methanol (%)	Flow rate (µL min^{-1})	Solvent A water (%)	Solvent B methanol (%)	Flow rate (µL min^{-1})	
0.0	25	75	1000	50	50	200	Load
6.0	25	75	1000	↓	↓	↓	Inject
6.1	0	100	720	↓	↓	↓	
7.0	↓	↓	↓	50	50	↓	
8.0	↓	↓	↓	0	100	↓	
12.0	↓	↓	↓	0	100	↓	
14.0	0	100	720	50	50	↓	
14.1	0	100	1000	↓	↓	↓	Load
15.0	0	100	↓	↓	↓	↓	
15.1	100	0	↓	↓	↓	↓	
20.0	100	0	↓	50	50	200	

Table 10a. Ions of analytes in ESI

Analyte	ESI parameter								
	Precursor ion	m/z	SRM1	CE1	SRM2	CE2	SRM3	CE3	tube lens
acetaminophen	[M+H]+	150.0	107.2	22	118.1	33	132.1	24	49
Caffeine	[M+H]+	195.1	138.2	18	110.2	22	83.3	26	67
Primidone	[M+H]+	219.1	91.2	28	162.2	12	117.2	23	68
Estriol	[M-H]-	287.1	145.2	41	171.1	37	159.2	40	94
Carbamazepine	[M+H]+	237.1	194.1	19	192.1	25			61
DEET	[M+H]+	192.1	91.3	25.0	119.2	11.0	65.3	36.0	72
Equilenin	[M-H]-	265.1	193.1	47	221.1	35	181.1	40	81
Naproxen	[M-H]-	229.0	170.1	18	185.2	11			60
17α-ethynylestradiol	[M-H]-	295.2	145.1	23	143.2	45	267.2	15	99
Equilin	[M-H]-	267.1	143.1	36	115.2	47	145.1	30	70
17β-estradiol	[M-H]-	271.1	145.1	45	146.2	35	158.0	33	79
estrone	[M-H]-	269.1	145.1	40	159.1	37	143.2	56	78
Testosterone	[M+H]+	289.2	109.2	25	97.2	25	79.3	39	109
Androsterone	[M+H]+	291.2	273.2	8	255.2	13	199.1	19	97

Analyte	ESI parameter								
Progesterone	[M+H]+	315.2	109.2	25	97.2	24	79.3	41	98
Mestranol									
coprostan-3-one	[M+H]+	387.3	369.4	11	95.2	30	119.3	32	129
coprostan-3-ol									
bisphenol A	[M-H]-	227.1	212.4	20	133.3	28	158.7	11	94
Ibuprofen	[M-H]-	205.1	161.3	10					51
Triclosan	[M-H]-	286.9	142.1	34	160.9	38	35.4	11	111
4-tert-octylphenol	[M-H]-	205.1	133.1	26	134.1	21	117.1	72	99
Gemfibrozil	[M-H]-	249.1	121.2	20	106.1	49	120.1	44	65

Table 10b. Ions of analytes in APCI

Analyte	APCI parameter								
	Precursor ion	m/z	SRM1	CE1	SRM2	CE2	SRM3	CE3	tube lens
Acetaminophen	[M+H]+	152.0	110.1	16	93.1	24	65.3	29	82
Caffeine	[M+H]+	195.1	138.0	19	110.1	23	123.0	31	87
Primidone	[M+H]+	219.1	91.2	28	162.0	12	119.1	16	83

Analyte	APCI parameter								
Estriol	[M-H2O+H]+	271.1	253.0	12	157.0	21	133.0	23	76
Carbamazepine	[M+H]+	237.0	193.9	19	192.9	33	191.9	24	93
DEET	[M+H]+	192.1	119.1	17	91.2	28	65.3	43	76
Equilenin	[M+H]+	267.1	209.0	18	249.0	12	165.0	50	70
Naproxen	[M+H]+	231.0	184.9	14	170.0	25	141.0	44	91
17α-ethynylestradiol	[M-H2O+H]+	279.1	133.0	16	159	21.0	105.1	32	78
Equilin	[M+H]+	269.1	251.0	13	211.0	18	181.0	40	78
17β-estradiol	[M-H2O+H]+	255.2	159.1	18	133.1	22	144.1	37	76
estrone	[M+H]+	271.2	253.0	12	157.0	19	159.0	22	78
Testosterone	[M+H]+	289.2	97.1	24	109.1	26	253.0	16	83
Androsterone	[M-H2O+H]+	273.2	255.2	10	91.129	38	105.1	35	90
Progesterone	[M+H]+	315.2	109.1	27	97.1	25	297.0	14	81
Mestranol	[M-H2O+H]+	285.2	267.0	14	171.0	19	173.0	23	66
coprostan-3-one	[M+H]+	387.3	369.3	10	95.12	33	147.0	25	94
coprostan-3-ol	[M-H2O+H]+	371.3	95.1	30	81.2	31	109.1	27.0	90
bisphenol A	[M-H]-	227.1	211.9	21	133.0	31	210.9	34	88

Analyte	APCI parameter								
Ibuprofen	[M-H]-	205.2	161.2	10					58
Triclosan	[M-Cl-2H]-	250.9	214.9	26	186.9	32.0	159.0	31.0	75
4-tert-octylphenol	[M-H]-	205.1	133.1	29	134.1	21	117.1	64	94
Gemfibrozil	[M+H]+	251.1	232.8	5	129.0	8	83.2	13	87

Table 10c. Ions of analytes in APPI

Analyte	RT (min)	APPI parameter							
		Precursor ion	m/z	SRM1	CE1	SRM2	CE2	SRM3	CE3	tube lens
Acetaminophen	8.2	[M+H]+	152.1	110.1	15	65.3	31	93.2	23	100
Caffeine	11.46	[M+H]+	195.1	138.1	19	110.2	22	123.1	31	100
Primidone	12.44	[M+H]+	219.1	162.1	13	91.2	34	119.1	18	98
Estriol	13.28	[M-H2O+H]+	271.1	253.1	12	133	30	159.1	30	75
Carbamazepine	13.66	[M+H]+	237.1	194.1	18	192.1	23	179.1	35	87
DEET	14.09	[M+H]+	192.1	119.1	17	91.2	31	65.3	43	88
Equilenin	14.59	[M+H]+	267.1	209.1	18	249.1	11	165.1	52	93

Analyte	RT (min)	APPI parameter								
Naproxen	14.62	[M-COOH]+	185.1	170.1	17	141.1	33	115.1	49	84
17α-ethynylestradiol	14.66	[M-H2O+H]+	279.1	133.1	17	159	19	105.1	37	82
Equilin	14.67	[M+H]+	269.1	251.1	14	211.1	18	181	38	103
17β-estradiol	14.69	[M-H2O+H]+	255.1	159	17	133.1	19	144	36	76
estrone	14.76	[M+H]+	271.1	2531.1	12	159	21	157	19	90
Testosterone	15.06	[M+H]+	289.2	97.1	23	109.1	24	253.2	16	107
Androsterone	15.96	[M-H2O+H]+	273.2	255.2	13	105.2	39	161.1	23	104
Progesterone	15.64	[M+H]+	315.2	109.1	22	97.1	21	297.3	15	68
Mestranol	16.18	[M+H]+	293.2	147.1	18	173.1	21	158.1	34	91
coprostan-3-one	22.04	[M+H]+	387.3	369.3	16	95.2	35	109.1	28	104
coprostan-3-ol	22.09	[M-H2O+H]+	371.3	95.2	31	81.2	34	109.1	26	103
bisphenol A	12.14	[M-H]-	227.1	212	22	211	35	133.1	34	87
Ibuprofen	13.20	[M-H]-	205.1	161.1	11					56
Triclosan	13.43	[M-Cl-2H]-	250.9	215	22	187	31	159	29	100
4-tert-octylphenol	13.59	[M-H]-	205.1	133.1	28	134.1	20	117.2	62	98
Gemfibrozil	13.69	[M-H]-	249.1	121.1	17	127.1	14	106.2	45	73

To avoid introducing additional variability, ionization techniques were performed using a common Ion MAX source housing (Thermo Scientific, San Jose, CA, USA). Source-dependent parameters for optimal HESI detection were as follows: capillary temperature (300 °C), vaporizer temperature (350 °C), sheath gas pressure (35 arbitrary units), aux gas pressure (5 arbitrary units), ion sweep gas pressure (8 arbitrary units) and spray voltage (+4000 V and -3500 V).

Source-dependent parameters for optimal APCI detection were as follows: capillary temperature (270 °C), vaporizer temperature (400 °C), sheath gas pressure (30 arbitrary units), aux gas pressure (5 arbitrary units), ion sweep gas pressure (0 arbitrary units) and discharge current (4 μA) for both positive and negative polarity.

The photoionization lamp used for APPI was a Syagen krypton UV lamp which emits photons at 10 eV and 10.6 eV (Syagen Technology Inc., Tustin, CA, USA). The source-dependent parameters for optimal detection were as follows: capillary temperature (270 °C), vaporizer temperature (400 °C), sheath gas pressure (30 arbitrary units), aux gas pressure (5 arbitrary units), ion sweep gas pressure (0 arbitrary units) and discharge current (0 μA). Four different dopants, acetone, anisole, chlorobenzene and toluene, were tested to find the best one for the analytes.

4.2.7 Matrix effect

Signal suppression or enhancement effects have been widely reported in the literature when complicated matrices are tested in API (Viglino et al., 2008). Therefore, matrix effects were evaluated using the following equation 1.

$$matrixeffect(\%) = (\frac{RWs - RWns}{DIs}) \times 100\% \quad (1)$$

RWs is the analyte peak area in the spiked reclaimed water, RWns is the analyte peak area in the non-spiked reclaimed water and DIs is the analyte peak area in DI water spiked with a known amount of analytes. Signal enhancement is indicated by matrix effect values more than 100%, while signal suppression is indicated by matrix effect values less than 100% (Garcia-Ac et al., 2009). Three replicate samples were run to determine the relative standard deviation (RSD). Spike level of analytes were four times of the spike level used to calculate MDLs, which is shown in table 11.

4.2.8 Extraction recovery and method detection limit

Analyte recovery was evaluated by measuring 5 mL spiked tap water samples since it is more comparable to environmental waters than DI water. The spiked level of analytes for method detection limits is shown in table 11. For each compound, three different spike levels from low to high were tested to evaluate how different spike amounts would affect recovery of compounds. Method detection limits (MDLs) were determined according to EPA guidelines (Ripp, 1996). Eight replicate tap water samples spiked with all compounds with

concentration ranging from one to eight times of the tested detection limit (DL). Standard deviations (SD) were calculated from replicates and MDL = 2.998 SD. MDLs were calculated for 5 mL sample size and 20 mL sample size.

4.2.9 QA/QC

Blanks were run with each batch to check for potential contamination and assess background levels of native analytes. Spiked blanks (LBS) and matrix spikes (MS) were also run with each analytical batch to check the recovery of analytes. Isotopic dilution was used to increase the precision and accuracy of analytsis. A five-point calibration curve was constructed with each batch of 20 or less samples to check for linearity and analytical sensitivity. Calibration range is shown in table 11.

4.3 Results and discussion

4.3.1 Online procedure optimization

Optimization of key parameters is essential in the development of a robust online procedure: mobile phase flow rate, sample volume, loading flow rate, wash flow rate, wash volume and organic composition of the wash solvent were all tested. Based on previous study (Lien et al., 2009), the analytical mobile phase flow was set at 200 µL/min to produce better analyte signal strength. 1 mL/min was chosen as the optimum loading flow rate since it allowed good overall recoveries of analytes and higher flow rates have shown to affect the long-term functioning of loading columns (Garcia-Ac et al., 2009). The presence of dissolved organic

substances in environmental waters could introduce severe interference and make quantitation complicated (Viglino et al., 2008). Thus, a wash step was used after the loading step. To avoid introducing pressure change, the washing flow rate was kept the same as the loading step (1 mL/min). The amount of washing time (0.5 and 1.0 mins) and percentages of methanol (25%, 50%, 75% and 100%) were changed for all target compounds using 5 mL sample size. Results from this optimization are shown in Figure 11. When washing with a volume of 500 µL (0.5 min), all the compounds except acetaminophen were effectively retained on the loading column independent of the percentage of methanol used (Figure 11a). Acetaminophen is very water soluble and was not well retained on the column even after washing with only 500 µL of pure water (recovery was 56% ± 5%). Therefore, the Hypersil aQ column is not a good choice to retain acetaminophen. When the preconcentration column was washed with a volume of 1000 µL (1.0 min), caffeine and primidone recoveries decreased for methanol content above 25% (Figure 11b). Quantification of hormones is also difficult because of the organic matter interference background when the wash step was eliminated, but it improves significantly after the preconcentration column is washed. Therefore, a wash step of 1000 µL water with 25% methanol was finally used because it represents the best overall performance for recoveries and sensitivities, except for acetaminophen, that is poorly recovered under most conditions tested. In negative mode, analytes were retained on the preconcentration column very well and 1000 µL water with 75% methanol was used as the wash step.

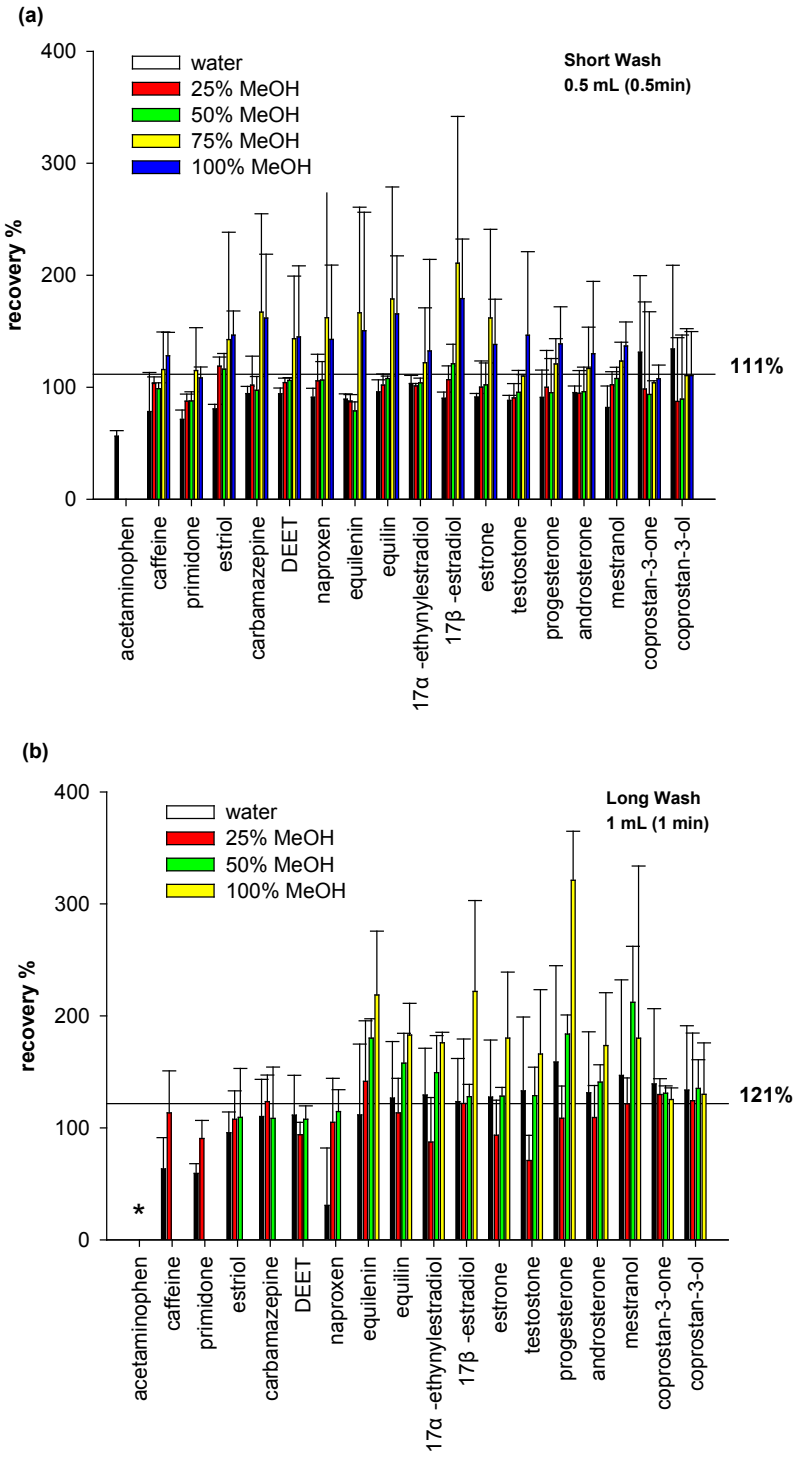


Figure 11. Recovery of analytes during the washing step after loading. (a) 500 µL wash with increasing methanol composition And (b) 1000 µL wash with increasing methanol composition. Asterisk (*) indicates not recovered. Spike levels are shown in table 11.

4.3.2 Comparisons of different ion sources based on MDLs

Analytes detected in positive mode were separated into three categories according to their chemical functionalities and general properties: the pharmaceuticals, the hormones and the sterols. Because MDLs were dramatically different between categories, MDLs were compared for compounds within each category with 5 mL sample size. The best ionization source was determined by direct comparison of the MDLs. Analytes detected in the negative ionization mode were separated into a fourth category. In each group, compounds were ordered based on the average MDL of the different ionization methods. Results are summarized in Figure 12,13 and 14.

4.3.3 Pharmaceuticals and personal care products

The first three compounds of the six compounds in this category, carbamazepine, DEET and caffeine, are easily detected in all ion sources (Figure 12). Previous studies indicate that these three compounds are better detected using ESI (Castiglioni et al., 2005; Trenholm et al., 2008). Our results indicate that APPI with toluene is also an excellent alternative. MDLs for the next three compounds, naproxen, acetaminophen and primidone, were higher than the first three compounds. For naproxen, acetaminophen and primidone, the best detection methods were APPI with toluene (15.4 ng/L), APCI (1.92 ng/L) and APPI with toluene (38.3 ng/L), respectively. Despite the fact that ESI and APCI showed advantage for certain compounds in the category, APPI with toluene is a good overall choice to detect them.

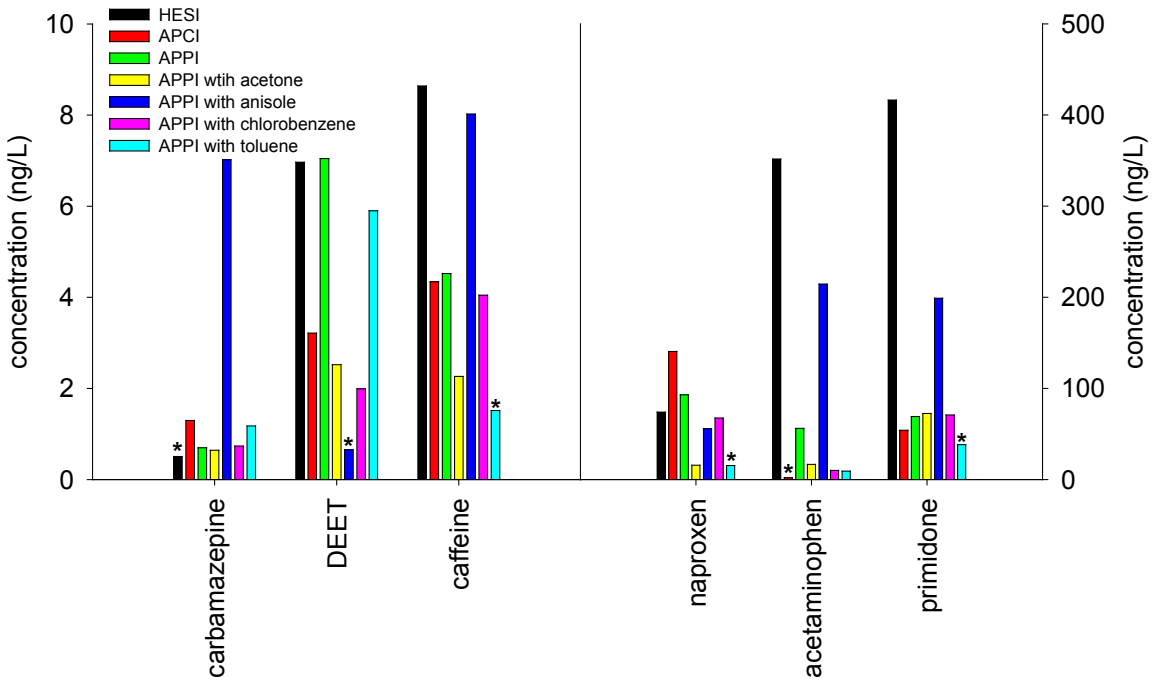


Figure 12. MDLs of pharmaceuticals for all ionization mechanisms tested. Note the different axes. Asterisk(*) indicates best performance

4.3.4 Hormones

Ten natural and synthetic hormones were investigated in this category. Seven out of ten compounds, testosterone, equilenin, equilin, 17α-ethynylestradiol, androsterone, estriol and mestranol, were best detected using APPI with toluene (Figure 13). Two out of ten compounds, progesterone, 17β-estradiol were best detected using APPI with chlorobenzene. One out of ten compounds, estrone, was best detected using APPI with acetone. However, for this category, APPI significantly outperformed all other ionization modes.

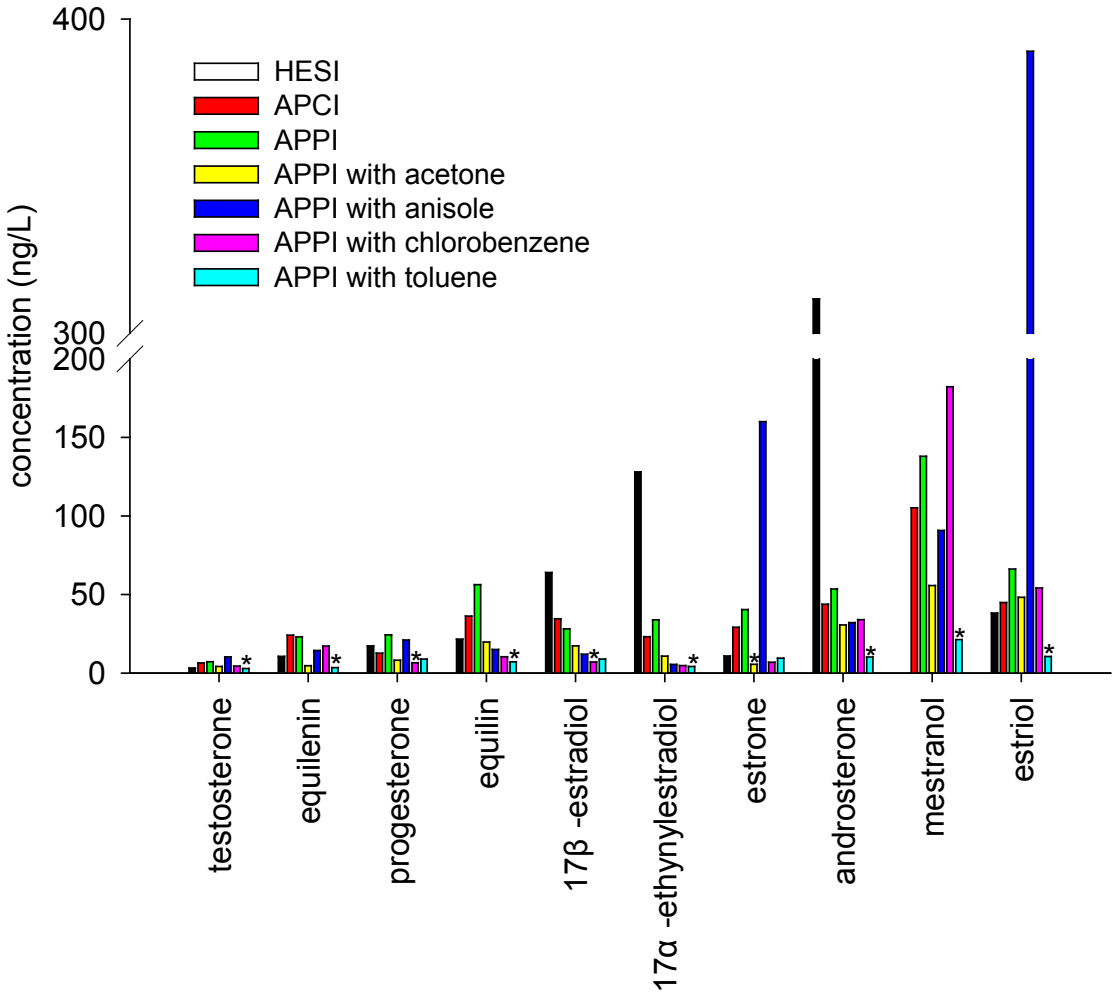


Figure 13. MDLs of hormones for all ionization mechanisms tested

Testosterone and progesterone behaved slightly differently. The two compounds showed overall low detection limits for all ion sources tested. A one way ANOVA comparison showed that there was no significant difference between ion sources. Equilenin and equilin required presence of dopants to improve the MDLs. The best dopant for them was toluene with MDLs of 3.44 ng/L and 7.15 ng/L, respectively. 17β-estradiol, 17α-ethynylestradiol (EE2), androsterone and

mestranol had similar results. These four analytes were extremely hard to ionize in HESI and mestranol even did not yield a signal in HESI. MDLs improved slightly when APCI and APPI were used but dramatically decreased when dopants were added. Toluene was the best dopant for all of them, except for 17β-estradiol. Based on the results, it is clear that dopant chemistry and proton affinity are important for this compound category.

4.3.5 Sterols and sterones

Two model compounds were investigated in the sterol category. Sterols showed much higher MDLs compared with the other two categories (Figure 14). The Conventional ESI method is not well suited to ionize sterols because their highly lipophilicity and the lack of multiple polar functional groups. APCI however has been successfully used for the detection of sterols. A recent study showed that APPI was particularly sensitive for cholesterol, sitosterol and sitostanol (Palmgren et al., 2005). Coprostan-3-ol could not be ionized in HESI. APPI, however, was much better, and MDLs using chlorobenzene (36.7 ng/L) and toluene (43.9 ng/L) were low enough for trace analysis. Coprostan-3-one was marginally ionized by HESI with an MDL of 2090 ng/L, but APPI with acetone dramatically decreased MDL to 118 ng/L. All these results point out again the benefit of using a single ionization method for trace environmental analysis.

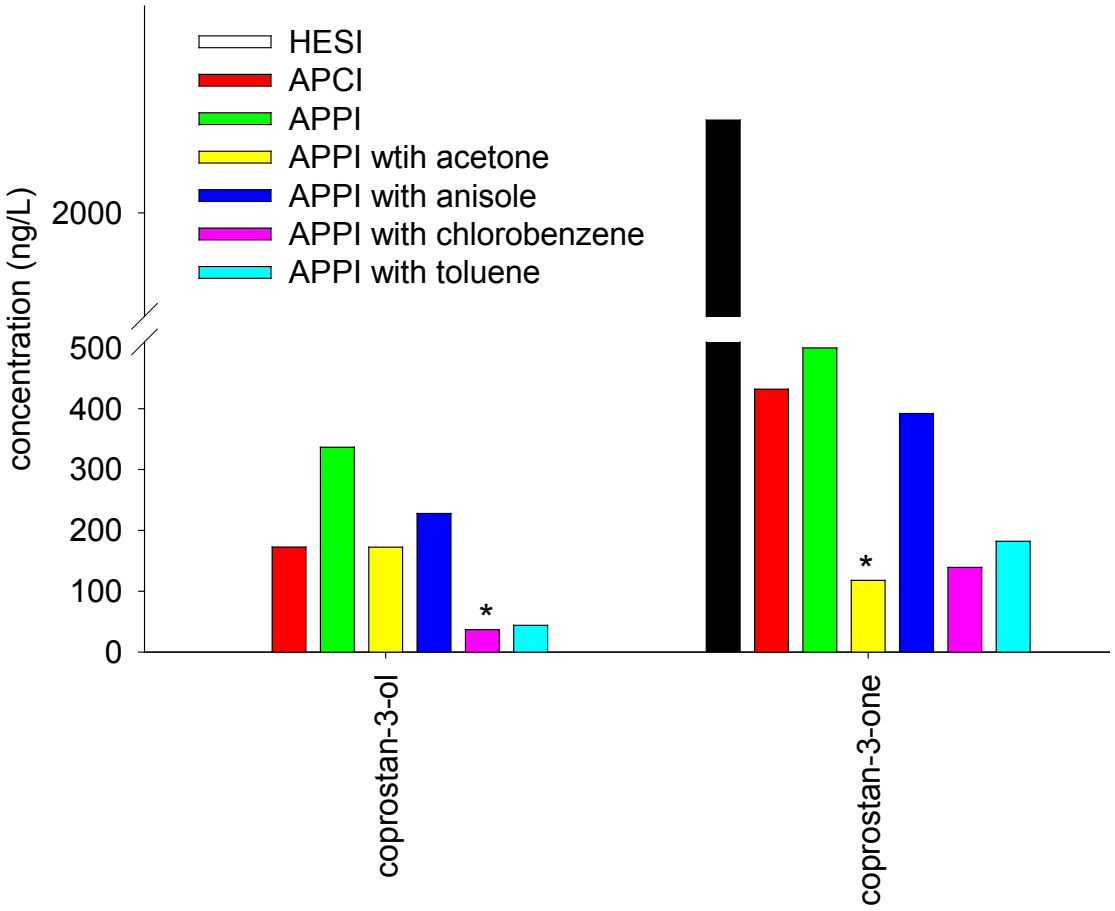


Figure 14. MDLs of sterols and sterones for all ionization mechanisms tested

4.3.6 Negative ionization mode

Five compounds were detected in the negative ionization mode: bisphenol A, ibuprofen, triclosan, gemfibrozil and 4-tert-octylphenol. All of them were detected by HESI, APCI and APPI with dopants. Results are shown in Figure 15.

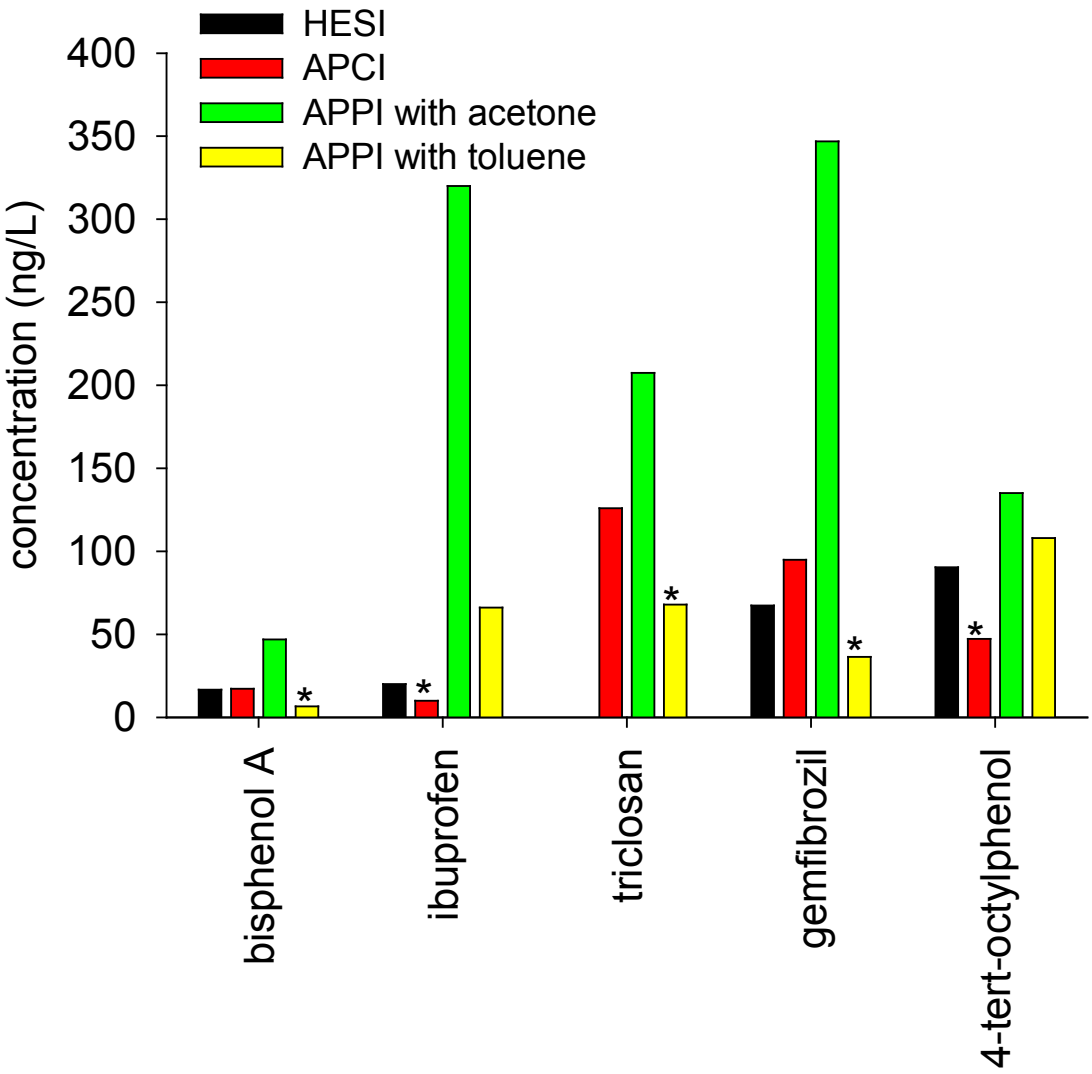


Figure 15. MDLs of analytes in negative mode for all ionization mechanisms tested

For bisphenol A, APPI with toluene was the best ionization with an MDL of 6.56 ng/L. For ibuprofen, both HESI and APCI were better choices. Ibuprofen is a very polar and small compound; therefore, Atmospheric pressure photoionization did not provide an effective method. Triclosan could not be ionized by HESI. APCI

improved the ionization of triclosan yielding an MDL of 126 ng/L. APPI with toluene as dopant lowered the MDL by half to 67.9 ng/L. Gemfibrozil was detected in the negative ionization mode in all the ion sources except for APCI. APPI with toluene was the best ionization method, with an MDL of 36.4 ng/L. The best ionization method for 4-tert-octylphenol was APCI with an MDL of 47.3 ng/L.

Three of the compounds were best detected in APPI with toluene as dopant. However, for polar compounds like ibuprofen or medium polar compounds like 4-tert-octylphenol, APPI was not the best choice. This is one of the few examples where HESI or APCI outperform the APPI source.

4.3.7 Final choice of dopant

A key goal of this study was to compare ionization sources and provide an overall solution in a single run. APPI with toluene as dopant was the choice because it provided the lowest MDLs for most analytes. Our results indicate that dopant flow is critical in controlling source stability but does not influence signal intensity. Different flows of toluene were tested, ranging from 5 µL/min to 50 µL/min in a constant mobile phase flow rate of 200 µL/min. Analyte signals did not increase as the flow rate of the dopant increased for the positive mode; however, analyte signals became more stable when the flow was set at 25 µL/min. In the negative mode, we observed that the signals of bisphenol A, ibuprofen and 4-tert-octylphenol were negatively affected when adding more than 10 µL/min toluene. Therefore, flow rate was kept under 5 µL/min. Dopants have

been used since 1994 to enhance photoionization performance (Marchi et al., 2009). The ionization mechanism usually depends on the PA of the dopant, solvent and analyte. There are two possible mechanisms: charge transfer, dominated by electron affinity (EA) and proton transfer. In addition, solvent molecules having the right proton affinities actively participate in the reaction to enhance the ionization efficiency (Equation 2 and 3). Based on the results shown in table 10c, where all the target analytes preferentially produced protonated or deprotonated molecular ions, the likely mechanism seen in the APPI source is probably dominated chemical ionization by solvent molecules (equation 3) with the dopant being used to promote the transfer of energy from krypton lamp to the solvent rather than to produce direct ionization of the analytes by proton transfer.

$$D^{\bullet+} + nS \rightarrow [D\text{-}H]^{\bullet} + [S_n + H]^+ \quad \text{if } PA_S > PA_{[D\text{-}H]^{\bullet}} \tag{2}$$

$$[S_n + H]^+ + M \rightarrow nS + [M+H]^+ \quad \text{if } PA_M > PA_S \tag{3}$$

The IE of four dopants, anisole (8.2 eV), toluene (8.3 eV), chlorobenzene (9.07 eV) and acetone (9.7 eV), are clearly below the IE provided by the lamp and lower than of water (12.6 eV) and methanol (10.8 eV), thus formation of the radical cation from the dopants will dominate in the source. The PA of four dopants are anisole (839.6 kJ/mol) > acetone (812.0 kJ/mol) > toluene (784.0 kJ/mol) > chlorobenzene (753.1 kJ/mol) (Marchi et al., 2009). Although PAs for the compounds targeted in this study are not available in the literature, it is likely that will range above the PAs for both water and methanol. Because most analytes formed $[M+H]^+$ ions under the optimized conditions tested, the

predominant mechanism for our analytes was likely the reaction of dopant photoions with solvent molecules, followed by solvent based ionization of the analytes by proton transfer as suggested by previous studies for a water-methanol-toluene system (Kauppila et al., 2002; Kauppila et al., 2004). Although acetone have a high IE, its high PA (812.0 kJ/mol) cause it less effective than toluene (784.0 kJ/mol) in proton transfer mechanism. Chlorobenzene has often been described as the best APPI dopant. However, in this study, toluene generated better results and has the advantage of being less toxic than chlorobenzene (Marchi et al., 2009). Therefore, toluene was chosen as the dopant for all analytes

4.3.8 Recovery comparison

Three spike levels (1 × MDL level, 5 × MDL level and 10 × MDL level) were used to assess recoveries. A summary of the results are present in Figure 16. Analyte recoveries in the positive mode ranged from 70% to 152% when spike levels were close to MDLs. The recoveries ranged from 86% to 121% when the spike level was increased to the 5 × MDL level and ranged from 79% to 126% when spike level was increased to the 10 × MDL level. No significant differences were seen between the 3 spiking concentrations (average recovery 105%). Results indicated that analytes were retained at the preconcentration column very well. When spike levels were close to the detection limit, recoveries were more variable than at high spike levels. RSD's of all the spiked samples were less than 20%, except testosterone and equilenin that spiked at the MDL level (24% and

21%). Recoveries of analytes detected in negative mode were less reproducible and clearly affected by the spike levels. Analyte recoveries in the negative mode ranged from 44% to 216% except for ibuprofen when spike levels were close to MDLs. The recoveries ranged from 76% to 163% when the spike level was increased to the 5 × MDL level and ranged from 67% to 155% when spike level was increased to the 10×MDL level. There is no difference between the 5 × and 10 × MDL fortification levels but method performance was less robust at concentrations near the MDLs.

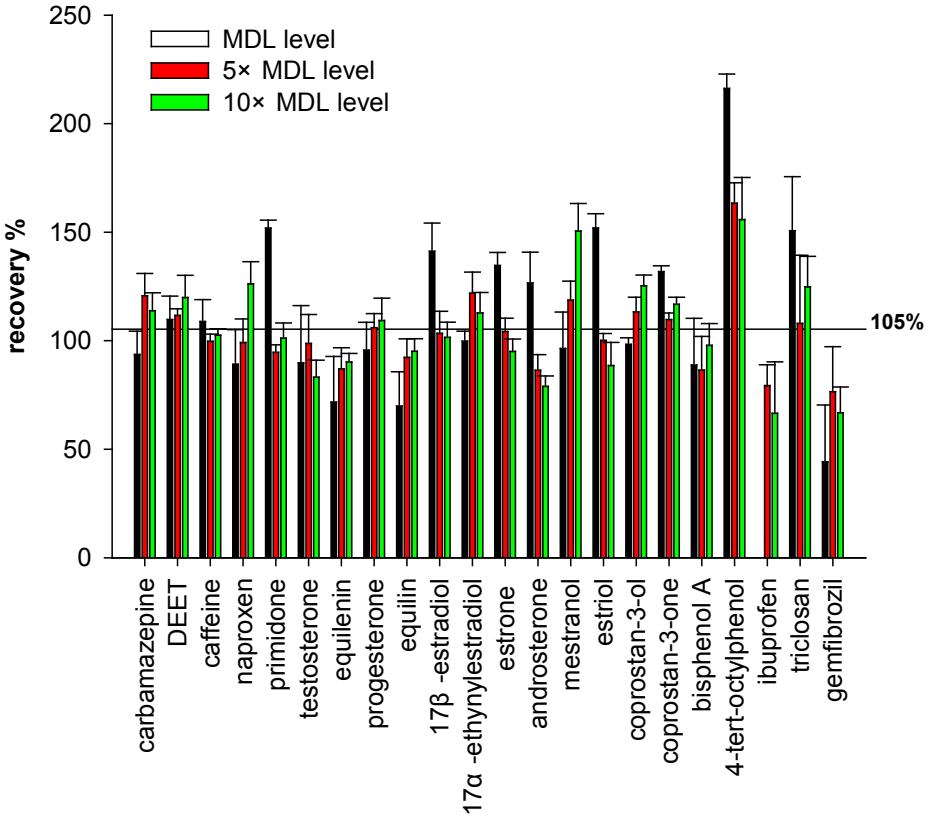


Figure 16. Recovery of analytes at different spike levels

4.3.9 Matrix effects evaluation

Results in Table 11 indicated that 70% of the analyte's signals were enhanced because of matrix effects in APPI, while 30% of analyte's signals were suppressed because of matrix effects. Two compounds, androsterone and ibuprofen, were not detected in the spiked reclaimed water sample because of high background interference. Compared to other ionization sources (ESI and APCI), APPI is not generally considered susceptible to the matrix effect (Hanold et al., 2004). However, results in our study showed otherwise, since signal intensity were significantly influenced by matrix for most analytes (127% average). For example, the enhancement for mestranol and triclosan were more than 200% and suppression of 4-tert-octylphenol was 55%. Enhancement and suppression were observed in both positive and negative mode. This is a clearly indication that the contribution of the solvent and other ionization producing procedure described in equation 2 is likely the controlling factor while the participation of the dopant is a secondary process. Results also pointed out that the wash procedure was essential in eliminating the interference from wastewater samples but was a compromise for early eluting highly-polar compounds that are more influenced by rinsing with a stronger organic phase.

Table 11. Evaluation of the APPI Matrix effect

Analyte	matrix effect (%)	RSD
	PI mode	
Caffeine	179	7
Primidone	131	12
Estriol	118	1
carbamazepine	172	4
DEET	120	11
Equilenin	70	8
17α-ethynylestradiol	117	15
Equilin	95	12
17β-estradiol	106	13
Estrone	113	3
Naproxen	121	15
Testosterone	113	17
Progesterone	116	5
Mestranol	247	7
coprostan-3-one	69	17
coprostan-3-ol	96	21
	NI mode	
bisphenol A	126	8
Triclosan	222	60
Gemfibrozil	156	11
4-tert-octylphenol	55	12

4.3.10 Method validation

The method was validated by using APPI with toluene as a dopant. Calibration range, linearity, spike levels and MDLs of 5 mL and 20 mL are shown in Table 12. The linearities of all analytes were above 0.99, except gemfibrozil, triclosan and 4-tert-octylphenol, which were all detected in the negative ionization mode. DEET was commonly detected in the reagent water at a concentration of about 2 ng/L. DEET can be transferred via dirty hands or gloves (Capdeville and Budzinski, 2011). Therefore, 10 ng/L was used as the starting calibration point of DEET and

MDLs were corrected to 5.9 ng/L. When sample size was increased from 5 mL to 20 mL, some analytes lost their recovery on column since they were washed away at loading procedure. Acetaminophen and caffeine cannot be detected when sample size was increased to 20 mL. The MDLs of bisphenol A and triclosan of 20 mL were higher than 5 mL because of the recovery lost. Statistical MDLs were comparable to previous research (Viglino et al., 2008) for estradiol, estrone, 17α-ethinylestradiol and progesterone, however the method reported here expanded significantly the list of analytes that could be detected simultaneously by APPI. Despite the fact that without using dopant (Viglino et al., 2008), the improvement for specific compounds like estriol (15 ×) combined with the detection of fecal sterols at low ng/L levels constitue an important improvement justifying the use of dopant.

Table 12. Calibration range, linearity, spike levels and MDLs

	calibration range(ng/L)	linearity	spike level (ng/L)	MDLs(ng/L) 5 mL	MDLs(ng/L) 20 mL
acetaminophen	100-800	0.9935	200	9.24	NA
caffeine	2-2048	0.9946	8	1.51	NA
primidone	62.5-4000	0.9905	250	38.3	14.9
estriol	32-1024	0.9905	64	10.5	1.64
carbamazepine	1-256	0.9927	1	1.18	0.30
DEET	10-2560	0.9912	10	5.90	2.83
equilenin	4-1024	0.9985	16	3.44	3.39
naproxen	80-5120	0.9943	160	15.4	8.45
17α-ethynylestradiol	4-1024	0.9903	16	4.22	1.26
17β-estradiol	4-1024	0.9935	16	8.94	2.57
equilin	10-1280	0.9939	40	7.15	1.17
estrone	10-1280	0.9951	20	9.47	1.59
testosterone	2-1024	0.9916	4	2.87	2.30
progesterone	10-160	0.9901	10	8.93	1.72
androsterone	32-512	0.9968	32	10.2	37.0
mestranol	25-800	0.9902	100	21.3	7.89
coprostan-3-one	400-6400	0.9941	1600	182	11.2
Coprostan-3-ol	200-3200	0.9901	200	43.9	5.44
bisphenol A	20-5120	0.9925	20	6.56	79.1
ibuprofen	80-5120	0.9994	80	69.3	NA
triclosan	25-800	0.9817	100	67.9	115
4-tert-octylphenol	30-3840	0.9631	60	108	54.7
gemfibrozil	40-5120	0.9866	80	36.4	NA

NA: acetaminophen and caffeine lost recovery when sample size was increased to 20 mL. Gemfibrozil and ibuprofen can be detected by HESI method.

4.4 Conclusion

A fully automated online preconcentration HPLC-APPI-MS/MS method for simultaneous detection of PPCPs, hormones and sterol steroid was developed. Results indicate that APPI produce great ionization capability for a broad range of compounds, in particular for ionization efficiency of hormones compared to APCI and HESI. The advantages of APPI made it a great alternative for consistent detection of trace level organic microconstituents in the environment. The online preconcentration method minimized the sample preparation procedure, thus producing a reliable and robust method that can be used for routine analysis of clean and complex matrix water samples, such as ground, surface and reclaimed waters.

CHAPTER 5

Assessing different ionization techniques for the detection of pharmaceuticals in aqueous samples using online SPE-LC-MS/MS

5.1 Introduction

Liquid chromatography mass spectrometry is one of the fastest growing analytical techniques because of its attribution to new application on life science and biopharmaceuticals (Hans H, 1998; Hanold et al., 2004; Diaz and Barcelo, 2005; Hernández et al., 2005; Petrovic et al., 2005; Nunez et al., 2011). Currently, ESI and APCI are the most widely used ionization techniques coupled to Liquid chromatography mass spectrometry. Electrospray ionization and APCI for ionization of polar compounds and have been applied successfully in the environmental analysis of aqueous samples (Cahill et al., 2004; Castiglioni et al., 2005; Martnez Bueno et al., 2007; Gros et al., 2009; Laven et al., 2009; Lopez-Serna et al., 2010). However, ESI and APCI are not very efficient for ionization of nonpolar compounds. A new ionization technique, atmospheric pressure photoionization (APPI), has been introduced to mass spectrometry (Robb et al., 2000). The application and principle of APPI have been reviewed and the rapidly growing number of publications in this area clearly demonstrates the advantages of APPI (Hanold et al., 2004; Bos et al., 2006). Atmospheric pressure photoionization has proven to be a valuable tool for analytes, which are poorly or not ionized by ESI and APCI or in the presence of complex analytical condition (Itoh et al., 2006; Yamamoto et al., 2006; Viglino et al., 2008; Cai et al., 2009). Even though many studies have been conducted to investigate the ability of APPI

to detect nonpoplar compounds, very little research has focused on the performance of APPI on analytes which are sufficiently ionized by ESI and APCI (Cai et al., 2005; Garcia-Ac et al., 2011).

In the present study, first, I developed an online SPE method for accurate and reliable detection of 52 pharmaceuticals simultaneously in water samples. The online SPE method minimized the sample preparation procedures, and saves solvent, time and labor. Second, I compared the ionization efficiency of two ionization techniques (HESI and APPI) on the basis of absolute signal intensity and also method detection limits (MDLs). It is the first time to compare the efficiency of different ionization techniques based on calculated MDLs, which provide a useful tool for comparing analytical methods.

5.2. Experimental

5.2.1 Chemicals

Ketoprofen, naproxen, ibuprofen, indomethacin, mefenamic acid, acetaminophen, salicylic acid, antipyrine, gemfibrozil, bezafibrate, fenofibrate, atorvastatin, mevastatin, pravastatin sodium salt hydrate, fluoxetine (hydrochloride), paroxetine (maleate), carbamazepine, primidone, ranitidine (hydrochloride), diphenhydramine (hydrochloride), cimetidine, atenolol, (±) metoprolol (+) tartrate, propranolol, betaxolol (hydrochloride), pindolol, nadolol, clenbuterol (hydrochloride), enalapril (maleate), hydrochlorothiazide, lisinopril, furosemide, tamoxifen, clotrimazole, glibenclamide were purchased from Sigma-

Aldrich (Allentown, PA, USA). Diclofenac, propyphenazone, phenylbutazone, clofibric acid, famotidine, timolol, salbutamol, metronidazole were purchased from Fisher Scientific (Pittsburgh, PA, USA). Codeine, diazepam, lorazepam, butalbital, phenobarbital, pentobarbital, were purchased from Cerilliant (Round Rock, TX, USA). Loratadine, sotalol (hydrochloride), carazolol (hydrochloride) were purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). Isotopically labelled compounds, used as surrogates, were diclofenac-d4 (phenyl-d4), (±)-ibuprofen-d3, N-(4-hydroxyphenyl-d4) acetamide, 2-hydroxybenzoic-d4 acid, phenylbutazone-d10, clofibric-d4 acid, cimetidine-d3, (±)-atenolol-d7, (±)-albuterol-d3, enalaprilat-d5, tamoxifen-d5, clotrimazole-d5, norfloxacin-d5 were purchased from C/D/N Isotopes Inc. (Quebec, Canada). Diazepam-d5, lorazepam-d4, codeine-d6, phenobarbital-d5, pentobarbital d5 were purchased from Cerilliant (Round Rock, TX, USA). Mefenamic acid-d3, fenirofibrate-d6, atorvastatin-d5 (sodium salt), pravastatin-d3 (sodium salt), rac-trans paroxetine-d4 (hydrochloride), ranitidine-d6 (hydrochloride), loratadine-d4, rac timolol-d5 (maleate), hydrochlorothiazide-^{13}C, d2, furosemide-d5, metronidazole-d4, glyburide-d11 were purchased from Toronto Research Chemicals, Inc. (Ontario, Canada). The detail information of analytes is shown in Table 13.

5.2.2 Sample preparation

Water samples were filtered through glass fiber filters with a pore size of 0.5 μm. Samples were analyzed within 14 days in order to avoid potential degradation

and transformation of analytes. pH was adjusted to 2 when sample was analyzed in the negative mode.

5.2.3 Instrumentation

An Environmental Quantition (EQuan^{TM}) system manufactured by Thermo Fisher Scientific was used to preconcentrate microconstituents from reclaimed water. The EQuan^{TM} system is based on a dual switching-column system, which consists of a sample delivery system, a switching-column array and an LC-MS/MS system. Its sample delivery system consists of an autosampler manufactured by CTC analytics AG (Zwingen, Switzerland) and an Accela 600 loading pump (Thermo Fisher Scientific, San Jose, CA, USA). Its column-switching array is composed of a Rheodyne 7750E-205 six-port switching valve system made by IDEX (Oak Arbor, WA, USA), a preconcentration column and an analytical column. A Thermo Hypersil Gold aQ (20 mm × 2.1 mm, 12 μm particle size) was used as the loading column and a Thermo Hypersil Gold aQ (50 mm × 2.1 mm, 1.9 μm particle size) was used as the analytical column. Mass spectrometry analysis was performed using a TSQ Quantum Access triple quadrupole Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA).

Table 13. Information of pharmaceuticals

GROUP	No	Name	CAS Number	Pka	log Kow	Surrogate
Analgesics and anti-inflammatories	1	Ketoprofen	22071-15-4	5.95	3.12	Ibuprofen-d3
	2	Naproxen	22204-53-1	4.2	3.18	Ibuprofen-d3
	3	Ibuprofen	15687-27-1	4.3	3.97	Ibuprofen-d3
	4	Indomethacin	53-86-1	4.5	4.27	Carbamazepine-d10
	5	Diclofenac	15307-86-5	4	4.5	Diclofenac-d4
	6	Mefenamic acid	61-68-7	4.2	5.12	Mefenamic acid-d3
	7	Acetaminophen	103-90-2	9.51	0.46	Acetaminophen-d4
	8	Salicylic Acid	69-72-7	2.97	2.26	2-hydroxybenzoic acid d4
	9	Antipyrin	60-80-0	1.4	0.38	Carbamazepine-d10
	10	Propyphenazone	479-92-5	4.5	1.94	Phenylbutazone-d10
	11	Phenylbutazone	50-33-9	4.5	2.5	Phenylbutazone-d10

GROUP	No	Name	CAS Number	Pka	log Kow	Surrogate
	12	Codeine	76-57-3	8.2	1.14	Codeine-d6
Antihyperlipidemics - Lipid Regulators	13	Clofibric Acid	882-09-7	2.84	2.57	Clofibric Acid-d4
	14	Gemfibrozil	25812-30-0	4.75	4.77	Diclofenac-d4
	15	Bezafibrate	41859-67-0	3.29	4.25	Fenirofibrate-d6
	16	Fenofibrate	49562-28-9	5	5.19	Fenirofibrate-d6
	17	Atorvastatin	134523-00-5	4.5	6.36	Atorvastatin-d5
	18	Mevastatin	73537-88-3	14.89	3.95	Pravastatin-d3
	19	Pravastatin	81131-70-6	4.7	3.95	Pravastatin-d3
Antidepressants and anticonvulsants	20	Fluoxetine	54910-89-3	9.5	3.82	Fluoxetine-d6
	21	Paroxetine	61869-08-7	9.9	3.95	Fluoxetine-d6
	22	Diazepam	439-14-5	3.3	2.19	Diazepam-d5
	23	Lorazepam	846-49-1	0.03	2.42	Lorazepam d4

GROUP	No	Name	CAS Number	Pka	log Kow	Surrogate
	24	Carbamazepine	298-46-4	13.9	2.46	Carbamazepine-d10
	25	Primidone	125-33-7	11.6	0.91	Carbamazepine-d10
Antihistaminies	26	Famotidine	76824-35-6	6.89	-0.64	Ranitidine-d6
	27	Ranitidine	66357-35-5	2.7/8.2	0.27	Ranitidine-d6
	28	Cimetidine	51481-61-9	6.8	0.4	Cimetidine-d3
	29	Loratadine	79794-75-5	5	5.2	Loratadine-d4
	30	Diphehydramine	88637-37-0	9	3.27	Carbamazepine-d10
Barbiturates - Anticonvulsants	31	Butalbital	77-26-9	12.15	1.87	Phenobarbital-d5
	32	Phenobarbital	50-06-6	7.4	1.47	Phenobarbital-d5
	33	Pentobarbital	76-74-4	8	2.1	Phenobarbital-d5
Beta-blockers - cardiac arrhythmias	34	Atenolol	29122-68-7	9.6	0.36	Atenolol-d7
	35	Sotalol	3930-20-9	9.55	0.24	Atenolol-d7

GROUP	No	Name	CAS Number	Pka	log Kow	Surrogate
	36	Metoprolol	37350-58-6	9.7	1.8	Atenolol-d7
	37	Propranolol	525-66-6	9.49	3	Atenolol-d7
	38	Timolol	26839-75-8	9.2	1.83	Timolol-d5 maleate
	39	Betaxolol	63659-18-7	9.4	2.81	Atenolol-d7
	40	Carazolol	57775-29-8	9.52	3.59	Atenolol-d7
	41	Pindolol	13523-86-9	8.8	1.75	Atenolol-d7
	42	Nadolol	42200-33-9	9.67	0.71	Atenolol-d7
Bronchodilators - Beta agonists	43	Salbutamol	18559-94-9	9.3	0.64	Albuterol-d3
	44	Clenbuterol	37148-27-9	17.84	2	Albuterol-d3
Antihypertensives	45	Enalapril	75847-73-3	3/5.5	0.07	Enalaprilat-d5
	46	Hydrochlorothiazide	58-93-5	7.9	-0.1	Hydrochlorothiazide-13C, d2
	47	Lisinopril	83915-83-7	2.5	-1.1	Carbamazepine-d10

GROUP	No	Name	CAS Number	Pka	log Kow	Surrogate
Diuretics	48	Furosemide	54-31-9	2.03	2.03	Furosemide-d5
Cancer treatment	49	Tamoxifen	10540-29-1	8.85	6.3	Tamoxifen-d5
Antifungals	50	Metronidazole	443-48-1	2.6	-0.1	Metronidazole-d4
	51	Clotrimazole	23593-75-1	6.7	1.33	Clotrimazole-d5
Antidiabetic	52	Glibenclamide	10238-21-8	5.3	2.4	Glyburide-d11

5.2.4 Direct injection

Standard solution (25 µL) at 100 ng/mL was injected to compare the ionization efficiency of different techniques. The mobile phase flow rate was 200 µL/min. The mobile phase used for HESI positive mode was methanol/acetonitrile (50:50, v/v) and 0.1% formic acid in water. The proportion of the organic solvent was 1% in the first 2 min, and then organic proportion was increased to 100% in 5 min and held for 5 min. After that, organic proportion was decreased to 1% in 2 min and held for another 2 min. The mobile phase used for HESI negative mode was methanol and water. The proportion of the organic solvent was 1% in the first 2 min, and then organic proportion was increased to 100% in 5 min and held for 5 min. After that, organic proportion was decreased to 1% in 2 min and hold for another 2 min. The mobile phase used for APPI positive mode was the same as HESI positive mode but the gradient was different. The proportion of the organic solvent was 10% in the first 2 min, and then organic proportion was increased to 100% in 5 min and held for 5 min. After that, organic proportion was decreased to 10% in 2 min and held for another 2 min. The mobile phase used for APPI negative mode was methanol and water. The proportion of the organic solvent was 10% in the first 2 min, and then organic proportion was increased to 100% in 5 min and held for 3 min. After that, organic proportion was decreased to 10% in 2 min and held for another 2 min.

5.2.5 On-line SPE

Water samples (5 mL) were loaded to the preconcentration column at a flow rate of 1 mL/min. The preconcentration column was washed by 1000 µL of water and connected to the analytical column after the valve had switched to inject position. After washing, the loading column and analytical column underwent the same gradient in both positive mode and negative mode.

5.2.6 Mass spectrometry

Mass spectrometry analysis was performed using a TSQ Quantum Access triple quadrupole QqQ Mass Spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) equipped with an Ion MAX source housing capable of operating HESI and APPI. Quantitation for all sources was performed using selected reaction monitoring (SRM) mode. Instrument control and data acquisition were performed using Xcalibur software (rev. 2.1, Thermo Fisher Scientific, San Jose, CA, USA). Source parameters for analytes were optimized using Heated Electrospray Ionization (HESI) and Atmospheric Pressure Photoionization (APPI) independently using flow injection with a carrier stream of mobile phase. A mobile phase of 0.1% fomic acid in water/MeOH (50:50, v/v) was used for positive mode and water/MeOH (50:50, v/v) was used for negative mode. Each analyte and surrogate was injected to the ion source at a concentration of 10 µg/mL. Compound-dependent parameters such as tube lens and collision energy were optimized to obtain maximum signals in the QqQ system. The precursor ion, fragment ions and mass-dependent parameters are listed in table 14.

Source-dependent parameters for optimal HESI detection were as follows: capillary temperature (350 °C), vaporizer temperature (250 °C), sheath gas pressure (30 arbitrary units), aux gas pressure (20 arbitrary units), ion sweep gas pressure (5 arbitrary units) and spray voltage (4000 V for positive polarity and 4000 V for negative polarity).

The photoionization lamp used for APPI was a Syagen 10 eV krypton UV lamp (Syagen Technology Inc., Tustin, CA, USA). The source-dependent parameters for optimal detection were as follows: capillary temperature (300 °C), vaporizer temperature (400 °C), sheath gas pressure (50 arbitrary units), aux gas pressure (35 arbitrary units), ion sweep gas pressure (0 arbitrary units), discharge current (0 μA). Four different dopants, acetone, anisole, chlorobenzene and toluene, were tested to find the best one for the analytes.

Table 14. Accurate mass and mass spectrometry parameters of analytes

	HESI									APPI							
compounds	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Ketoprofen	[M-H]-	253.1	65	209.1	11					[M+H]+	255.0	70	209.0	13	105.1	22	77.2	39
Naproxen	[M-H]-	229.0	60	170.1	18	185.2	11			[M+H-H2O-CO]+	185.1	84	170.1	17	141.1	33	115.1	49
Ibuprofen	[M-H]-	205.1	51	161.3	10					[M-H]-	205.1	161.1	11.0					
Indomethacin	[M-H]-	356.1	65	312.1	12	297.1	21	282.1	32	[M+H]+	357.9	61	138.9	20	111.0	43	174.0	12
Diclofenac	[M-H]-	294.0	60	250.0	14	214.0	22	178.1	28	[M+H]+	295.9	63	213.9	34	249.9	13	277.9	7
Mefenamic acid	[M-H]-	240.1	65	196.1	19	192.1	28	180.1	29	[M-H2O+H]+	224.0	97	209.0	28	180.0	40	208.0	36
Acetaminophen	[M-H]-	150.0	49	107.2	22	118.1	33	132.1	24	[M+H]+	152.1	100	110.1	15	65.3	31	93.2	23
Salicylic Acid	[M-H]-	137.1	48	93.1	19	65.2	31	75.2	37	[M-H]-	136.9	47	93.4	20				
Antipyrin	[M+H]+	189.1	72	77.2	36	56.3	36	131.1	22	[M+H]+	189.0	68	77.2	37	131.1	21	146.1	20
Propyphenazone	[M+H]+	231.1	71	201.1	24	189.1	20	56.3	35	[M+H]+	231.1	76	189.0	20	146.0	25	77.2	41
Phenylbutazone	[M+H]+	309.2	71	120.1	19	188.1	15	211.1	16	[M+H]+	309.1	82	210.9	13	132.2	29	77.3	42
Codeine	[M+H]+	300.2	86	215.1	25	152.1	65	165.1	42	[M+H]+	300.0	81	165.0	35	215.0	25	153.0	44
Clofibric Acid	[M-H]-	213.1	56	127.1	19	85.2	12	91.3	47	[M-H]-	213.0	79	127.2	20	85.4	13		

	HESI									APPI								
compounds	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Gemfibrozil	[M-H]-	249.1	65	121.2	20	106.1	49	120.1	44	[M-H]-	249.1	73	121.1	17	127.1	14	106.2	45
Bezafibrate	[M-H]-	360.1	63	274.1	20	154.0	32	85.2	19	[M+H]+	362.1	71	316.0	13	276.0	13	138.9	25
Fenofibrate	[M+H]+	361.2	72	233.0	16	139.0	31	121.0	32	[M+H]+	361.0	73	232.9	16	138.9	30	111.0	50
Atorvastatin	[M+H]+	559.3	88	440.3	21	250.0	42	276.1	40	[M+H]+	559.1	84	440.0	21	249.9	41	276.0	45
Mevastatin	[M+H]+	391.3	74	185.1	14	229.1	13	159.1	26	[M+Na]+	413.1	94	311.1	21	296.0	26	325.3	23
Pravastatin	[M+Na]+	447.3	97	327.1	19	309.2	22			[M+Na]+	447.1	101	327.0	19	309.0	24	429.3	24
Fluoxetine	[M+H]+	310.1	60	44.3	13	148.1	5	183.1	45	[M+H]+	310.1	70	44.4	13	148.1	5	259.0	5
Paroxetine	[M+H]+	330.2	71	192.1	20	70.2	31	135.1	37	[M+H]+	330.0	88	192.0	20	70.2	33	135.0	34
Diazepam	[M+H]+	285.1	77	193.1	32	154.1	26	222.1	26	[M+H]+	285.0	83	193.0	31	222.1	25	257.1	21
Lorazepam	[M+H]+	321.1	74	275.0	22	303.0	15	229.1	31	[M+H]+	320.9	76	274.9	20	302.9	14	228.9	29
Carbamazepine	[M+H]+	237.1	61	194.1	19	192.1	25			[M+H]+	237.1	87	194.1	18	192.1	23	179.1	35
primidone	[M+H]+	219.1	68	91.2	28	162.2	12	117.2	23	[M+H]+	219.1	83	91.2	28	162.0	12	119.1	16
Famotidine	[M+H]+	338.1	55	189.0	20	259.1	11	155.1	32	[M+H]+	338.2	68	321.2	12	303.2	11	97.1	20
Ranitidine	[M+H]+	315.1	65	176.0	18	130.1	25	102.1	34	[M+H]+	315.0	57	176.0	17	130.0	25	270.0	12

	HESI									APPI								
compounds	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Cimetidine	[M+H]+	253.1	67	159.1	14	117.2	16	95.2	29	[M+H]+	253.0	61	159.0	13	117.1	16	95.1	30
Loratadine	[M+H]+	383.1	82	337.1	23	267.1	31	259.1	30	[M+H]+	383.0	86	337.0	22	266.9	31	259.0	31
diphenhydramine	[M+H]+	256.2	54	167.1	14	165.2	37	152.1	37	[M+H]+	256.1	51	167.0	15	152.0	33	165.0	44
Butalbital	[M-H]-	223.1	60	180.1	14	42.3	20	85.2	15	[M+H]+	225.1	70	171.1	12	154.1	13	169.1	14
Phenobarbital	[M-H]-	231.1	61	188.2	14	42.5	15	85.3	15	[M-H]-	231.1	24	188.4	13	42.6	18		
Pentobarbital	[M-H]-	225.1	60	182.1	15	42.4	20	138.2	19	[M-H]-	225.1	62	182.3	15				
Atenolol	[M+H]+	267.2	78	145.1	26	190.1	18	133.1	31	[M+H]+	267.1	72	145.1	26	190.1	18	225.1	16
Sotalol	[M+H]+	273.2	77	255.1	11	213.1	18	133.1	27	[M+H]+	273.1	68	255.1	12	213.1	18	133.1	29
Metoprolol	[M+H]+	268.2	77	159.1	21	191.1	17	133.1	26	[M+H]+	268.1	80	116.2	17	191.1	17	159.1	20
Propranolol	[M+H]+	260.2	78	183.1	17	155.1	25	157.1	20	[M+H]+	260.1	68	116.2	18	183.0	18	155.1	26
Timolol	[M+H]+	317.2	84	261.1	16	244.1	21	188.0	25	[M+H]+	317.1	66	261.0	16	244.0	20	188.0	26
Betaxolol	[M+H]+	308.2	82	121.1	26	133.1	26	91.1	39	[M+H]+	308.2	82	116.2	19	98.2	22	72.3	22
Carazolol	[M+H]+	299.1	70	222.1	19	184.0	25	194.1	29	[M+H]+	299.1	81	116.2	21	222.0	20	194.1	29
Pindolol	[M+H]+	249.2	72	116.1	17	172.1	17	144.1	25	[M+H]+	249.1	66	116.2	17	172.1	17	146.0	19

	HESI									APPI								
compounds	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3	precursor ion	m/z	tube lens	SRM1	CE 1	SRM2	CE 2	SRM3	CE 3
Nadolol	[M+H]+	310.2	73	254.1	17	201.1	22	236.1	19	[M+H]+	310.1	70	254.1	17	236.1	19	201.0	23
Salbutamol	[M+H]+	240.2	62	148.1	18	222.1	10	121.1	29	[M+H]+	240.1	66	148.1	19	166.0	12	222.1	8
Clenbuterol	[M+H]+	277.1	71	203.0	16	259.1	10	132.1	30	[M+H]+	277.0	60	202.9	16	259.0	9	132.1	28
Enalapril	[M+H]+	377.2	74	234.1	19	303.2	17	117.1	36	[M+H]+	377.1	71	234.1	19	303.1	17	117.1	36
Hydrochlorothiazide	[M-H]-	296.0	78	269.0	20	205.0	23	126.1	33	[M-H]-	295.9	60	269.0	19	205.1	23	126.1	23
Lisinopril	[M+H]+	406.2	88	84.2	33	246.1	22	309.2	18	[M+H]+	406.1	78	84.2	31	246.1	19	291.1	8
Furosemide	[M-H]-	328.9	75	285.0	17	204.9	23	126.0	36	[M-H]-	328.9	69	205.0	24	285.2	20	78.2	34
Tamoxifen	[M+H]+	372.2	84	72.2	23	129.1	26	70.2	36	[M+H]+	372.1	86	72.3	23	129.1	26	70.3	36
Metronidazole	[M+H]+	172.1	63	128.1	13	82.2	23	111.1	20	[M+H]+	172.1	61	128.1	12	82.2	25	111.2	18
Clotrimazole	[M-C3H3N2]+	277.1	60	165.1	27	199.0	31	242.1	20	[M-C3H3N2]+	277.0	60	165.0	23	241.0	27	239.0	50
Glibenclamide	[M+H]+	494.3	70	369.1	14	169.0	33	304.1	25	[M+Na]+	516.0	101	390.9	19	416.9	19	419.4	19

5.2.7 Dopant delivery system

Dopant was delivered by Fusion Touch 100 syringe pump from Chemyx (Stafford, TX, USA). Syringe pump was connected to the auxiliary gas and dopant was nebulized to gas phase. The dopant gas delivery system is shown in Figure 17. Compared to mixing dopant with mobile phase, this dopant gas delivery system increased reaction between dopant and analytes.

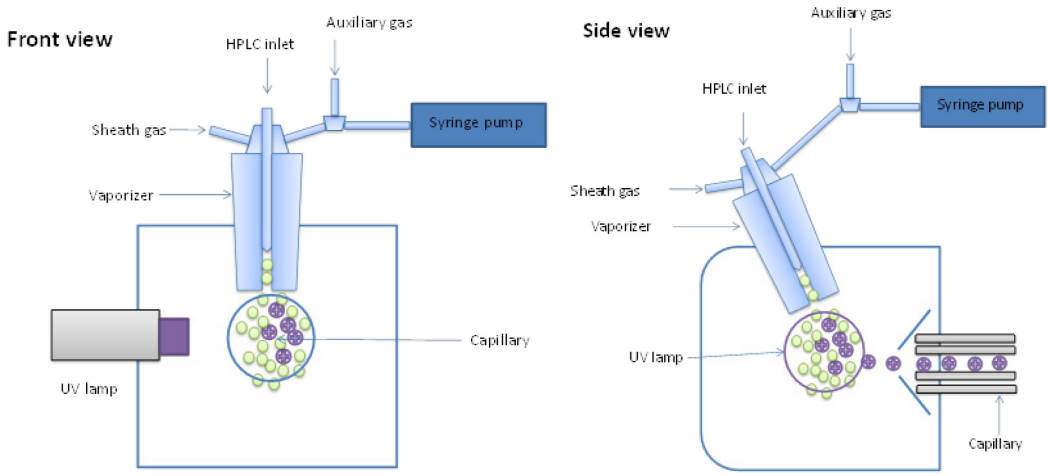


Figure 17. Dopant gas delivery system, modified from (Hanold et al., 2004)

5.3 Results and discussion

5.3.1 Optimization of online SPE procedure

Water samples (5 mL) were loaded to the preconcentration column at different flow rates: 500 µL/min, 1000 µL/min, 1500 µL/min and 2000 µL/min. Absolute recovery of analytes (based on the response only) detected in the positive ion mode is shown in Figure 18a and absolute recovery of analytes detected in the negative mode is shown in Figure 18b. Loading speed of 2000 µL/min and 1000 µL/min was chosen for positive mode and negative mode respectively since

analytes were recovered most at these two flow rates. In the negative mode, the pH of samples was adjusted to 2 to increase the recovery of salicylic acid and clofibric acid in the loading column.

In order to reduce matrix effects, a wash step was applied after samples were loaded to the preconcentration column. Only water was used to wash the preconcentration column because some analytes are very soluble in organic solvents. Three different volumes of water (1 mL, 2 mL and 3 mL) were tested in both positive mode and negative mode and results are shown in figure 19a and figure 19b, respectively. In the positive mode, Metronidazole, lisinopril and primidone started to be lost when wash volume was more than 1 mL. Therefore, 1 mL was chosen as the wash volume in the positive mode. In the negative mode, acetaminophen was not retained in the preconcentration column when the wash volume was more than 1 mL and the result was consistent with the result shown in 4.3.1. Therefore, the Hypersil aQ column is not a good choice to retain acetaminophen. Hydrochlorothiazide lost its recovery on the preconcentration column when wash volume was more than 1 mL. Thus, 1 mL was also chosen as the wash volume in the negative mode.

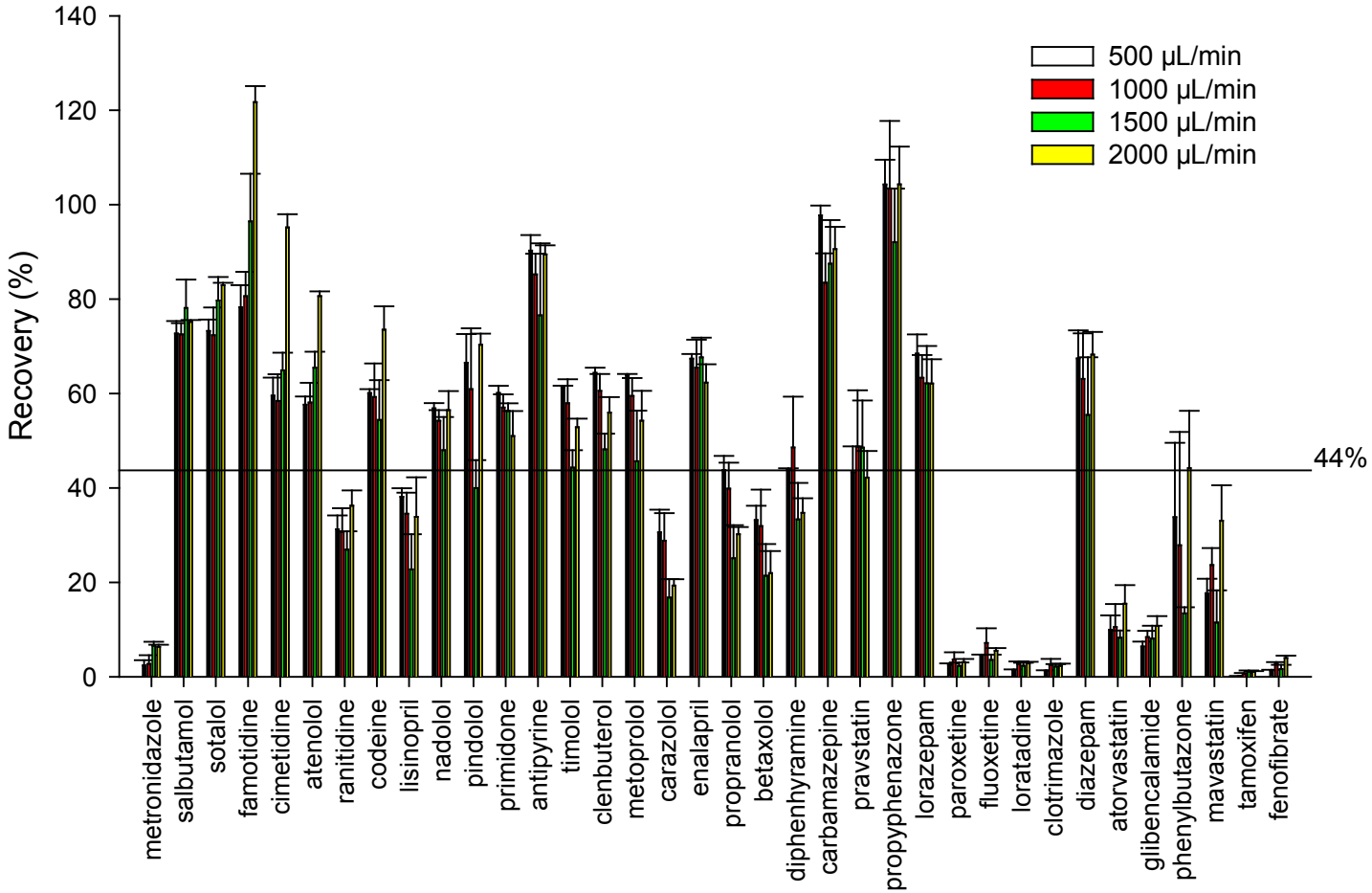


Figure 18a. Absolute recovery of analytes at different load speeds in the positive mode

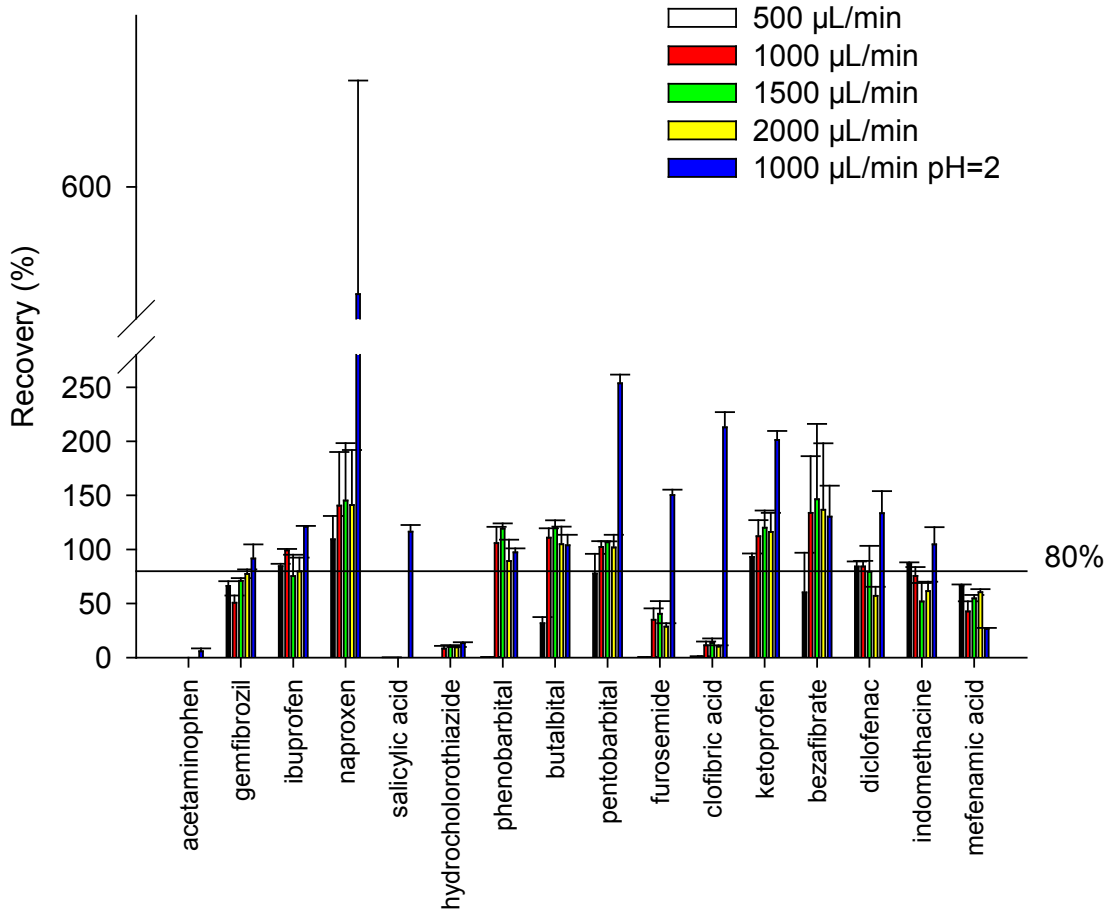


Figure 18b. Absolute recovery of analytes at different load speeds in the negative mode

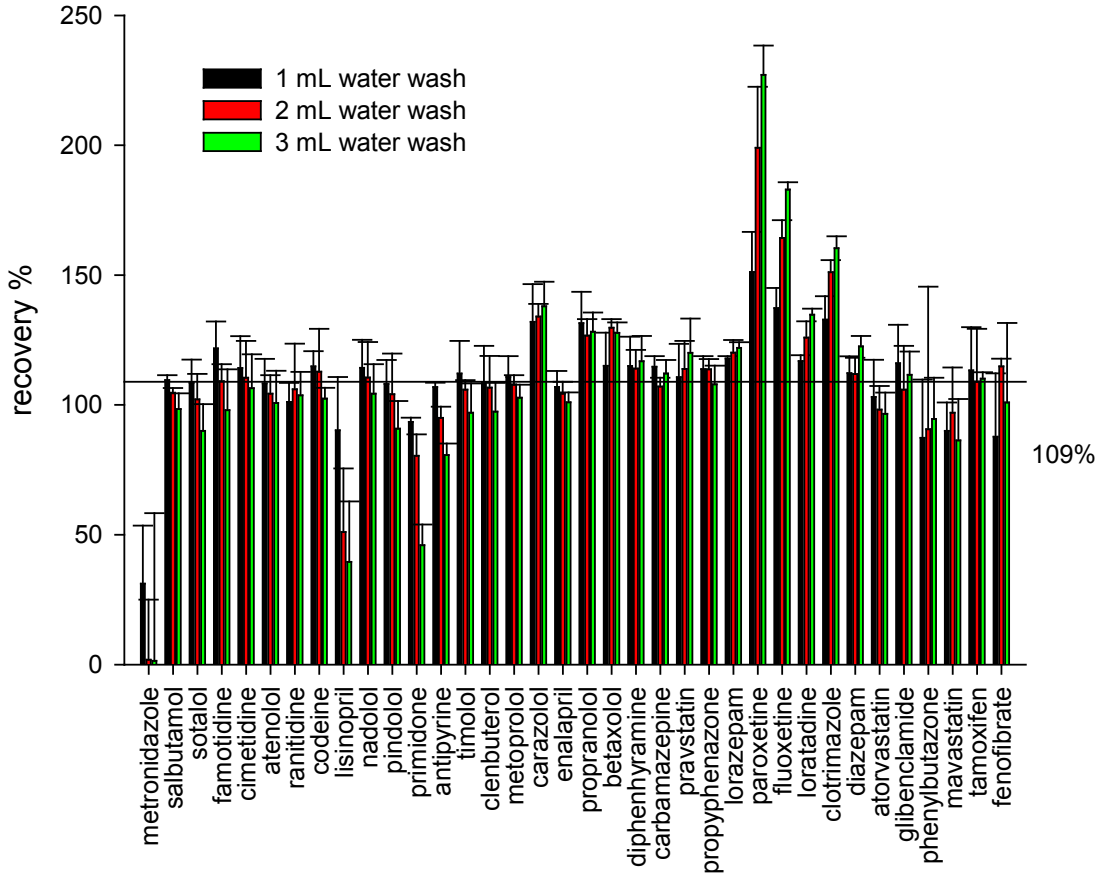


Figure 19a. Absolute recovery of analytes in positive mode with different wash volume

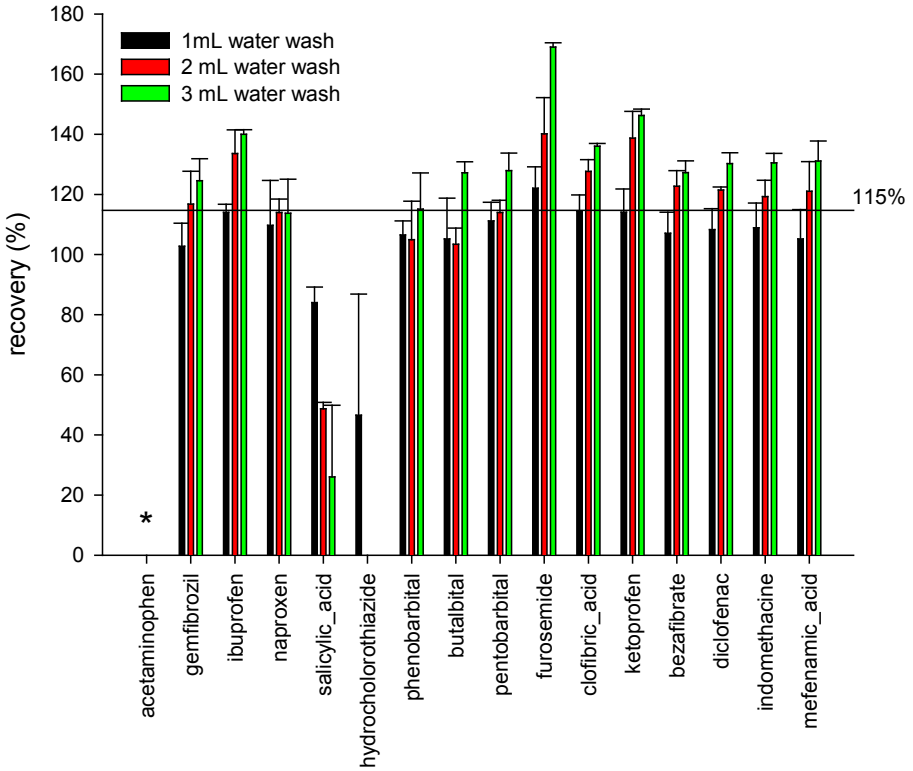


Figure 19b. Absolute recovery of analytes in negative mode with different wash volume. Asterisk (*) indicates not recovered.

5.3.2 Comparison of different API techniques

5.3.2.1 Comparison based on absolute signal intensity

Several studies compared the absolute signal intensity of pharmaceuticals using different ionization methods (Cai et al., 2005; Garcia-Ac et al., 2011). We compared the signal intensity of different ionization methods by direct injection of 2.5 ng standard compounds. The signal intensity of compound detected in the HESI method was treated as 100%. The signal intensity of compounds detected in other ionization methods was compared with the HESI method. Results of comparison are shown in Table 15. The best ionization method is shown in green. For 32 out of 52 compounds, HESI was the best ionization method. 14 out of 52 compounds were best detected by APPI with toluene as dopant. Only 3 analytes were best detected using chlorobenzene as dopant and 3 were best detected in APPI with acetone as dopant. Results indicated that HESI was still a very effective ionization method for the selected target compounds. For some of the compounds usually analyzed in the negative mode in ESI, signal intensity increased remarkablely when they were ionized in the positive mode in APPI. Ketoprofen ([M-H]- → [M+H]+), naproxen ([M-H]- → [M+H-H2O-CO]+), indomethacin ([M-H]- → [M+H]+), diclofenac ([M-H]- → [M+H]+), mefenamic acid ([M-H]- → [M+H-H2O]+), acetaminophen ([M-H]- → [M+H]+) and bezafibrate ([M-H]- → [M+H]+) were in that case (figure 20). For acetaminophen, signal intensity increased 80 times when it was ionized by APPI with toluene as dopant. Therefore, APPI with toluene as dopant has two major advantages, firstly, it

increases the sensitivity and secondly it avoids the need for positive and negative runs for the same sample.

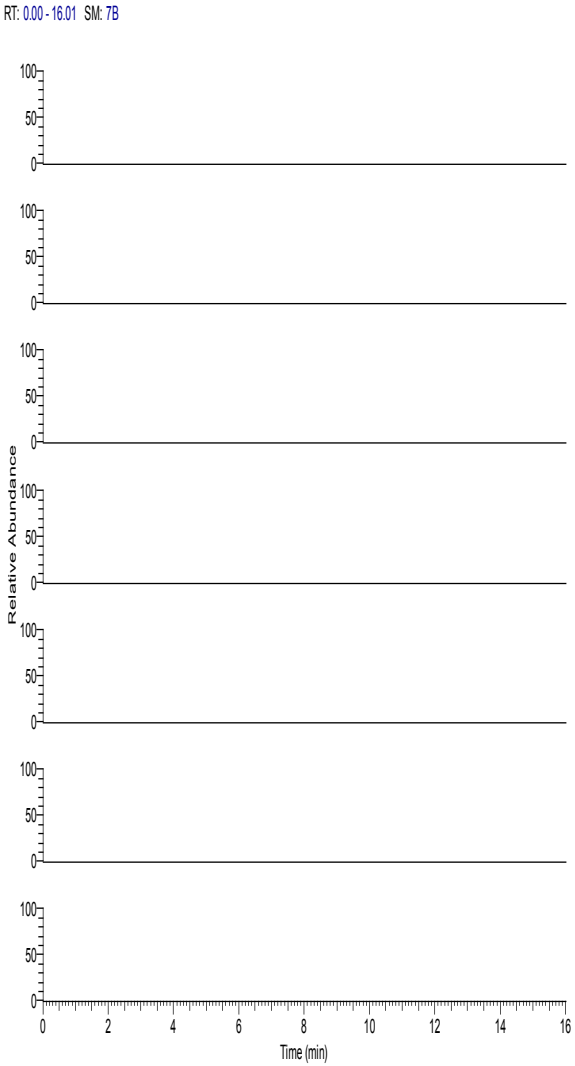


Figure 20. Chromatography of compounds in HESI- and APPI+

Table 15. Comparison of analyte responses under different ionization conditions

	HESI		RSD	APPI		RSD	APPI toluene		RSD	APPI acetone		RSD	APPI anisole		RSD	APPI chlor oben zene		RSD
ketoprofen	100	+ - +	10	1	+ -	8	234	+ - +	8	55	+ - +	5	41	+ -	9	25	+-	9
Naproxen	100	- +	22	ND			73	- +	5	38	- +	14	ND	+		425	+-	11
Ibuprofen	100	- +	13	ND			6	- +	8	3	- +	16	7	- +	2	5	+-	6
Indometacin	100	- +	11	1	+ - +	22	136	- +	7	80	- +	3	58	- +	3	51	+-	5
Diclofenac	100	- +	10	1	- +	24	112	- +	1	49	- +	4	27	- +	7	20	+-	13
Mefenamic acid	100	- +	16	8	- +	28	228	- +	2	168	- +	4	67	- +	1	53	+-	2
Acetaminophen	100	- +	19	124	-	9	8059	-	9	6682	-	13	1373	-	9	1111	+-	12
Salycilic Acid	100	- +	11	ND			ND			ND			ND			ND		
Antipyrin	100	- +	5	4	+ - +	20	166	- +	19	170	- +	5	85	+ - +	23	ND		
Propyphenazone	100	- +	4	24	- +	11	316	- +	4	257	- +	3	64	- +	3	54	+-	12
Phenylbutazone	100	- +	8	1	- +	20	115	- +	5	54	- +	3	19	- +	5	13	+-	33
Codeine	100	- +	3	4	-	12	61	- +	7	87	- +	3	32	- +	14	28	+-	17
Clofibric Acid	100	- +	4	ND			8	- +	7	5	- +	2	11	- +	8	13	+-	11
Gemfibrozil	100	- +	15	ND			27	- +	2	17	- +	5	31	- +	2	23	+-	5
Bezafibrate	100	- +	8	1	+ - +	8	67	- +	4	49	- +	3	13	- +	10	9	+-	20
Fenofibrate	100	- +	0	2	- +	20	128	- +	3	84	- +	5	25	- +	8	21	+-	14
Atorvastatin	100	- +	5	ND	- +	56	3	- +	13	3	- +	12	1	- +	15	1	+-	24
Mevastatin	100	- +	17	14	-	6	5	- +	13	4	-	15	2	-	14	2	+-	14
Pravastatin	100	-	4	ND			10	-	18	ND			ND			ND		

	HESI		RSD	APPI		RSD	APPI toluene		RSD	APPI acetone		RSD	APPI anisole		RSD	APPI chlor oben zene		RSD
		+			+			+			+			+				
Fluoxetine	100	-	3	ND	-	16	6	-	4	4	-	6	1	-	9	1	+-	26
		+			+			+			+			+				
Paroxetine	100	-	3	4	-	21	24	-	6	19	-	7	5	-	1	5	+-	8
		+			+			+			+			+				
Diazepam	100	-	4	3	-	18	48	-	3	34	-	4	17	-	1	16	+-	8
		+			+			+			+			+				
Lorazepam	100	-	1	2	-	18	91	-	3	69	-	13	27	-	7	21	+-	13
		+			+			+			+			+				
Carbamazepine	100	-	1	1	-	8	32	-	6	31	-	3	8	-	3	6	+-	15
		+						+			+			+				
Primidone	100	-	9	ND			334	-	2	74	-	4	61	-	1	223	+-	1
		+									+			+				
Famotidine	100	-	3	ND			ND			380	-	2	138	-	4	110	+-	7
		+			+			+			+			+				
Ranitidine	100	-	6	2	-	13	84	-	12	83	-	9	11	-	8	9	+-	8
		+			+			+			+			+				
Cimetidine	100	-	5	9	-	13	336	-	8	418	-	10	72	-	7	59	+-	11
		+			+			+			+			+				
Loratadine	100	-	2	4	-	18	30	-	4	22	-	5	7	-	2	6	+-	16
		+						+			+			+				
diphenhydramine	100	-	2	ND			5	-	4	3	-	12	1	-	4	1	+-	16
		+						+										
Butalbital	100	-	15	ND			65	-	8	ND			ND			ND		
		+						+			+			+				
Phenobarbital	100	-	2	ND			135	-	15	77	-	5	160	-	5	170	+-	23
		+						+			+			+				
Pentobarbital	100	-	25	ND			349	-	15	179	-	21	344	-	8	379	+-	7
		+			+			+			+			+				
Atenolol	100	-	8	3	-	21	176	-	9	148	-	10	24	-	11	22	+-	12
		+			+			+			+			+				
Sotalol	100	-	2	5	-	26	228	-	8	202	-	5	36	-	6	31	+-	11
		+			+			+			+			+				
Metoprolol	100	-	4	3	-	8	15	-	19	13	-	14	2	-	20	2	+-	4
		+			+			+			+			+				
Propranolol	100	-	4	4	-	27	20	-	10	16	-	15	3	-	11	2	+-	16
		+			+			+			+			+				
Timolol	100	-	1	2	-	22	16	-	16	12	-	19	3	-	20	2	+-	27
		+			+			+			+			+				
Betaxolol	100	-	4	19	-	15	72	-	5	59	-	6	14	-	9	14	+-	17
		+			+			+			+			+				
Carazolol	100	-	5	5	-	42	28	-	8	21	-	14	4	-	20	3	+-	14

	HESI		RSD	APPI		RSD	APPI toluene		RSD	APPI acetone		RSD	APPI anisole		RSD	APPI chlor oben zene		RSD
		+			+			+			+			+				
Pindolol	100	-	7	12	-	7	445	-	15	383	-	11	61	-	5	45	+-	16
		+			+			+			+			+				
Nadolol	100	-	3	6	-	8	190	-	6	134	-	12	26	-	9	19	+-	21
		+			+			+			+			+				
Salbutamol	100	-	2	1	-	24	30	-	11	17	-	17	5	-	4	3	+-	10
		+						+			+			+				
Clenbuterol	100	-	4	ND			14	-	11	9	-	11	2	-	25	1	+-	18
		+			+			+			+			+				
Enalapril	100	-	3	3	-	17	27	-	12	15	-	10	4	-	11	3	+-	8
		+						+			+			+				
Hydrochlorothiazide	100	-	13	ND			5	-	53	2	-	18	8	-	21	14	+-	11
		+																
Lisinopril	100	-	12	ND			ND			ND			ND			ND		
		+						+			+			+				
Furosemide	100	-	9	ND			25	-	7	15	-	19	30	-	3	32	+-	8
		+			+			+			+			+				
Tamoxifen	100	-	2	9	-	24	14	-	2	15	-	4	5	-	1	4	+-	8
		+			+			+			+			+				
Metronidazole	100	-	3	5	-	8	248	-	18	149	-	11	45	-	17	37	+-	25
		+			+			+			+			+				
Clotrimazole	100	-	2	7	-	19	20	-	2	11	-	3	22	-	3	21	+-	1
		+						+			+							
Glibenclamide	100	-	1	ND			1	-	17	1	-	21	ND			ND		

5.3.2.2 Comparison based on MDLs

For further study, we also compared the MDLs of target compounds. Statistically determined MDLs using fortified matrix matched samples can be used to compare among method and laboratory and truly reflect the performance of the detection method. The study only reported the MDLs comparison for compounds that were detected in the positive mode in APPI because for compounds detected in the negative mode HESI had certainly a better sensitivity. MDLs for target compounds under the different ionization methods are shown in Table 16 and number of compounds with MDLs in 0-5 ng/L, 5-10 ng/L and >10 ng/L range are shown in figure 21. For some of the compounds MDLs of APPI were much higher than HESI. Take pravastatin for example, MDLs of HESI was 6.9 ng/L while MDLs of APPI was more than 500 ng/L. Thus, we still should use HESI as ionization method for these compounds. For some compounds like propyphenazone, gemfibrozil and fenofibrate etc, calculated MDLs didn't decrease much from HESI to APPI with dopant. Therefore, we can use HESI or APPI as the ionization method for these compounds. For compounds that were ionized in the negative mode in HESI but the positive mode in APPI, even thorough MDLs didn't decrease much in APPI, a much better background were observed in APPI. Take ketoprofen for example, MDLs of HESI and APPI with toluene were 10.0 ng/L and 14.7 ng/L, respectively. However, in the negative ionization mode of HESI, there were two interference peaks very close to the ketoprofen peak. In the positive ionization mode of APPI, there were no interference peaks for ketoprofen at all (figure 22). Thus, we used APPI as

ionization method for detection of these compounds. In figure 23, analytes in blue cycle is properly to be ionized by HESI and the analytes in the orange cycle is properly ionized by APPI, while the analytes in overlapped part of two cycles indicated that these analytes can be both ionized by both HESI and APPI. HESI is still an ideal ionizatioin technique for polar compounds except for acetaminophen. APPI is also a competitive ionization method for many polar analytes (overlap part in figure 23). APPI is an excellent ionization method for less polar and nonpolar compounds (analytes in orange cycle from chapter 4). If there is a need to detect polar and non-polar com pounds simultaneously, APPI will be the best ionization technique.

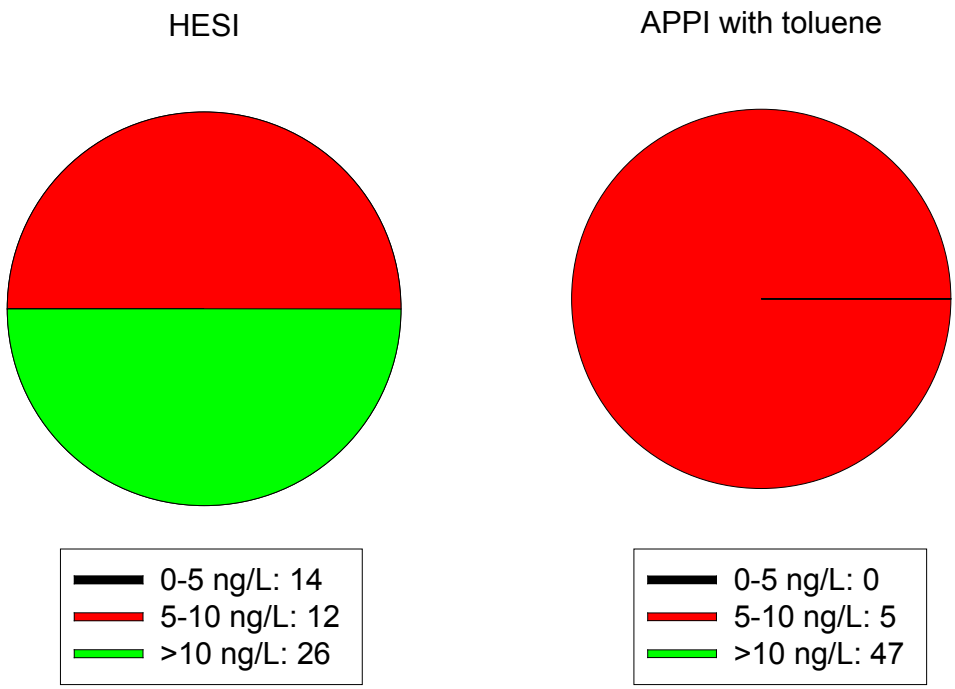


Figure 21. Number of compounds with MDL in 0-5 ng/L, 5-10 ng/L and >10 ng/L in HESI and APPI with toluene as dopant

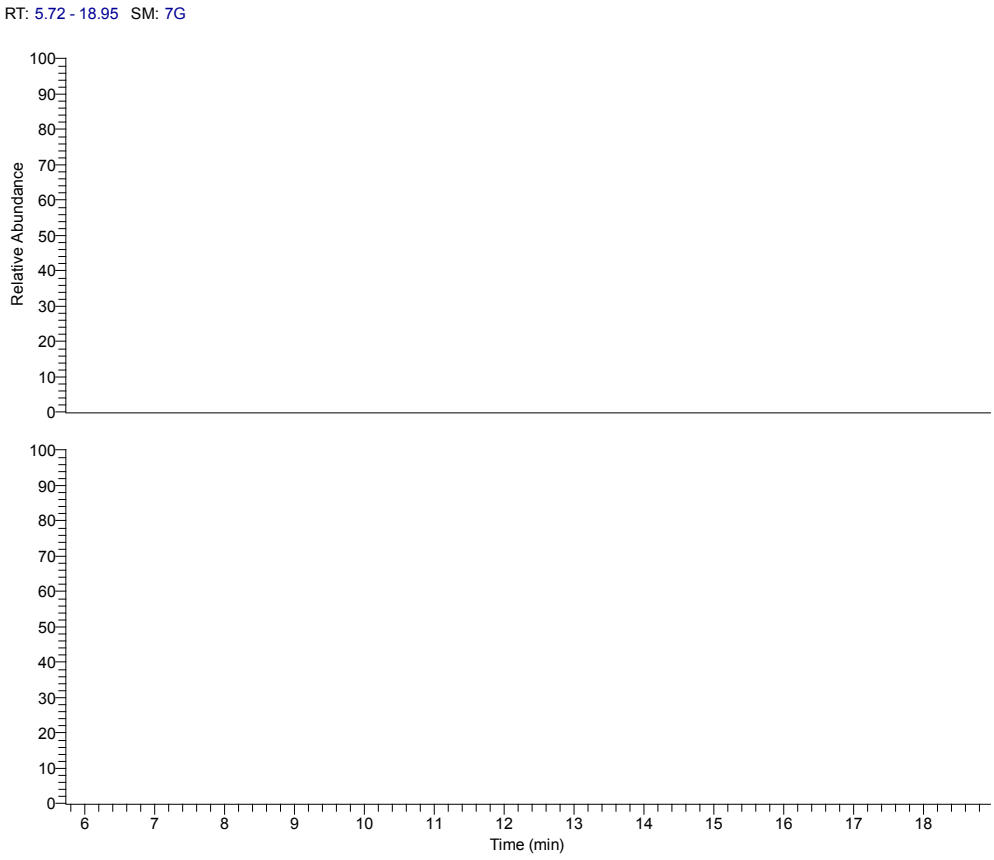


Figure 22. Chromatography of ketoprofen in HESI- and APPI+

Table16. MDLs comparison of different API

		HESI	APPI toluene	APPI acetone
1	ketoprofen	10.0	14.7	250
2	naproxen	74.0	15.4	15.7
3	ibuprofen	11.9	NA	NA
4	indomethacine	19.2	8.6	51.0
5	diclofenac	8.7	9.2	382
6	mefenamic_acid	2.5	6.0	339
7	acetaminophen	351	9.24	16.6
8	salicylic_acid	23.6	NA	NA
9	antipyrine	4.5	148	>250
10	propyphenazone	5.0	12.3	217
11	phenylbutazone	147	500	500

		HESI	APPI toluene	APPI acetone
12	codeine	6.2	354	500
13	clofibric_acid	23.6	NA	NA
14	gemfibrozil	14.4	36.4	367
15	bezafibrate	26.0	11.2	24.1
16	fenofibrate	14.2	21.8	5.1
17	atorvastatin	6.9	>500	156
18	mavastatin	75.1	>500	>500
19	pravstatin	9.0	>500	>500
20	fluoxetine	12.3	166	388
21	paroxetine	12.2	235	514
22	diazepam	1.8	10.9	206
23	lorazepam	6.4	20.5	31.9
24	carbamazepine	2.8	19.9	8.5
25	primidone	28.9	38.3	72.5
26	famotidine	1.8	>500	>500
27	ranitidine	3.5	500	500
28	cimetidine	4.6	154	118
29	loratadine	5.6	13.0	24.4
30	diphenhyramine	4.3	33.0	500
31	butalbital	189	NA	NA
32	phenobarbital	39.7	NA	NA
33	pentobarbital	32.4	NA	NA
34	atenolol	7.0	218	29.6
35	sotalol	3.5	125	387
36	metoprolol	13.1	29.6	24.9
37	propranolol	13.6	22.5	341
38	timolol	3.9	202	10.5
39	betaxolol	19.3	224	487
40	carazolol	10.0	38.2	156
41	pindolol	10.2	136	152
42	nadolol	5.7	107	6.9
43	salbutamol	3.8	357	500
44	clenbuterol	4.1	15.7	141
45	enalapril	3.7	6.8	20.3
46	hydrocholorothiazide	250	NA	NA
47	lisinopril	25.6	>500	>500
48	furosemide	8.2	NA	NA
49	tamoxifen	42.7	324	500
50	metronidazole	98.2	>500	>500
51	clotrimazole	6.3	29.3	140
52	glibenclamide	22.1	381	>500

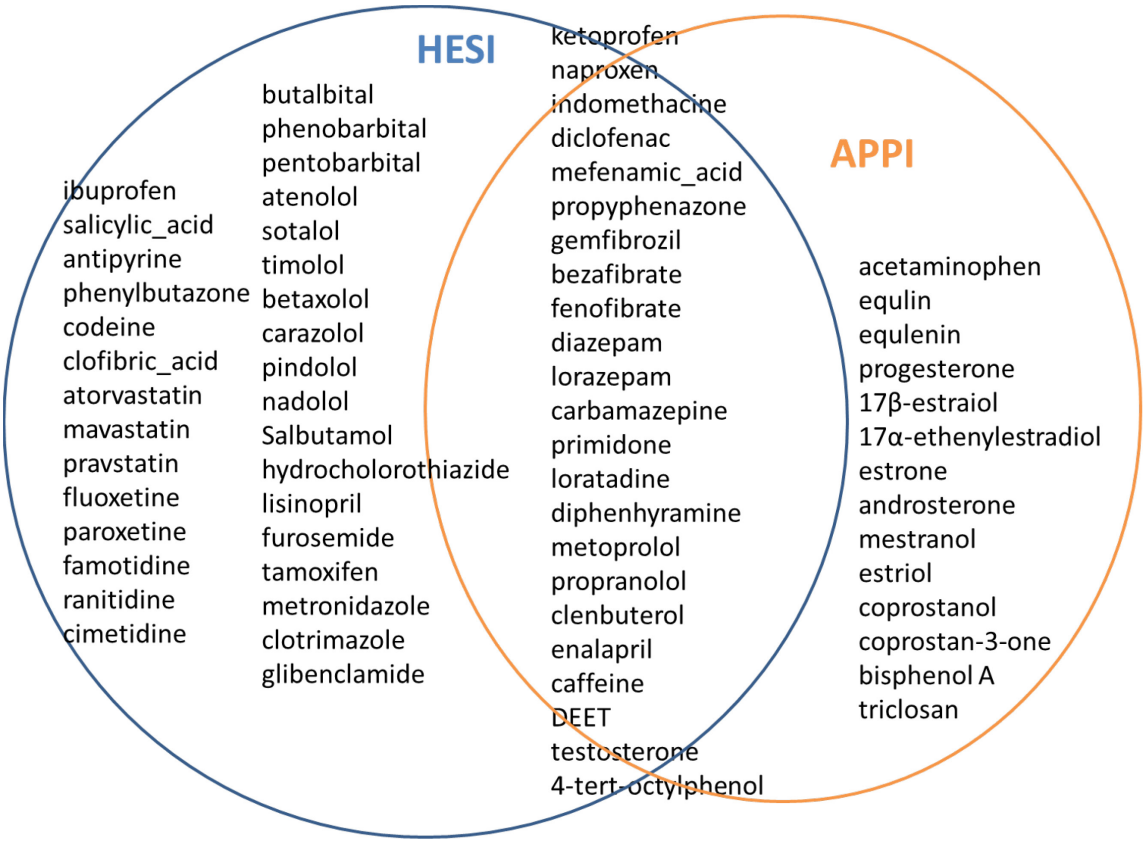


Figure 23. Analytes that can be ionized in HESI, APPI or both

5.4 Conclusion

An online-SPE-LC-MS/MS method was developed to detect 52 pharmaceuticals simultaneously. The online-SPE method was able to retain target compounds very well and interference was reduced by the wash step. HESI and APPI were compared by both absolute intensity and MDLs for ionization of 52 pharmaceuticals. Results indicated that HESI was an ideal ionization technique for polar compounds. However, APPI is an alternative ionization technique for some polar compounds that were ionized in positive mode. For simultaneous detection of compounds with various polarities, APPI will be a better choice since

it showed comparable performance to HESI for some polar compounds and excellent performance for less polar or non-polar compounds.

A summary is shown in table 17 on the basis of comparison of methods developed in previous chapters for analytes. Proper methods were recommended for the detection of analytes. In general, GC/MS is good for nonpolar compounds. For less polar compounds, a derivation step is very important for the analysis of GC/MS. Heated electrospray ionization of LC/MS is appropriate for the detection of polar compounds. APPI with dopant is capable to ionize compounds with various polarities.

Table 17. Comparison of GC/MS, LC/MS with different ionization sources for analytes

	GC/MS	LC/MS					
Name	HESI	APCI	APPI	APPI acetone	APPI anisole	APPI chlorobenzene	APPI toluene
Ketoprofen	YES						YES
Naproxen	YES						YES
Ibuprofen	YES	YES					
Indomethacin	YES						YES
Diclofenac	YES						YES
Mefenamic acid	YES						YES
Acetaminophen		YES					YES
Salicylic Acid	YES						
Antipyrin	YES						
Propyphenazone	YES						YES
Phenylbutazone	YES						
Codeine	YES						
Clofibric Acid	YES						
Gemfibrozil	YES						YES

	GC/MS	LC/MS					
Name	HESI	APCI	APPI	APPI acetone	APPI anisole	APPI chlorobenzene	APPI toluene
Bezafibrate	YES						YES
Fenofibrate	YES						YES
Atorvastatin	YES						
Mevastatin	YES						
Pravastatin	YES						
Fluoxetine	YES						
Paroxetine	YES						
Diazepam	YES						YES
Lorazepam	YES						YES
Carbamazepine	YES	YES	YES	YES	YES	YES	YES
Primidone	YES						YES
Famotidine	YES						
Ranitidine	YES						
Cimetidine	YES						
Loratadine	YES						YES

Name	GC/MS	LC/MS						
	HESI	APCI	APPI	APPI acetone	APPI anisole	APPI chlorobenzene	APPI toluene
Diphehydramine	YES						YES
Butalbital	YES						
Phenobarbital	YES						
Pentobarbital	YES						
Atenolol	YES						
Sotalol	YES						
Metoprolol	YES						YES
Propranolol	YES						YES
Timolol	YES						
Betaxolol	YES						
Carazolol	YES						
Pindolol	YES						
Nadolol	YES						
Salbutamol	YES						
Clenbuterol	YES						YES

	GC/MS	LC/MS						
Name		HESI	APCI	APPI	APPI acetone	APPI anisole	APPI chlorobenzene	APPI toluene
Enalapril		YES						YES
Hydrochlorothiazide		YES						
Lisinopril		YES						
Furosemide		YES						
Tamoxifen		YES						
Metronidazole		YES						
Clotrimazole		YES						
Glibenclamide		YES						
Caffeine	YES	YES	YES	YES	YES	YES	YES	YES
Triclosan	YES							YES
Triclocarban								YES
DEET	YES	YES	YES	YES	YES	YES	YES	YES
Bisphenol -A	YES	YES	YES					YES
4-n-nonylphenol	YES		YES					YES
4-t-octylphenol	YES							YES

	GC/MS	LC/MS						
Name		HESI	APCI	APPI	APPI acetone	APPI anisole	APPI chlorobenzene	APPI toluene
Androsterone	YES							YES
Estrone (E1)	YES				YES			YES
Equilin	YES						YES	YES
Testosterone	YES	YES	YES	YES	YES	YES	YES	YES
Equilenin	YES				YES			YES
17-β-Estradiol (E2)	YES						YES	YES
17-α-Estradiol	YES							YES
Ethynyl Estradiol (EE2)	YES						YES	YES
Progesterone	YES						YES	YES
Mestranol	YES							YES
Estriol (E3)	YES							YES
Coprostanol	YES						YES	YES
Coprostanone	YES				YES			YES
Coprostane	YES							
Cholesterol	YES							

CHAPTER 6

Occurrence of PPCPs in reclaimed water

6.1 Introduction

Water stress has become a serious problem worldwide because of the rapid population growth on the earth. Properly managed water resources are critical for sustainable development of water supply. In order to improve the management efficiency of water resources, treated waters are commonly reused worldwide for landscape, agriculture, irrigation, recharging, etc. In the United States, treated water has been used in more than 3000 application sites. Over 40 $\times 10^6$ m^3 of reclaimed water is used in California every year (Wu et al., 2010) (Xu et al., 2009).

However, potential adverse effects persist when reusing treated water. It is proved that current WWTPs with primary treatment and secondary treatment processes could not remove PPCPs completely as PPCPs have been detected in the effluent of WWTPs (Deblonde et al., 2011). Tertiary treatments such as granular active carbon adsorption, ozonation and catalytic oxidation showed a relatively high removal efficiency of PPCPs in the wastewater, but these treatments are seldom used in current WWTPs (Yoon et al., 2006; Broséus et al., 2009; Pisarenko et al.). Therefore, when reusing treated water, PPCPs could enter the ecosystem and water supply, which may have potential adverse effects to human beings. When treated water is used for irrigation, PPCPs may enter surface water by runoff and cause adverse effects on organism in the aqueous

environment (Xu et al., 2009). In addition, compounds with strong sorption that are recalcitrant to degradation may remain on the surface of the soil and be uptaken by plants. Research about uptake of human pharmaceuticals in plants grown in soil suggested that compounds introduced by irrigation may be more available for plant uptake and translocation than by biosolid application (Wu et al., 2010). When treated water is used for deep well injection, PPCPs may contaminate ground water, which may be used as a source of drinking water. Thus, it is essential to monitor the presence of PPCPs in treated water targeted for reuse or recharge.

At Florida International University's Biscayne Bay Campus, treated wastewater from the North District WWTP is used for irrigation. The North District WWTP is located at NE 154 Street and is east of Biscayne Boulevard, and it receives wastewater from the North District of Miami-Dade County. The wastewater treatment plant was designed to have a flow of 120 million gallons per day (MGD) with average daily flow around 112.5 MGD. The facilities include screening, grit removal, primary sedimentation, activated sludge treatment by oxygenation and chlorination. Extra filtration (DynaSand Filtration, Leopold Filtration and Tetra filtration) and disinfection are applied to effluents before release to make the reclaimed water ready for use in irrigation. The following study monitored the reclaimed water between January 2011 and December 2011.

6.2 Experimental

Analyte information is shown in table 18. Sample preparation, online SPE procedure and detection method were described in part 4.2 and 5.2. Reclaimed water was collected directly from sprinkler system during January 2011 and December 2011 at Florida International University, Biscayne Bay Campus.

Table 18. Analytes in reclaimed water samples

GROUP	Name	CAS Number
Analgesics and anti-inflammatories	Ketoprofen	22071-15-4
	Naproxen	22204-53-1
	Ibuprofen	15687-27-1
	Indomethacin	53-86-1
	Diclofenac	15307-86-5
	Mefenamic acid	61-68-7
	Acetaminophen	103-90-2
	Salicylic Acid	69-72-7
	Antipyrin	60-80-0
	Propyphenazone	479-92-5
	Phenylbutazone	50-33-9
	Codeine	76-57-3
Antihyperlipidemics - Lipid Regulators	Clofibric Acid	882-09-7
	Gemfibrozil	25812-30-0
	Bezafibrate	41859-67-0
	Fenofibrate	49562-28-9

GROUP	Name	CAS Number
	Atorvastatin	134523-00-5
	Mevastatin	73537-88-3
	Pravastatin	81131-70-6
Antidepressants and anticonvulsants	Fluoxetine	54910-89-3
	Paroxetine	61869-08-7
	Diazepam	439-14-5
	Lorazepam	846-49-1
	Carbamazepine	298-46-4
	Primidone	125-33-7
Antihistaminies	Famotidine	76824-35-6
	Ranitidine	66357-35-5
	Cimetidine	51481-61-9
	Loratadine	79794-75-5
	Diphehydramine	88637-37-0
Barbiturates - Anticonvulsants	Butalbital	77-26-9
	Phenobarbital	50-06-6
	Pentobarbital	76-74-4
Beta-blockers -cardiac arrhythmias	Atenolol	29122-68-7
	Sotalol	3930-20-9
	Metoprolol	37350-58-6
	Propranolol	525-66-6
	Timolol	26839-75-8
	Betaxolol	63659-18-7
	Carazolol	57775-29-8

GROUP	Name	CAS Number
	Pindolol	13523-86-9
	Nadolol	42200-33-9
Bronchodilators - Beta agonists	Salbutamol	18559-94-9
	Clenbuterol	37148-27-9
Antihypertensives	Enalapril	75847-73-3
	Hydrochlorothiazide	58-93-5
	Lisinopril	83915-83-7
Diuretics	Furosemide	54-31-9
Cancer treatment	Tamoxifen	10540-29-1
Antifungals	Metronidazole	443-48-1
	Clotrimazole	23593-75-1
Antidiabetic	Glibenclamide	10238-21-8
Wastewater Indicators	Caffeine	58-08-2
	Triclosan	3380-34-5
	Triclocarban	101-20-2
	DEET	134-62-3
	Bisphenol -A	80-05-7
	4-n-nonylphenol	104-40-5
	4-t-octylphenol	27193-28-8
Hormones	Androsterone	53-41-8
	Estrone (E1)	53-16-7
	Equilin	474-86-2
	Testosterone	58-22-0
	Equilenin	517-09-9

GROUP	Name	CAS Number
	17-β-Estradiol (E2)	50-28-2
	Ethynyl Estradiol (EE2)	57-63-6
	Progesterone	58-83-0
	Mestranol	72-33-3
	Estriol (E3)	50-27-1
Fecal Sterols and sterones	Coprostanol	360-68-9
	Coprostanone	601-53-6

6.3 Result and discussion

One or more compounds were found in 100% of the reclaimed water samples. The reason for the high detection frequency is that the treatment processes in the North District WWTP only include primary and secondary treatments that are not designed to remove microconstituents. Even though extra filtration and chlorination are applied to effluent, PPCPs and hormones still cannot be removed completely. In this one year study period, 33 out of 72 target compounds were detected more than once. The detected concentrations of the target compounds are shown in figure 24. About 15% of the concentrations were more than 1 μg/L and 80% of the high concentrations (>1 μg/L) were derived from gemfibrozil, atenolol, caffeine and bisphenol A.

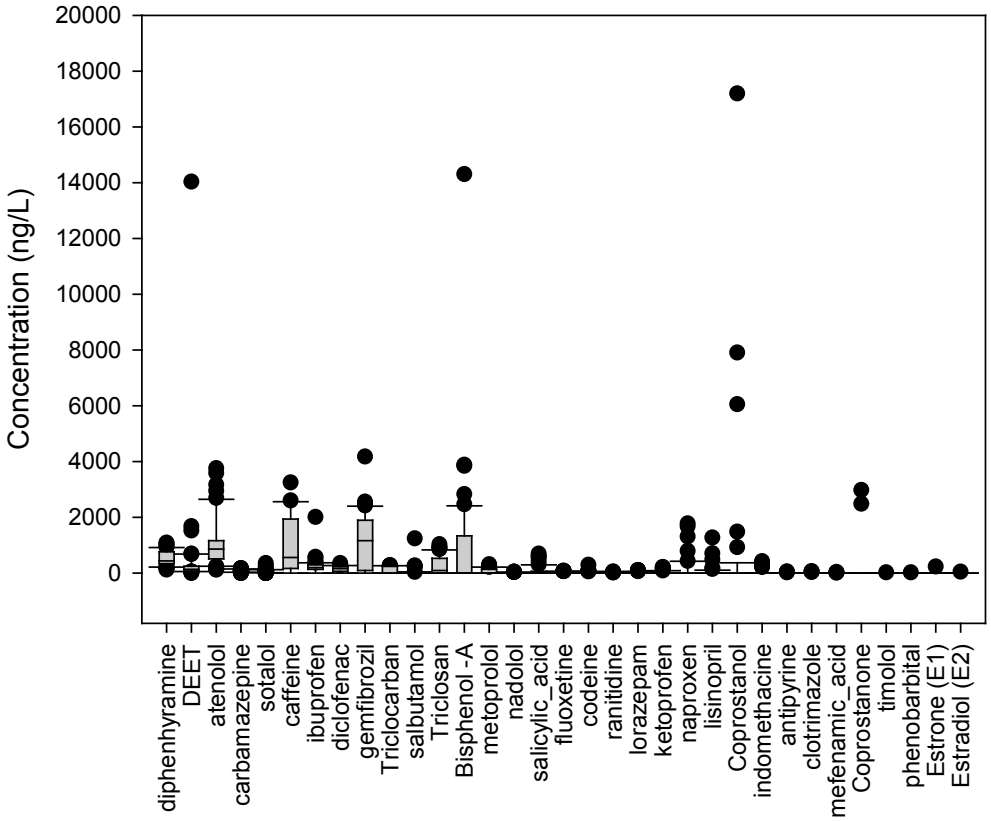


Figure 24. Detected concentration ranges for all compounds in reclaimed water samples.

Among the high concentration compounds, coprostanol, bisphenol A and DEET are the three compounds with detected maximum concentrations that exceeded 10 µg/L (figure 25). Bisphenol A is known as a weak environmental estrogen, more recent research has demonstrated that bisphenol A may be similar to estradiol in stimulating some cellular responses (Beverly S, 2011). DEET's chronic aquatic toxicity data is available for fish (8.42 mg/L), daphnia (5.13 mg/L) and algae (9.65 mg/L) (Aronson et al., 2012). The reported observed effect

concentrations were hundreds of times higher than the detected concentrations. Only two hormones (estrone and estradiol) were detected in the reclaimed water samples. The detected concentrations of estrone (50.8 ng/L) and estradiol (58.5 ng/L) were relatively high compared to lowest observed effect concentration (usually few ng/L) (Larsen et al., 2008), but the detection frequency is only 2%. Actually, acute toxicity to aquatic organisms is unlikely to occur because acute effect concentrations are 100-1000 higher than the detected concentrations in the environmental samples.

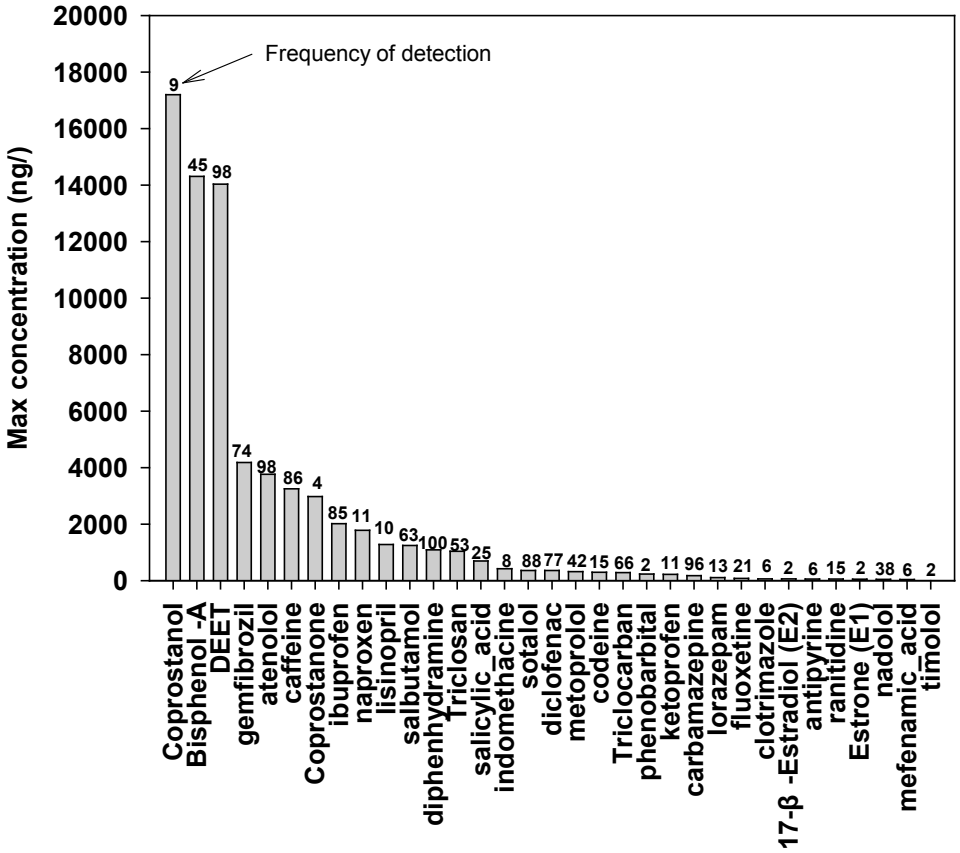


Figure 25. Maximum concentration and detection frequency of compounds in reclaimed water samples

In addition, the detection frequency is a critical factor since long-term exposure to PPCPs especially compounds with endocrine disruption effects may cause problems even though their concentrations are low. The four most frequently detected compounds were diphenhydramine (100%), DEET (98%), atenolol (98%) and carbamazepine (96%). Diphenhydramine has been reported in water, sediment and fish but the effects of diphenhydramine on aquatic organisms is still lacking. In the reclaimed water samples, diphenhydramine was detected in all the samples all year long with a maximum concentration of 1091 ng/L. A previous study indicated that no-observed-effect concentration of diphenhydramine on reproduction of D.magna is 0.8 μg/L, while 17% of concentrations detected in reclaimed water exceeded 0.8 μg/L and there is no indication of what the effect level could be. Atenolol was detected at a maximum concentration of 3761 ng/L in 98% of reclaimed water samples. In reproduction test with *Daphnia magna,* the most sensitive no-observed-effect concentration of atenolol was 1.8 mg/L (Küster et al., 2009). Winter and his coworker used fathead minnows as test species and undertook embryo-larval development (early life stage or ELS) and short-term adult reproduction studies. The results of the ELS study showed that $NOEC^{growth}$ and $LOEC^{growth}$ of atenolol were 3.2 and 10 mg/L, respectively. In the short-term reproduction study, $NOEC^{reproduction}$ and $LOEC^{reproduction}$ of atenolol were 10 and >10 mg/L, respectively (Winter et al., 2008). Compared to the toxicity test results, the detected concentrations of atenolol in reclaimed water samples are much lower than the concentration that will cause chronic effect to fish. Carbamazepine is an anticonvulsant pharmaceutical that is commonly found in

effluent of WWTPs, surface water and drinking water (Heberer et al., 2002; Deblonde et al., 2011). In this study, carbamazepine was detected in 96% of reclaimed water samples with a maximum concentration of 173 ng/L. To test the chronic effect of carbamazepine, rainbow trout were exposed to three concentrations of carbamazepine (1.0 μg/L, 0.2 mg/L and 2.0 mg/L) for 42 days. Result indicated that at 2.0 mg/L, both physiological condition status and muscle-based biomarkers were significantly affected (Li et al., 2009; Li et al., 2010). By comparing with toxicity study, the detected concentrations of compounds in the reclaimed water were generally lower than the lowest-observed-effect concentrations of chronic effects, thus the risk associated with the their occurrence was probably minimal.

Although for a single compound, the detected concentrations was lower than the lowest-observed-effect concentration, most of the time more than one analytes was found in the environmental samples. The resulting additive effects of PPCP mixture may cause observed effects to organisms eventually. In the one year study, more than one target compounds were found in all reclaimed water samples and 13% of reclaimed water samples had a total concentration of > 10 μg/L. However, the effect and interactions of PPCP mixture in the environmental samples is still unclear, and further investigations are required.

To obtain a broader view of the results, target compounds were divided into 15 groups on the basis of their general application or origins. The percent of

detection frequencies of each group are shown in figure 26. The number of compounds in the group does not reflect the detection frequency. Most of the time, detection frequency was influenced by the usage of compounds and removal rate of the WWTPs. Wastewater indicators, β-blockers and analgesics/anti-inflammatories were the three most detected groups, which covered 67% of detection frequency. The three groups also covered 69% of the total concentration (figure 27). Another group that should be of concern is the lipid regulator group. Even though the percent of detection frequency is relatively low (6%), compounds in the lipid regulator group covered 20% of the total concentration.

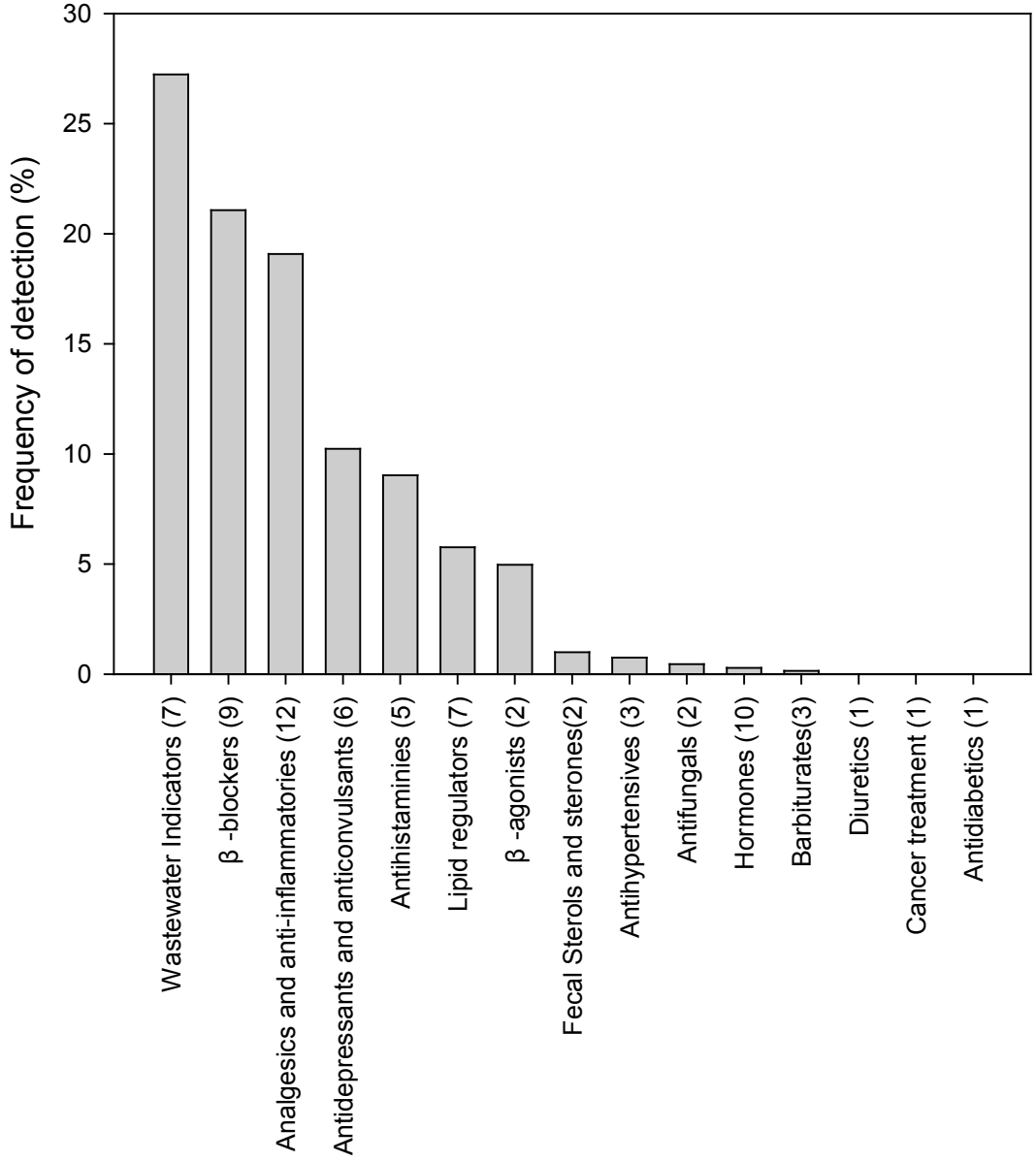


Figure 26. Detection frequency as a percent of different classes in reclaimed water samples

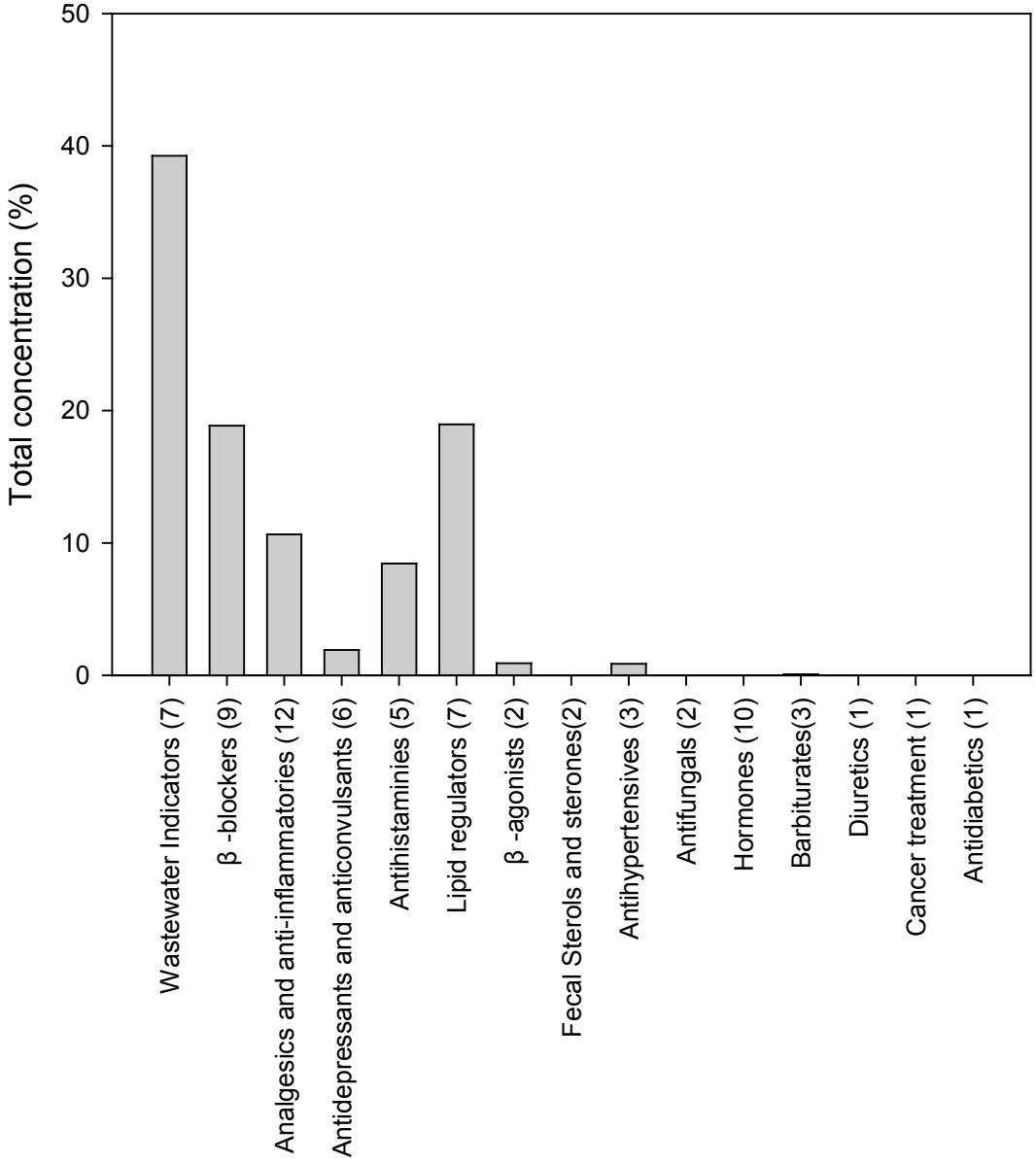


Figure 27. Percent of total measured concentration for each group of compounds in reclaimed water samples

6.4 Conclusion

Methods developed in chapter 4 and 5 were successful applied on simultaneously detection of 71 compounds in reclaimed water samples. The online SPE method was robust, sensitive and reliable, making it suitable for routine analysis of environmental water samples. Reclaimed water samples were collected from the sprinkler system for a year-long period in Florida International University Biscayne Bay Campus, where reclaimed water was reused for irrigation. Analysis results showed that more than one analytes were detected in all reclaimed water samples. About 15% of the detected concentrations were more than 1 μg/L. Among the detected compounds with high concentrations (>1 μg/L), coprostanol, bisphenol A and DEET's maximum concentration exceeded 10 μg/L. The four most frequently detected compounds were diphenhydramine (100%), DEET (98%), atenolol (98%) and carbamazepine (96%). Wastewater indicators, β-blockers and analgesics /anti-inflammatories were the three most detected groups and these three groups covered 67% of detection frequency and 69% of the total concentration. The one-year study confirmed that current primary treatment, secondary treatment, extra filtration and chlorination in North District WWTP could not remove PPCPs completely from effluent. The microconstituents were continuously released to the environment through water reuse. More seriously, the releasing of microconstituents will continue for a long period of time until effective treatment processes are incorporated into the WWTPs. Although the detected maximum concentration of a single compound may not cause acute effect to organisms and the frequently detected compound

may not cause chronic effect at the detected concentrations, it is still uncertain that treated water reuse is safe for the environment. The reason is that for all reclaimed water samples, more than one compounds was detected and very little is known about the combination effect of PPCPs mixture to environmental organisms. Therefore, instead of doing experiments about the acute and chronic effect of a single compound, more research should focus on the investigation of the combination effects of PPCP mixture at environmental relevant concentrations. The one-year study of reclaimed water provided lots of critical information about compounds that persist in the effluent of WWTPs and their environmental concentrations, which are very valuable for toxicity research in the future.

CHAPTER 7

Occurrence of PPCPs in drinking water

7.1 Introduction

Pharmaceuticals and personal care products have been widely reported in wastewater and surface waters worldwide. The occurrence of PPCPs in the environment is a partially result of wastewater discharges. Other sources of PPCPs in the environmental waters include: runoff from agriculture fields, application of veterinary drugs, landfill leachates, etc. Issues concerning the quality of drinking water are important because the sources of drinking water (surface waters, ground water, etc.) might be impacted by the intrusion of wastewaters (Focazio et al., 2008; Fram and Belitz, 2011). The removal efficiency of PPCPs in the drinking water treatment plant varies both among chemicals and between different processes employed in the treatment plants. Advanced technologies such as ozonation (Broséus et al., 2009; Pisarenko et al., 2012) and granular activated carbon (GAC) (Stackelberg et al., 2007b) can remove many compounds, but they cannot eliminate all the contaminations. In addition, advanced technologies are not universally applied to the treatment of potable supplies, even in developed counties. Consequently, several studies have shown the positive detection of PPCPs in the tap water that people are drinking (Mompelat et al., 2009). Although it is not clear yet whether drinking water containing PPCPs at the detected levels is a risk to humans, drinking water will always be a major public concern because it is a direct route for PPCPs to

enter the human body. Therefore, a better understanding of occurrence of PPCPs in the drinking water is critical for public health.

The following study summarized results from a comprehensive survey of 720compounds in 54 tap water samples collected from homes located in the Miami-Dade County area. The results provided a preliminary but informative assessment of the actual concentrations of PPCPs to which people are exposed from drinking water.

7.2 Experimental

Chemicals, sample preparation, online SPE procedure and detection method were described in parts 4.2 and 5.2. Briefly, drinking water samples were loaded to the Hypersil Gold aQ preconcentration column and then analytes were back flushed to the Hypersil Gold analytical column. Analytes were then separated and analyzed by tandem mass spectrometry. Personal care products, steroid hormones, sterols and sterones were analyzed with an APPI source, while pharmaceuticals were analyzed with the HESI source. Drinking water samples were collected from the Miami-Dade area between August and October 2011. Information of sampling location is shown in table 19 and figure 28. Water samples were collected by volunteers at their place of residence. Samplers were instructed to open the water valve, let it run for about five minute and to collect the water in a new PET container. Participants were asked to provide an approximate location of their home but not a physical address (street crossings

only). All samples were immediately frozen or kept refrigerated while transported to the lab.

Table 19. Sample name, sampling date and location

Sample name	Sampling date	Latitude	Longitude
DW001	8/22/2011	25°46'20.91"N	80°22'6.15"W
DW002	8/22/2011	26° 1'15.59"N	80° 8'30.79"W
DW003	8/22/2011	25°54'20.54"N	80° 9'31.89"W
DW004	8/23/2011	25°57'3.51"N	80° 9'58.40"W
DW005	8/23/2011	26° 0'45.46"N	80° 9'0.06"W
DW006	8/23/2011	25°45'34.04"N	80°21'54.13"W
DW007	8/23/2011	25°52'33.72"N	80° 7'32.80"W
DW008	8/24/2011	25°33'36.96"N	80°21'4.39"W
DW009	8/28/2011	25°55'53.30"N	80° 7'30.34"W
DW010	9/6/2011	25°53'59.72"N	80° 9'0.36"W
DW011	9/13/2011	25°56'35.70"N	80° 8'21.82"W
DW012	9/13/2011	25°54'16.94"N	80°11'31.66"W
DW013	9/13/2011	25°51'49.99"N	80° 7'28.05"W
DW014	9/13/2011	25°51'4.95"N	80°17'54.93"W
DW015	9/13/2011	25°46'17.43"N	80°22'19.79"W
DW016	9/13/2011	25°45'6.11"N	80°12'5.22"W
DW017	9/13/2011	25°54'31.34"N	80°18'31.18"W
DW018	9/13/2011	25°45'46.76"N	80°22'53.87"W
DW019	9/13/2011	25°45'31.28"N	80°21'56.43"W
DW020	9/13/2011	25°40'11.52"N	80°26'32.74"W
DW021	9/13/2011	25°45'39.59"N	80°22'34.44"W
DW022	9/13/2011	25°46'1.62"N	80°22'26.60"W
DW023	9/13/2011	25°59'33.45"N	80°15'7.17"W
DW024	9/13/2011	25°45'56.37"N	80°23'46.87"W
DW025	9/13/2011	25°45'44.44"N	80°12'9.24"W
DW026	9/13/2011	25°46'58.22"N	80° 8'23.03"W
DW027	9/13/2011	25°53'50.73"N	80°18'47.40"W
DW028	9/13/2011	25°46'36.45"N	80°22'27.53"W
DW029	9/13/2011	25°41'37.49"N	80°21'54.24"W
DW030	9/13/2011	25°45'44.70"N	80°15'49.34"W
DW031	9/13/2011	25°46'14.82"N	80°11'51.36"W
DW032	9/20/2011	25°45'16.06"N	80°13'13.16"W
DW033	9/29/2011	25°54'14.88"N	80°17'57.88"W

Sample name	Sampling date	Latitude	Longitude
DW034	10/1/2011	25°53'53.45"N	80°13'53.75"W
DW035	10/1/2011	25°47'0.12"N	80°20'11.02"W
DW036	10/1/2011	25°48'43.74"N	80°22'12.05"W
DW037	10/1/2011	25°50'21.18"N	80°22'14.67"W
DW038	10/1/2011	25°50'24.33"N	80°19'7.10"W
DW039	10/1/2011	25°53'57.52"N	80°11'39.34"W
DW040	10/2/2011	26° 7'16.37"N	80°10'47.11"W
DW041	10/2/2011	25°29'39.38"N	80°24'58.93"W
DW042	10/2/2011	25°28'39.66"N	80°27'56.37"W
DW043	10/2/2011	25°40'10.68"N	80°25'57.50"W
DW044	10/2/2011	25°37'36.85"N	80°24'53.63"W
DW045	10/2/2011	25°28'44.81"N	80°25'50.04"W
DW046	10/2/2011	25°33'56.77"N	80°22'56.38"W
DW047	10/2/2011	25°36'50.11"N	80°18'58.73"W
DW048	10/2/2011	25°43'2.62"N	80°16'41.26"W
DW049	10/2/2011	25°38'40.40"N	80°20'19.40"W
DW050	10/2/2011	25°40'20.88"N	80°19'21.63"W
DW051	10/16/2011	25°53'6.04"N	80° 9'56.22"W
DW052	10/16/2011	25°50'49.93"N	80°11'4.68"W
DW053	10/16/2011	25°47'47.93"N	80°11'23.91"W
DW054	10/12/2011	25°58'18.44"N	80° 9'12.11"W

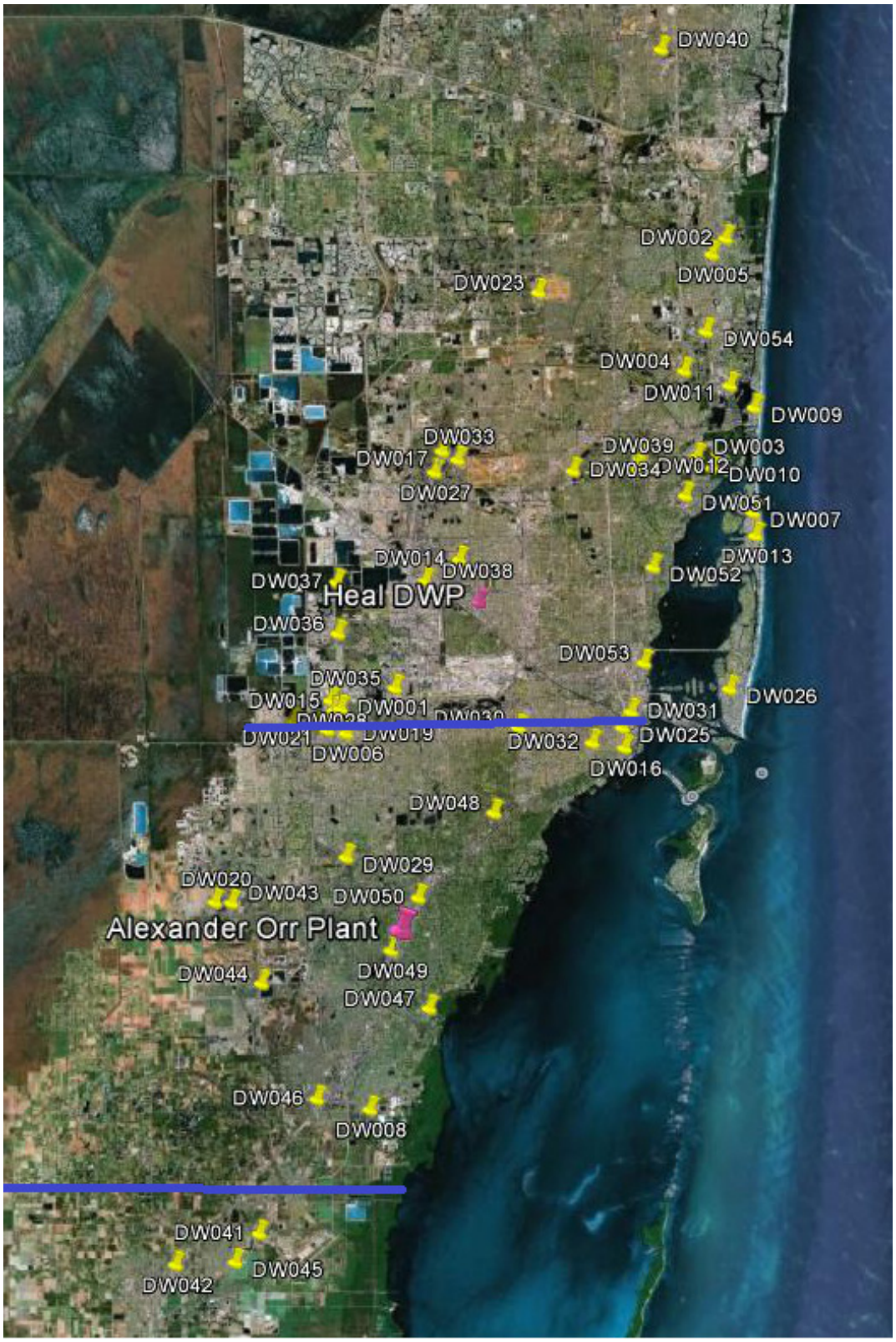


Figure 28. Map showing the collection sites of drinking water samples

7.3 Result and discussion

The water source for the Miami-Dade County Water and Sewer Department (WASD) is the Biscayne Aquifer, which is located just below the surface in South Florida. Approximately 330 million gallons per day (mgd) of water are withdrawn from the aquifer to meet the needs of the community. There are three water treatment facilities in the Miami-Dade County area: the Hialeah and Preston Plant, Alexander Orr Plant and the South Dade Water Supply System. The Hialeah and Preston plant provides drinking water for residents who live north of Flagler Street Up to the Miami-Dade/ Broward line. The Alexander Orr Plant serves people who live from Flagler Street to S.W. 248 Street. The South Dade Water Supply System serves residents south of S.W. 248 Street. The blue line in figure 28 delimits the areas served by different water supply systems. The most common water treatment processes are filtration, flocculation and sedimentation, and disinfection (URL3).

A total of 15 analytes were detected in the drinking water samples and the concentrations are shown in figure 29. At least one analyte was found in 96% of the drinking water samples. Compared to the concentrations of analytes detected in the reclaimed water or the surface waters, the concentrations detected in the drinking water were much lower. Only the concentrations of salicylic acid, ibuprofen and DEET were found to be more than 200 ng/L. The maximum concentrations of salicylic acid, ibuprofen and DEET were 521 ng/L, 301 ng/L and 290 ng/L, respectively (table 20). The occurrence of PPCPs in the drinking

water has been reported in other places in the world. A previous study showed that the maximum concentrations of ibuprofen detected in tap water were 3 ng/L, 0.6, 8.5 and 1350 ng/L in Germany, France, Finland and the USA, respectively (Mompelat et al., 2009). The maximum concentration of DEET found in drinking water samples in the USA is 63 ng/L (Benotti et al., 2009) and this number is significantly lower than maximum value reported in this study (521 ng/L). However, DEET is a common environmental contaminant and is very difficult to avoid cross-contaminating samples if the compound is present in the sampling area (kitchens, bathrooms, etc).

Table 20. Maximum concentration of analytes detected in Miami-Dade area and other parts of the world (ng/L)

	Miami Dade	Finland	France	Germany	USA	Canada	UK	Italy
ketoprofen	179	8.0[a]	3.0[b]					
ibuprofen	301	8.5[a]	0.6[b]	3[d]	1350[g]			
diclofenac	ND		2.5[b]	35[e]				
acetaminophen	ND		210[b]					
salicylic_acid	521		19[c]					
propyphenazone	9.72			240[e]				
codeine	ND				30[h]			
clofibric_acid	65.1			270[f]				
gemfibrozil	ND					70[k]		
bezafibrate	ND			27[d]				
fluoxetine	58.4							
paroxetine	65.9							
diazepam	ND						10[l]	23.5[m]
carbamazepine	27.8		43.2[b]		258[i]	24.0[k]		
primidone	ND			40[e]				
propranolol	9.58							
betaxolol	24.0							
carazolol	11.2							

salbutamol	13.1	
metronidazole	178	
clotrimazole	18.5	
Triclosan	ND	734[g]
DEET	290	63.0[j]

a (Vieno et al., 2005)
b (Togola and Budzinski, 2008)
c (Vulliet et al., 2011)
d (Stumpf et al., 1996)
e (Heberer et al., 2004)
f (Heberer et al., 1997)
g (Loraine and Pettigrove, 2006)
h (Stackelberg et al., 2007b)
i (Stackelberg et al., 2004)
j (Benotti et al., 2009)
k (Tauber, 2003)
l (Waggot, 1981)
m (Zuccato et al., 2000)

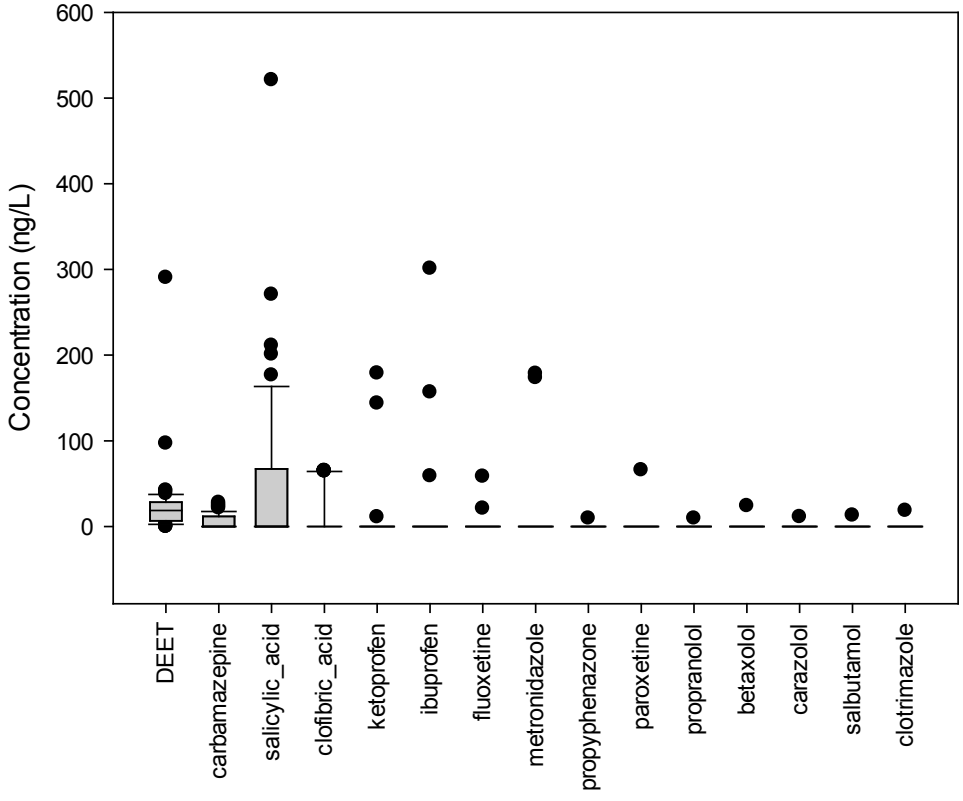


Figure 29. Concentrations of PPCPs in the drinking water samples collected from Miami-Dade County

The three most frequently detected compounds were DEET (93%), carbamazepine (43%) and salicylic acid (37%) (Figure 30). The high detection frequencies of the three compounds indicated that current drinking water treatment could not remove them completely from the finished water. In a previous study (Stackelberg et al., 2007a), DEET has been reported as one of the most persistent compounds during drinking water treatment. Even the application of ozone and GAC can only partially remove DEET from the finished water. Carbamazepine is another compound that is usually detected in drinking water samples. Research indicated that ozone is an efficient way to remove it but since the removal rate is not 100%, carbamazepine is still persistent in the finished water. DEET and carbamazepine can be used as indictors representing potential contamination of PPCPs. Carbamazepine was detected in 40% of the tap water samples in the U.S in Benotti's study and 33% of tap water samples in Montreal boroughs in Canada (Garcia-Ac et al., 2009), which is very close to the detection frequency of carbamazepine in our study (Benotti et al., 2009). DEET's detection frequency was lower in Benotti's study (27%) than our study (Benotti et al., 2009). Salicylic acid is used as anti-inflammatory drug because of its ability to ease aches and pains and reduce fevers. Salicylic acid is also the key ingredient in many skin care products, such as soap, cleanser, body wash and cream etc., to treat acne, seborrhoeic dermatitis, psoriasis, corns, etc. Currently, very little research is available about the presence of salicylic acid in drinking water. In Vulliet's study, salicylic acid is reported as the most frequently detected compounds in the drinking water samples, however, the maximum concentration

(19 ng/L) is much lower than the results in our study. Because salicylic acid was detected in the drinking water samples at relatively high concentrations and high detection frequencies, future research should focus more on salicylic acid.

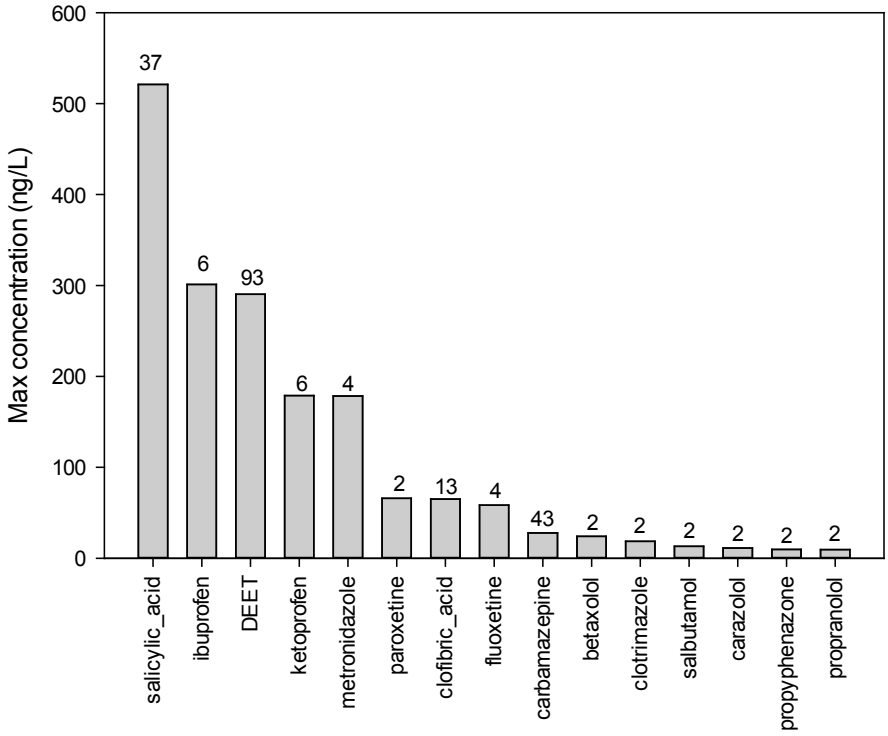


Figure 30. Maximum concentrations and detection frequencies of PPCPs in drinking water samples

To obtain a broader view of the results, target compounds were divided into 15 groups on the basis of their general application or origins. The three most frequently detected groups were wastewater indicators, analgesics and anti-inflammatories and antidepressants and anticonvulsants drugs, while the group

of analgesics and anti-inflammatories covered 56% of the total concentration (figure 31 and 32). The high detection frequency and high detection concentration resulted from the large amount of daily use and low removal efficiency of drinking water treatment (Benotti et al., 2009).

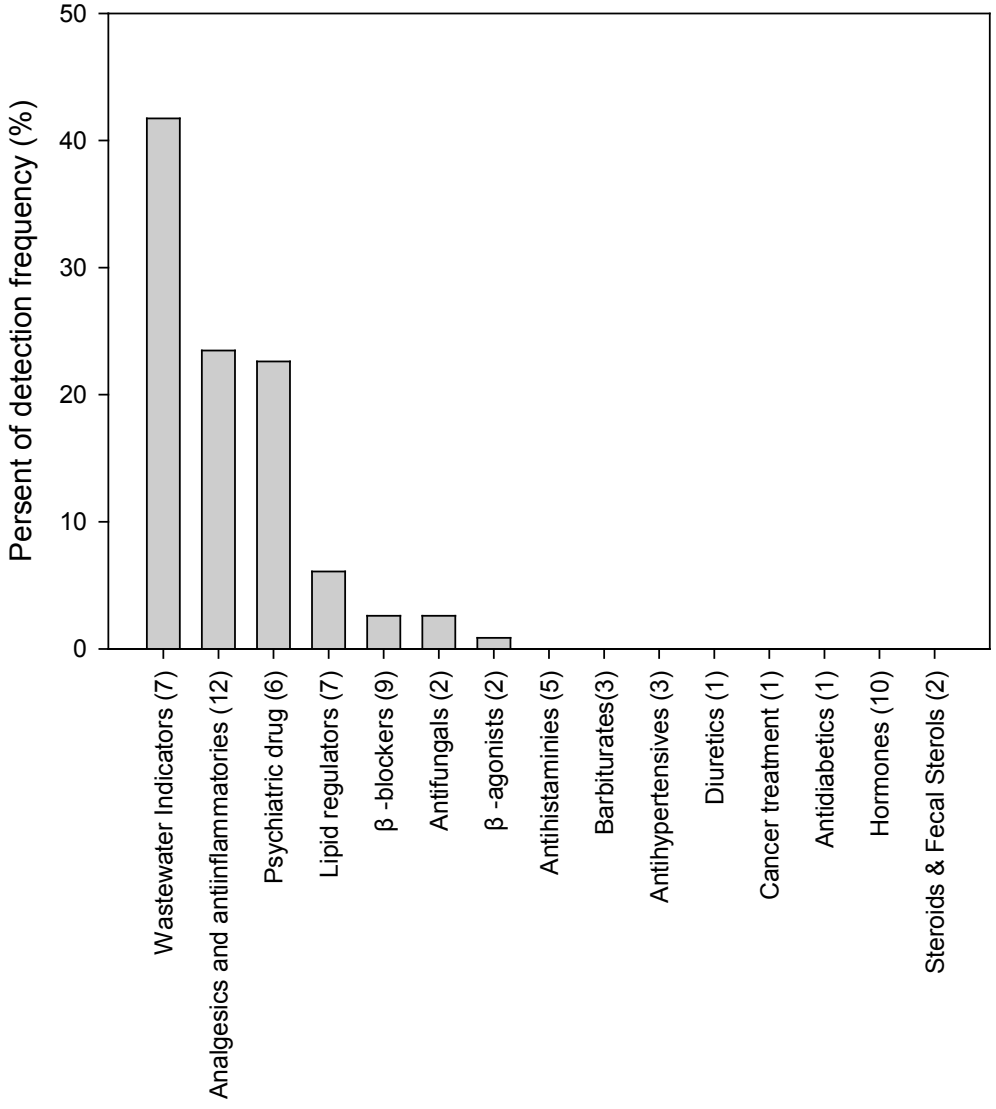


Figure 31. Detection frequency as percent of different classes in drinking water samples

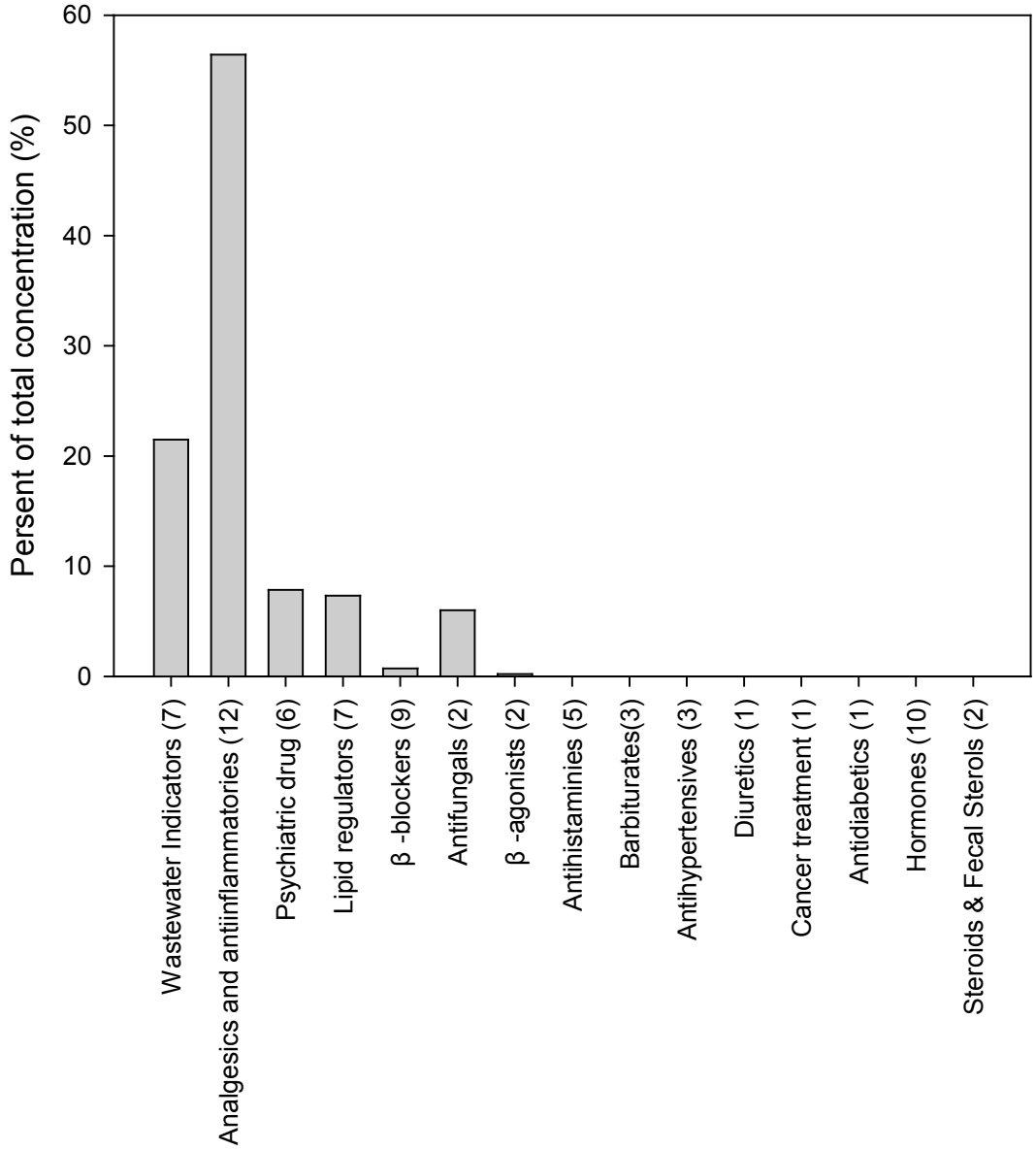


Figure 32. Percentage of total concentration of each class in drinking water samples

In order to understand the spatial distribution of the detection, sampling sites were shown in figure 33 as dots and the total concentration was indicated by different colors. Grey color represents no analytes detected at the sampling site. Blue color represents the total concentration detected at the sampling sites was between ND and 10 ng/L. Green color represents the total concentration detected at the sampling sites was between 10 and 50 ng/L. Yellow color represents the total concentration detected at the sampling sites was between 50 and 100 ng/L. Orange color represents the total concentration detected at the sampling sites was between 100 and 200 ng/L. Red color represents the total concentration detected at the sampling sites was between 200 and 500 ng/L. Purple color represents the total concentration detected at the sampling sites was more than 500 ng/L. The total concentrations were randomly disturbed at the samples sites. There is no trend in the relationship of the location of sampling sites and total concentrations. Although the total concentrations of three points were more than 500 ng/L, the high total concentrations were contributed by salicylic acid, which may enter drinking water sample after distribution. Results showed that the drinking water quality were similar in the whole Miami-Dade County area, which indicated that similar treatments were applied to source water in different drinking water facilities.

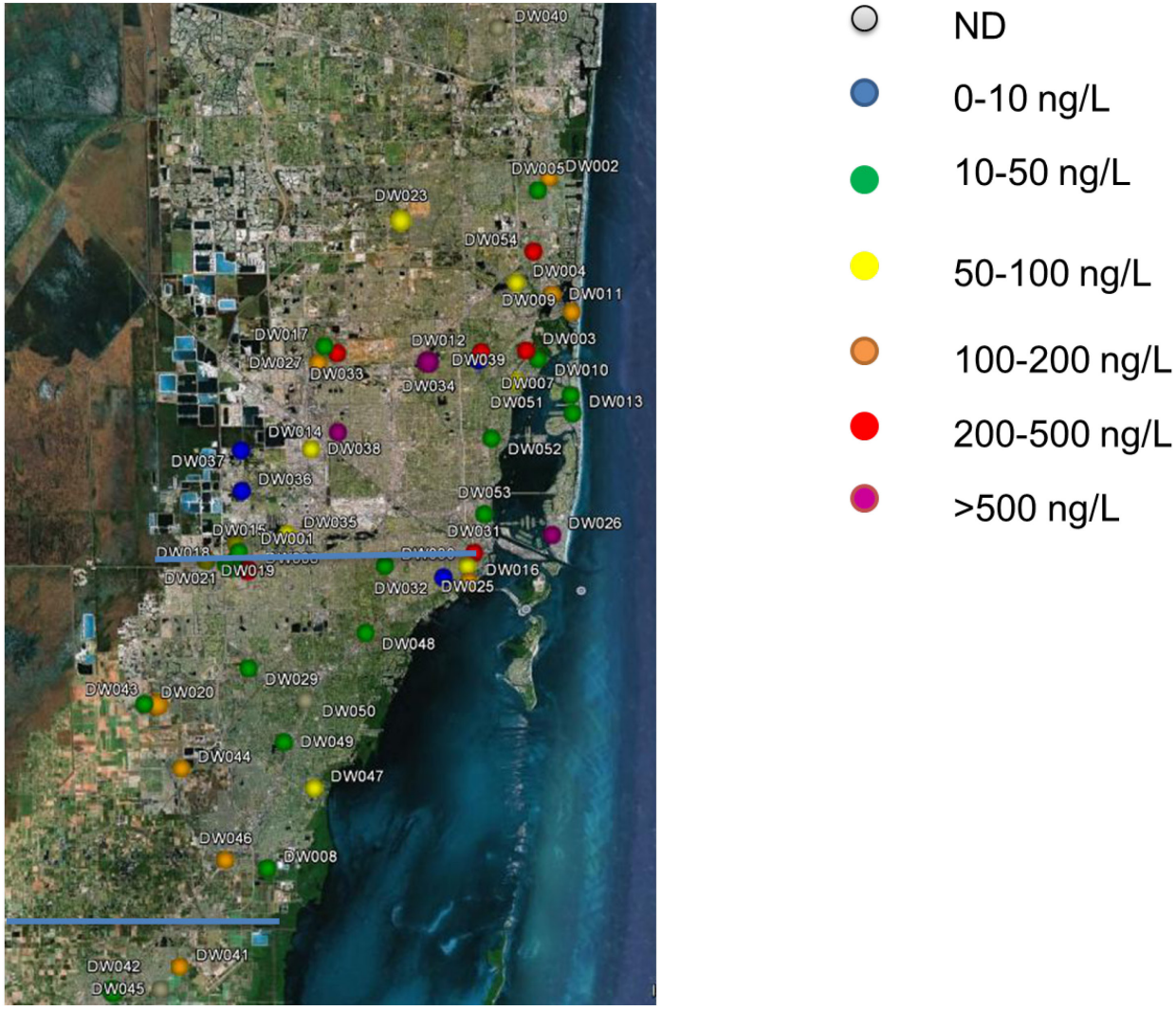


Figure 33. Distribution of total concentration of analytes in Miami-Dade County

Results of drinking water sample can be compared to reclaimed water results. Overall, fewer numbers of analytes were detected in the drinking water samples, only 15 analytes compared to 33 analytes in reclaimed water samples. The detected concentrations of analyte in the drinking water were much lower than the detected concentrations in the reclaimed water samples. The three highest maximum concentrations in the drinking water samples were 521 ng/L, 301 ng/L and 290 ng/L for salicylic acid, ibuprofen and DEET, respectively. While the three highest concentrations in reclaimed water for coprostanol, bisphenol A and DEET were more than 10,000 ng/L. In addition, many analytes were detected at relatively high average concentration in reclaimed water sample while average concentration were close to zero in the drinking water samples (cycled point in figure 34). Although, similar compounds were detected in the drinking water samples, the detection of frequency is lower compared to reclaimed water samples. DEET and carbamazepine were detected in 93% and 37% in drinking water samples respectively, while DEET and carbamazepine were detected in 98% and 98% of reclaimed water samples, respectively. DEET and carbamazepine could be used as the indicator of contamination of PPCPs because their persistence in both WWTPs and DWPs. However, more studies are needed to establish a potential relationship between reclaimed water and drinking water.

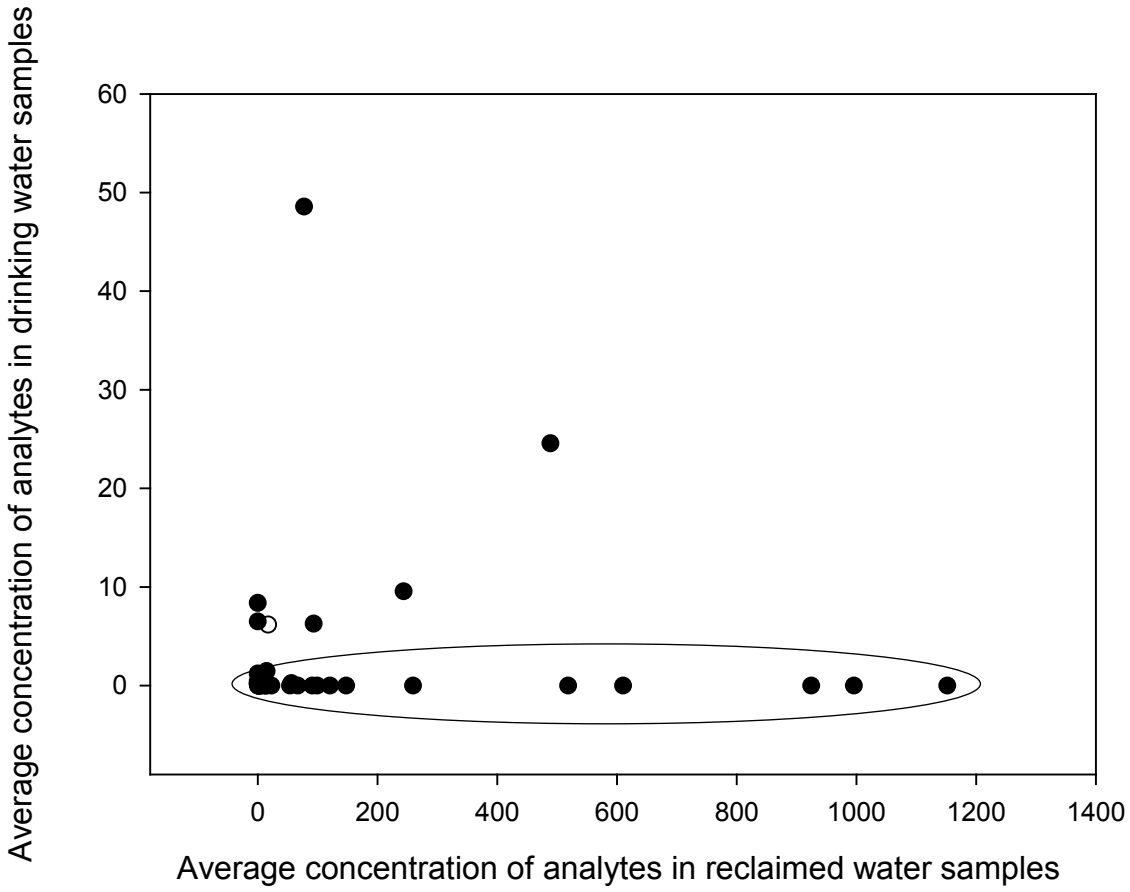


Figure 34. Comparison of average concentration of analytes in drinking water samples and reclaimed water samples

7.4 Conclusion

In the study of drinking water, 54 tap water samples collected from the Miami-Dade area were analyzed. 15 compounds were detected and more than one compound was detected in 93% of samples. The maximum concentration of salicylic acid, ibuprofen and DEET were 521 ng/L, 301 ng/L and 290 ng/L, respectively. The three most frequently detected compounds were DEET (93%), carbamazepine (43%) and salicylic acid (37%). Results indicated that drinking

water sources, such as ground water, may be impacted by discharge of wastewater, disposal of biosolids from WWTPs or landfilling. Current drinking water treatments cannot prevent certain PPCPs, such as DEET and carbamazepine, from entering the drinking water system. The quality of drinking water is a public concern since any compound present in the drinking water enters the human body directly. It is the largest threat to human health and long-term effects (80 years) might occur at a much lower concentration than the concentration of therapeutic effects. Adverse effects may be more obvious for children, elderly or pregnant women. However, no data are currently available regarding the risk because of the long period and difficulty of the research. The occurrence of PPCPs in the drinking water is a key issue for water quality. Although there are no regulations for PPCPs in drinking water so far, more research about the occurrence of PPCPs in the drinking water and more studies about the risk of long-term exposure of humans to PPCPs through drinking water are highly desirable.

CONCLUSIONS

The overall objective of the study was to detect trace level PPCPs and hormones in the South Florida environment (both aqueous phase and solid phase) using sensitive and reliable detection methods in order to gain a basic understanding on the quality of major environmental compartments with respect to a group of important unregulated emergent contaminants. Four methods were successfully developed to detect analytes in different matrices, including reclaimed waters, surface waters, drinking waters, sediments and soils. By conducting a comprehensive evaluation of a large number of environmental samples, we confirmed that reclaimed water from a typical secondary treatment WWTP routinely introduced a number of PPCPs to the environment at concentrations that could range into the μg/L during water reuse applications. In addition, the results clearly show that surface waters in South Florida are impacted by several PPCPs at trace level. Although contamination is not widespread, there are clear indications that wastewater intrusions exist in a number of coastal freshwater and saltwater environments. On a positive side, the presence of endocrine disrupting chemicals was less prevalent and usually at levels very close to the method detection limits so that their potential impacts to biological resources are limited both in temporal and geographical extent. Furthermore, some PPCPs were detected in drinking water samples from the Miami-Dade County distribution system. Concentrations were relatively low and some of the compounds may have alternative sources (i.e. salicylic acid) but the presence of low concentrations of carbamazepine with relatively high frequency of detection is a

conclusive indicator of a connection between wastewater streams and the drinking water sources or the distribution system itself. Because the presence of these compounds in drinking water constitutes a direct route to human exposure, a more detailed survey both in area and time should be conducted.

In summary this study shows that excretion by humans, disposal of unused medicine and application of veterinary drugs does cause PPCPs to enter the sewage collection systems. Low efficiency of removal in the WWTPs leads to the releasing of PPCPs into the environment, in particular in reclaimed water only treated to secondary standards. Despite the fact that most PPCPs are not acutely toxic, their chronic release at low levels combined with their environmental persistence may be cause of potential concern for aquatic organisms in receiving waters under the influence of poorly treated wastewaters. The presence of PPCPs in drinking water is not novel, however in the case of Miami-Dade County where the source for drinking water is the shallow Biscayne Aquifer additional studies should be planned to assure that the source remains isolated from surface or ground waters influenced by treated or untreated wastewater streams. With the increased population growth in South Florida and need for water reuse careful consideration should be given to protect the drinking water sources from further degradation .

In the future, more effective treatments need to be applied to both WWTPs and DWPs to remove PPCPs. Great progress has been accomplished with the use of

reverse osmosis and advanced oxidation processes. Until then, or when forced by a regulatory framework, the concentrations of PPCPs in wastewater effluents targeted for environmental release and drinking water need long term monitoring .

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